**UNIVERSIDADE DE SÃO PAULO
FACULDADE DE CIÊNCIAS FARMACÊUTICAS
Departamento de Alimentos e Nutrição Experimental
Programa de Pós-Graduação em Ciência dos Alimentos
Área de Nutrição Experimental**

Associação entre atividade antioxidante *in vitro* e características químicas, sensoriais, cromáticas e comerciais de vinhos tintos Sul-Americanos

Daniel Granato

Tese para obtenção do grau de
DOUTOR

Orientadora:
Prof^a. Dr^a. Inar Alves de Castro

São Paulo
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Tese para obtenção do grau de DOUTOR

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São Paulo, ____ de _____ de 2011

DEDICATÓRIA

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SUMÁRIO

1. INTRODUÇÃO.....	12
2. REVISÃO BIBLIOGRÁFICA	15
2.1 Estresse oxidativo e antioxidantes.....	15
2.1.1 <i>Estresse oxidativo</i>	15
2.1.2 <i>Antioxidantes</i>	18
2.2 Vinhos sul americanos: regiões produtoras.....	20
2.2.1 <i>Vinhos brasileiros</i>	20
2.2.2 <i>Vinhos argentinos</i>	23
2.2.3 <i>Vinhos chilenos</i>	25
2.3 Vinho e o Paradoxo Francês	28
2.4 Vinho tinto: composição de fenólicos.....	29
2.4.1 <i>Fenólicos não-flavonóides</i>	30
2.4.2 <i>Flavonóides</i>	31
2.4.2.1 <i>Flavonóis</i>	33
2.4.2.2 <i>Flavanóis</i>	34
2.4.2.3 <i>Antocianinas</i>	34
2.5 Metodologias para avaliação da atividade antioxidante.....	36
2.5.1 <i>Metodologia do 2,2-difenil-1-picrilhidrazila (DPPH)</i>	36
2.5.2 <i>Metodologia da capacidade de absorção do radical de oxigênio (ORAC)</i> ..	37
2.6 Análise sensorial de vinhos tintos.....	38
2.7 Quimiometria na ciência e tecnologia do vinho.....	39
2.7.1 <i>Análise por componentes principais (ACP)</i>	40
2.7.2 <i>Análise hierárquica de agrupamentos (AHA)</i>	40
2.7.3 <i>Aplicações de métodos quimiométricos na ciência e tecnologia do vinho</i> ..	41
3. OBJETIVOS	43
4. DESCRIÇÃO DOS CAPÍTULOS	44
5. CAPÍTULO 1.....	46
6. CAPÍTULO 2.....	75
7. CAPÍTULO 3.....	99
8. CONCLUSÕES FINAIS.....	126
9. REFERÊNCIAS BIBLIOGRÁFICAS	128

LISTA DE FIGURAS

Figura 1: Biossíntese geral dos compostos fenólicos	13
Figura 2: Espectro de doenças humanas que podem ser desencadeadas pelo excesso de espécies reativas de oxigênio.....	16
Figura 3: Principais regiões produtoras de uvas e vinhos do Brasil.....	21
Figura 4: Principais regiões produtoras de uvas e vinhos da Argentina.....	25
Figura 5: Principais regiões produtoras de uvas e vinhos do Chile.. ..	27
Figura 6: Principais ácidos fenólicos.....	31
Figura 7: Estruturas químicas de alguns grupos representativos dos flavonóides ..	32
Figura 8: Estrutura química das antocianinas encontradas em vinho tinto.....	35
Figura 9: Reação genérica entre o radical DPPH e o um antioxidante (AH), na formação do 2,2-difenilpicril-hidrazina (DPPH-H), de coloração amarela.....	37

LISTA DE TABELAS

Tabela 1: Produção de vinhos (tintos, brancos e rosê) no Brasil no período de 2004-2009.....	23
Tabela 2: Conteúdo de fenólicos totais, dado em miligramas de ácido gálico-equivalente por litro de vinho (GAE/L), em vinhos tintos de diversas origens.	30
Tabela 3: Quantidade de fenólicos, em mg/L, encontrados em diversos tipos de vinhos em diferentes localidades.....	30

LISTA DE ABREVIATURAS

AAPH	[2,2'-azobis (2-amidinopropano) dihidrocloreto]
ACP	Análise de componentes principais
AHA	Análise hierárquica de agrupamento
CAT	Catalase
CT	Colesterol total
DCNT	Doenças crônicas não-transmissíveis
DPPH	2,2-difenil-1-picrilhidrazila
FRAP	Poder antioxidante de redução do ferro
GPx	Glutathione peroxidase
GR	Glutathione reductase
GST	Glutathione S-transferase
G6DP	Glicose-6-fosfato desidrogenase
HDL	Lipoproteína de alta densidade
LDL	Lipoproteína de baixa densidade
MONICA	Sistema de monitoramento de doenças cardiovasculares
ORAC	Capacidade de absorção do radical de oxigênio
ROS	Espécies reativas de oxigênio
SOD	Superóxido dismutase
TG	Triacilgliceróis

ASSOCIAÇÃO ENTRE ATIVIDADE ANTIOXIDANTE *IN VITRO* E CARACTERÍSTICAS QUÍMICAS, SENSORIAIS, CROMÁTICAS E COMERCIAIS DE VINHOS TINTOS SUL-AMERICANOS

O vinho tinto é rico em compostos fenólicos com atividade antioxidante, capazes de inativar espécies reativas de oxigênio, minimizando danos celulares oriundos do estresse oxidativo, proporcionando uma redução de risco para doenças crônicas não transmissíveis. Assim, os objetivos desta pesquisa foram identificar associações entre a atividade antioxidante *in vitro* e fatores relacionados ao tipo de uva, região de produção, perfil sensorial, safra, valor comercial e concentração de compostos fenólicos de vinhos tintos produzidos no Brasil, Chile e Argentina. Inicialmente, os vinhos brasileiros (n=29) foram avaliados em relação à atividade antioxidante (ORAC e DPPH), cor instrumental e compostos fenólicos majoritários, no intuito de verificar qual classe de fenólicos estaria associada com a atividade antioxidante. Verificou-se que tanto os compostos fenólicos totais como os flavonóides totais, com destaque aos flavonóides não-antociânicos, se associaram significativamente ($p<0,05$) com a atividade antioxidante. Em um segundo passo, as características sensoriais, a cor, o valor comercial e a atividade antioxidante das 80 amostras de vinhos Sul-Americanos, distribuídas em Merlot (n=9), Pinot Noir (n=17), Malbec (n=11), Syrah (n=12), Cabernet Sauvignon (n=24), e vinhos de uvas americanas (*Vitis labrusca*) (n=7) foram avaliados usando estatística multivariada, objetivando-se verificar se a qualidade sensorial das amostras estaria associada com o valor comercial, cor, e à atividade antioxidante. De uma forma geral, os vinhos chilenos e argentinos apresentaram maior atividade antioxidante, valor comercial, intensidade de odor, qualidade sensorial, índice de acidez e taninos, ao passo que os vinhos brasileiros obtiveram os menores valores para os atributos sensoriais. Os vinhos de uvas americanas apresentaram menores valores para todas as variáveis. As variedades Syrah, Malbec e Cabernet Sauvignon apresentaram maior capacidade antioxidante e melhores características sensoriais, sendo que esse resultado foi independente da safra e de procedência. Como última etapa, as amostras de vinhos produzidas com uva *V. vinifera* (n=73) foram classificadas, usando análise hierárquica de agrupamentos, de acordo com o valor comercial, qualidade sensorial e capacidade antioxidante medida por ORAC e DPPH, e os grupos foram caracterizados em relação à sua composição fenólica. Os resultados indicaram que a atividade antioxidante se correlacionou positivamente com o conteúdo de fenólicos totais, flavonóides totais, ácido gálico, quercetina, catequina, ácido ferúlico, miricetina, caempferol, e rutina, sendo que apenas os conteúdos de miricetina, ácido gálico e quercetina foram diferentes ($p<0,05$) entre os grupos. Nenhum composto fenólico avaliado nesse estudo associou-se com as diferenças sensoriais dos grupos de vinho.

Palavras-chave: Vinho tinto. Capacidade antioxidante. Estatística multivariada. Compostos fenólicos.

ASSOCIATION BETWEEN *IN VITRO* ANTIOXIDANT ACTIVITY AND CHEMICAL, SENSORY, CHROMATIC AND COMMERCIAL CHARACTERISTICS OF SOUTH-AMERICAN RED WINES

Red wine is rich in phenolic compounds with antioxidant activity, being able to buffer reactive oxygen species, thus decreasing the risk of non-transmissible chronic diseases. In this regard, the objectives of this research aimed at identifying associations between the *in vitro* antioxidant activity and factors related to grape varietal, region of production, sensory profile, vintage, color, commercial value, and concentration of phenolic compounds of red wines produced in Brazil, Chile, and Argentina. Initially, the Brazilian red wines (n = 29) were assessed in relation to antioxidant activity, instrumental color, and major phenolic compounds with the objective to verify which phenolic class was associated with the antioxidant activity. Both the total phenolic compounds and total flavonoids, with special attention to non-anthocyanin flavonoids, were significantly associated with the antioxidant activity. In a second step, the sensory characteristics, color, commercial value, and antioxidant activity of the 80 red wine samples, which were distributed in Merlot (n=9), Pinot Noir (n=17), Malbec (n=11), Syrah (n=12), Cabernet Sauvignon (n=24), and table wines (*Vitis labrusca*) (n=7) were evaluated using multivariate statistical techniques, with the aim to verify how the overall perception of quality of wines was related to commercial value, color and antioxidant activity. In a general way, the Chilean and Argentinean red wines displayed a higher antioxidant activity, commercial value, intensity of odors, sensory perception of quality, acidity level, and tannin level, whereas the Brazilian samples obtained the lowest values for the sensory attributes. The table wines presented the lowest values for all response variables. Syrah, Malbec and Cabernet Sauvignon varieties presented the highest antioxidant activity and most favorable sensory features, and this result was independent of wine's vintage and origin. As a last step, the wines produced with *V. vinifera* grapes (n=73) were classified, using hierarchical cluster analysis, in relation to commercial value, sensory quality and antioxidant activity measured by ORAC and DPPH assays, and the groups were characterised in relation to the phenolic composition. The results showed that the antioxidant capacity correlated to the total contents of phenolic compounds, flavonoids, gallic acid, quercetin, catechin, ferulic acid, myricetin, kaempferol, and rutin, where only the contents of quercetin, gallic acid, and myricetin were different ($p < 0.05$) within clusters. None of the phenolic compounds analysed in this research was associated with sensory differences within clusters.

Keywords: Red wine. Antioxidant capacity. Multivariate statistics. Phenolic composition.

1. INTRODUÇÃO

O oxigênio molecular e seus radicais são os reagentes mais importantes na bioquímica dos radicais livres nas células aeróbicas. O termo "espécies reativas de oxigênio" (ROS) inclui os radicais livres contendo oxigênio, como o ânion superóxido (O_2^-), o radical hidroxila ($HO\cdot$), o radical peroxila ($ROO\cdot$) e espécies não radicalares como o peróxido de hidrogênio (H_2O_2) e o oxigênio singlete (1O_2), os quais são frequentemente gerados como subprodutos de reações biológicas ou por fatores exógenos (GÜLCIN et al., 2003). Estas ROS podem causar um grande número de desordens celulares ao reagir com lipídeos, proteínas, carboidratos e ácidos nucleicos, estando envolvidas tanto no processo de envelhecimento, como também em muitas complicações biológicas, incluindo inflamação crônica, problemas respiratórios, doenças neurodegenerativas, *Diabetes mellitus*, aterosclerose, doenças auto-imunes das glândulas endócrinas, doenças cardiovasculares, carcinogênese e mutagênese (GÜLCIN et al., 2003; CHANWITHEESUK; TEERAWUTGULRAG; RAKARIYATHAM, 2005).

Antioxidantes são substâncias que retardam ou previnem significativamente a oxidação de lipídios, proteínas ou de outras moléculas ao inibirem a iniciação ou a propagação da reação de oxidação em cadeia (MOREIRA et al., 2002; WU et al., 2005; LIMA et al., 2006), além de prevenirem ou repararem danos ocasionados às células pelas ROS (CHANWITHEESUK et al., 2005). As substâncias com núcleo fenólico, como os flavonóides e ácidos fenólicos, apresentam destaque especial como antioxidantes, por atuarem como eficientes captadores de ROS, além de reduzirem e quelarem íons férrico que catalisam a peroxidação lipídica através da degradação dos hidroperóxidos (NAHAR & SARKER, 2005; DELAZAR et al., 2006).

Os fenóis presentes nos vinhos como a miricetina, quercetina, rutina, e antocianinas apresentam comprovada atividade antioxidante, uma vez que podem seqüestrar radicais livres e, portanto, minimizar danos celulares oriundos do estresse oxidativo, explicando a proteção observada por epidemiologistas contra doenças degenerativas não-transmissíveis (DCNT) (VILLANO et al., 2005). Os fenólicos são produtos do metabolismo secundário das plantas derivados da fenilalanina (SHAHIDI &

NACZK, 2004). A fenilalanina é desaminada até ácido cinâmico, o qual entra na via fenilpropanol, sendo que o passo chave nesta via metabólica é a introdução de um ou mais grupos hidroxila no anel fenil, produzindo assim os fenóis (Figura 1). Como resultado, os compostos fenólicos são derivados de uma mesma estrutura: a unidade fenilpropanol (HOLLMAN, 2001).

Figura 2: Biossíntese geral dos compostos fenólicos. (HOLLMAN, 2001).

Os compostos fenólicos encontram-se basicamente nas cascas e nas sementes. Na casca de uvas tintas, as antocianinas são os compostos fenólicos mais abundantes, ao passo que nas sementes há predominância dos flavan-3-óis (catequina e epicatequina). A quantidade desses compostos no vinho varia tanto na uva como no seu processamento de acordo com alguns fatores, tais como: clima, quantidade de luz e água, estado nutricional e patogênese da videira, natureza e composição química do solo, altitude, incidência solar sobre a videira, variedade e grau de maturação da uva, pH do mosto, adição de dióxido de enxofre, e quantidade de etanol (PENNA; DAUDT; HENRIQUES, 2001; DOWNEY; DOKOOZLIAN; KRSTIC, 2006; CORTELL &

KENNEDY, 2006; PÉREZ-MAGARIN & GONZÁLEZ-SANJOSÉ, 2006). Outros fatores relacionados ao processamento da uva como tempo e temperatura de maceração, temperatura de fermentação e tipo de levedura também afetam quanti e qualitativamente a composição fenólica de vinhos (RAMOS et al., 1999; FREITAS, 2006; LACHMAN; SULC; SCHILLA, 2007; CADAHIA et al., 2009).

Apesar do elevado número de publicações que avaliam a correlação entre a atividade antioxidante e a concentração de diferentes fenólicos nos vinhos tintos, pouco se conhece sobre a diversidade desses compostos fenólicos nos vinhos Sul-Americanos, e como essa diversidade se associa às características sensoriais, cromáticas, e valor comercial desses produtos.

2. REVISÃO BIBLIOGRÁFICA

2.1 Estresse oxidativo e antioxidantes

2.1.1 *Estresse oxidativo*

O oxigênio é vital para a maioria dos organismos, mas, paradoxalmente pode ser fonte de moléculas capazes de danificar sítios biológicos essenciais. Espécies reativas de oxigênio (ROS), de carbono e nitrogênio são continuamente produzidas por meio da geração de energia através da cadeia transportadora de elétrons microssomal e mitocondrial. Fontes exógenas de formação de ROS são representadas pelo tabagismo, poluição, exercício físico intenso, consumo excessivo de álcool e agrotóxicos, dentre outros (FRANKEL, 2005).

As ROS são átomos, moléculas ou íons que contêm um elétron não pareado em seu último orbital. Apresentam grande instabilidade e, conseqüentemente, elevada reatividade (HALLIWEL & GUTTERIDGE, 2000). Tendem a ligar o elétron não pareado com outros presentes em estruturas próximas de sua formação, comportando-se como receptores ou como doadores de elétrons. As principais ROS são hidroxila ($\text{HO}^\bullet$), ânion superóxido ($\text{O}_2^{\bullet-}$), radical peroxila ($\text{ROO}^\bullet$), hidroperoxila ($\text{HO}_2^\bullet$) e alcoxila ($\text{RO}^\bullet$) e os não-radicalares: oxigênio singleto, e peróxido de hidrogênio (LAURINDO, 2005).

Quando presentes em excesso, os radicais livres podem promover alterações em moléculas de DNA, proteínas, carboidratos e lipídios. A oxidação das biomoléculas pode levar a apoptose ou a lesão em tecidos. Evidências epidemiológicas sugerem que essas alterações estão associadas ao desenvolvimento e evolução de diversas patologias como doença de Alzheimer, artrite reumatóide, esclerose, cataratogênese, diversas doenças cardiovasculares e alguns tipos de câncer (SUGIYAMA et al., 2003; NAISSIDES et al., 2004; KAUR et al., 2007; LEE & RENNAKER, 2010). Algumas outras doenças que podem ser associadas aos efeitos deletérios das ROS estão mostradas na Figura 2.

Figura 3: Espectro de doenças humanas que podem ser desencadeadas pelo excesso de espécies reativas de oxigênio. (Adaptada de HALLIWELL, 1987 e HALLIWELL & GUTTERIDGE, 2000).

A oxidação lipídica pode ser iniciada pelas ROS afetando ácidos graxos poliinsaturados. Na iniciação, espécies reativas ou iniciadores ($\text{In}^{\bullet}$) promovem a quebra homolítica da ligação hidrogênio-carbono na dupla ligação do ácido graxo, com a abstração de um hidrogênio alílico na extremidade carboxílico da cadeia, formando um radical acila ($\text{L}^{\bullet}$) (MIYASHITA, 2008). Na presença de oxigênio, este reage diretamente com o radical acil ($\text{L}^{\bullet}$), produzindo um radical peroxila ($\text{LOO}^{\bullet}$), caracterizando assim a etapa de propagação da oxidação. Esses radicais reagem posteriormente com moléculas de lipídios formando os hidroperóxidos (LOOH) ou podem ser neutralizados

por antioxidantes, como o tocoferol e os polifenóis. Alternativamente, eles podem reagir com outros radicais lipídicos formando produtos não-radicalares, caracterizando a reação de terminação (FRANKEL, 2005).

O esquema abaixo resume cada uma das etapas do processo de oxidação (CHAIYASIT et al., 2007):

A lipoperoxidação é provavelmente o processo induzido por radicais livres mais extensivamente investigado, sendo que os mecanismos, dinâmica e produtos da lipoperoxidação *in vitro* têm sido estudados, compreendidos e documentados. Entretanto, os efeitos biológicos e fisiológicos da lipoperoxidação e seus produtos ainda não foram completamente elucidados (NIKI, 2009). A presença abundante de fosfolipídios de membrana em locais onde os radicais livres são formados, tornam-os alvos endógenos facilmente acessíveis e rapidamente afetados pelos radicais livres (de ZWART et al., 1999). Dessa forma, a lipoperoxidação promove alterações na integridade, fluidez, e permeabilidade das membranas, levando à perda de sua função, além de gerar produtos potencialmente adversos à saúde (NIKI, 2009).

Quando os níveis de ROS oriundos da lipoperoxidação e de outras reações químicas no organismo estão aumentados ou quando os mecanismos antioxidantes não são acessíveis ou perdem a eficiência para tamponar o efeito dessas espécies, ocorre o estresse oxidativo (LAURINDO, 2005). O estresse oxidativo modifica o balanço redox por meio de potenciais oxidativos, podendo causar danos às moléculas biológicas e consequentes alterações da função celular ou mesmo a morte das células.

Em caso de aumento de ROS, as células respondem com um eficiente e sofisticado sistema de defesa antioxidante controlando a formação de radicais livres e

limitando seus efeitos prejudiciais. No citoplasma, os principais mecanismos de defesa são enzimáticos, enquanto no plasma, pequenas moléculas respondem pela maior parte do poder antioxidante (FÖRSTERMANN, 2008). Esse sistema de defesa conta com a participação de proteínas quelantes de metais, enzimas antioxidantes, as proteínas de choque térmico e agentes de baixo peso molecular que apresentam atividade “*scavenger*” de ROS, como glutathiona, α -tocoferol, ácido ascórbico, bilirrubina, ácido úrico e compostos polifenólicos, como os flavonóides e ácidos benzóicos (FÖRSTERMANN, 2008).

2.1.2 Antioxidantes

Antioxidantes são quaisquer substâncias que, quando presentes em baixas concentrações em relação ao substrato oxidável, retardam ou inibem a oxidação desse substrato, impedindo ou diminuindo o estresse oxidativo e o conseqüente dano celular (HALLIWELL & GUTTERIDGE, 2000). Compostos com atividade antioxidante estão presentes nas células e nos tecidos. Alguns são sintetizados pelo organismo, enquanto outros podem ser obtidos de fontes exógenas, tais como dieta. Entre os principais antioxidantes exógenos conhecidos estão o ácido ascórbico e os tocoferóis, os carotenóides, e os compostos fenólicos (MOREIRA et al., 2002; LI et al., 2008).

A atividade dos antioxidantes frente às ROS pode ser na forma de “*radical scavengers*”, “*chain-breaking*” ou mesmo os dois combinados, dependendo do composto e da matriz em oxidação (KALIORA & DEDOUSSIS, 2007). Compostos com ação “*radical scavenger*” atuam na inativação de espécies reativas na fase de iniciação, enquanto os “*chain-breaking*” reagem com os radicais lipídicos já formados, interrompendo a fase de propagação. Além de doar átomos de hidrogênio aos radicais livres, os antioxidantes podem atuar como quelantes de íons metálicos que também estão envolvidos na produção de ROS (CHAILLOU & NAZARENO, 2006).

A atividade antioxidante dos compostos fenólicos encontrados no vinho tinto deve-se principalmente às suas propriedades redutoras e estrutura química nucleofílica, sendo que alguns mecanismos têm sido propostos: interrupção da reação em cadeia da peroxidação lipídica, por meio da sua reação com algumas espécies radicalares; reação

com metais pró-oxidantes, tais como ferro e cobre, que são conhecidos por favorecer a formação dos radicais livres; supressão da peroxidação lipídica pela reciclagem de outros antioxidantes como o tocoferol e a preservação da atividade da paraoxonase associada à lipoproteína de alta densidade (HDL), a qual é capaz de proteger a lipoproteína de baixa densidade (LDL) contra a oxidação (KALIORA; DEDOUSSIS; SCHMIDT, 2006). Estas características desempenham um papel importante na neutralização ou seqüestro de radicais livres e quelação de metais de transição, agindo tanto na etapa de iniciação como na propagação do processo oxidativo. Os intermediários formados pela ação de antioxidantes fenólicos são relativamente estáveis, devido à ressonância do anel aromático presente na estrutura destas substâncias (SOUSA et al., 2007).

Os antioxidantes participam de reações adicionais que removem radicais livres do sistema. Isto significa que cada molécula de antioxidante pode inativar pelo menos dois radicais livres: o primeiro sendo inativado quando o antioxidante (AH) doa um hidrogênio para um radical peroxila ($LOO^{\bullet}$), e o segundo quando o radical antioxidante ($A^{\bullet}$) entra em uma reação de terminação com outro radical peroxila ($LOO^{\bullet}$), formando um composto estável, fazendo assim que essas espécies altamente deletérias sejam neutralizadas (DECKER, 2002). Flavonóides e outros compostos fenólicos como os ácidos cinâmicos e benzóicos, possuem pelo menos uma hidroxila ligada ao anel benzênico, o que caracteriza esses compostos como sendo nucleofílicos, ou seja, compostos fenólicos 'ricos em elétrons' doam elétrons aos compostos eletrofílicos, no caso os radicais livres, caracterizando os compostos fenólicos do vinho como hábeis antioxidantes (AL-AZZAWIE & MOHAMED-SAIEL, 2006).

Os organismos aeróbios somente conseguem sobreviver na presença do oxigênio devido às defesas antioxidantes. Essas defesas diferem de tecido para tecido e de célula para célula. As defesas antioxidantes podem ser induzidas por exposição do organismo às ROS e por sinais celulares de moléculas, tais como as citocinas. No entanto, essa defesa pode ser incompleta, ou seja, não previne completamente o dano oxidativo (BLOKHINA; VIROLAINEN; FAGERSTEDT, 2003).

Os mecanismos de defesa antioxidante endógenos são formados por enzimas, tais como a superóxido dismutase (SOD), a catalase (CAT), glutational peroxidase

(GPx), glutathione S-transferase (GST) e outras que não participam diretamente do processo, mas fornecem suporte à GPx, como a glicose-6-fosfato desidrogenase (G6PD) e a glutathione redutase (GR). Além dessa defesa enzimática, existem ainda antioxidantes não-enzimáticos, como o α -tocoferol (vitamina E), ácido ascórbico (vitamina C), flavonóides e outras moléculas, como o β -caroteno e N-acetilcisteína (RITTER et al., 2004).

2.2 Vinhos sul americanos: regiões produtoras

2.2.1 Vinhos brasileiros

A viticultura é uma atividade econômica recente no Brasil, quando comparada aos tradicionais países produtores da Europa, especialmente no que se refere aos vinhos finos. A produção de uvas, no Brasil, está localizada nas regiões Sul, Sudeste e Nordeste. Constitui-se em atividade consolidada, com importância sócio-econômica, no estado do Rio Grande do Sul, que responde por mais de 97% da produção nacional de vinhos. Cerca de 50% da produção nacional de uva é destinada à elaboração de vinhos, sucos, destilados e outros derivados, chegando a 190 milhões de litros de vinhos tintos produzidos somente no estado do Rio Grande do Sul (FREITAS, 2006).

Em relação à qualidade, o vinho brasileiro ainda tem dificuldade para alcançar o apelo sensorial dos consumidores mais exigentes. A raiz desse problema está, entre outros fatores, na pouca idade das parreiras, que influencia diretamente a composição química das uvas (FIELDEN, 2001). O plantio de uvas americanas em lugar de uvas européias (*Vitis vinifera*) e o consumo desse vinho são outros aspectos que influenciam até hoje o cenário da produção de vinhos no Brasil (VIOTTI, 2010). Se por um lado esses fatores formaram as bases para a produção de uvas em volume suficiente para a estruturação de uma indústria vinícola moderna, rentável e versátil, como a atual, por outro lado serviram para diferenciar e isolar ainda mais o vinho que se bebia no Brasil daquele que se fazia no restante do mundo. O Brasil é o único país que insiste em utilizar uvas americanas para a produção de vinho de mesa (VIOTTI, 2010).

Como zonas de viticultura temperada (Figura 3) destacam-se as regiões da Fronteira, Serra do Sudeste, Serra Gaúcha, Campos de Cima da Serra e regiões Central e Norte do Estado do Rio Grande do Sul; as regiões do Vale do Rio do Peixe, Planalto Serrano e Planalto Norte e Carbonífera, no Estado de Santa Catarina; a região Sudeste do Estado de São Paulo e, a região Sul do Estado de Minas Gerais. A região Norte do Paraná é tipicamente subtropical e as regiões Noroeste do Estado de São Paulo, Norte do Estado de Minas Gerais e Vale do Sub-Médio São Francisco (Pernambuco e Bahia), caracterizam-se como zonas tropicais, com sistemas de manejo adaptado às suas condições ambientais específicas. Além destes, novos pólos vitivinícolas estão surgindo em diferentes regiões do país, seja sob condições temperadas, tropicais ou subtropicais. A viticultura brasileira desenvolvida sob condições temperadas segue, em geral, os mesmos procedimentos utilizados em países tradicionais no cultivo da videira. Já nas regiões de clima quente, adaptaram-se técnicas de manejo a cada situação específica, sendo que os ciclos vegetativos e de produção são definidos em função das condições climáticas e das oportunidades e exigências do mercado (FIELDEN, 2001).

Figura 4: Principais regiões produtoras de uvas e vinhos do Brasil. (Academia do vinho, 2010).

Destaca-se como emergente da vitivinicultura brasileira de clima temperado a região do Planalto Catarinense, estruturada em três pólos produtores: São Joaquim, Campos Novos e Caçador. Situam-se entre as latitudes 26° e 28°S e entre as longitudes 50° e 52°W, com altitude variando entre 900 e 1.400 m. Este pólo produtor está voltado exclusivamente ao cultivo de castas de *Vitis vinifera*, para a produção de vinhos finos. Os primeiros vinhedos foram plantados na região em 2001, chegando, em 2007 a uma área aproximada de 180 hectares. Entre as principais variedades cultivadas encontram-se as tintas Cabernet Sauvignon, Merlot, Cabernet Franc, Pinot Noir e Malbec (IBRAVIN, 2010). Existem iniciativas vitícolas em várias regiões do Brasil tropical, com destaque para as regiões Nordeste, nos Estados de Pernambuco, Bahia, Ceará, Maranhão e Piauí, Centro-Oeste, nos Estados do Mato Grosso e Goiás e Sudeste, nos Estados de Minas Gerais e Espírito Santo. Em sua maioria são ainda empreendimentos de pequeno porte, voltados principalmente à produção de uvas de mesa (*Vitis labrusca*) (FIELDEN, 2001).

No Brasil, a serra gaúcha é a mais importante região vitivinícola destacando-se a região de Bento Gonçalves (Figura 3). Apresenta um clima úmido, temperado e quente, com noites temperadas e zonas caracterizadas por topoclimas determinados pelas diferenças de altitudes (cerca de 500 m) (FREITAS, 2006). Outra região muito importante é a Campanha, localizada na fronteira gaúcha, que é caracterizada por invernos rigorosos e solo com baixa fertilidade natural. A topografia também ajuda na produtividade em função de ser plana, possibilitando a mecanização das lavouras. As uvas são todas oriundas de cepas *V. vinifera* sendo as principais Cabernet Sauvignon, Merlot, Tannat, Tempranillo, e Touriga Nacional nas tintas (ACADEMIA DO VINHO, 2010). Como coadjuvantes há a Cabernet Franc (em declínio) sendo seguida por cepas que vêm ganhando bastante espaço como a Egidola, Ancelota, e Marselan. Ao todo, o Rio Grande do Sul possui cerca de 320 produtores de vinhos. Apesar das dificuldades climáticas e de solo, os produtores têm investindo expressivamente nos vinhedos, em tecnologia, em conhecimento e desenvolvimento de novos produtos, buscando competir no mercado internacional (IBRAVIN, 2010).

A produção de vinho de mesa, oriundo de cepas de *Vitis labrusca*, ainda constitui a maior parte de vinho produzido no Brasil (Tabela 1).

Tabela 1: Produção de vinhos (tintos, brancos e rosê) no Brasil no período de 2004-2009 (Adaptado de IBRAVIN, 2010).

Milhões de litros			
Ano	Vinhos finos (<i>Vitis vinifera</i>)	Vinhos de mesa (<i>Vitis labrusca</i>)	Total
2004	42,96	313,70	356,66
2005	45,45	226,08	271,53
2006	32,12	185,08	217,20
2007	43,18	275,25	318,43
2008	47,33	287,44	334,77
2009	39,90	205,42	245,32

De acordo com o Instituto Brasileiro de Vinho (IBRAVIN, 2010), o consumo per capita no Brasil, por ano, não chega a 2 litros, enquanto esse índice é cerca de dez vezes maior em países europeus.

2.2.2 Vinhos argentinos

Até o século XVIII, o vinho argentino era somente produzido com uvas americanas, mas a partir do século XIX, a indústria começou a crescer graças à influência dos imigrantes italianos e espanhóis que trouxeram ao país novas videiras e importantes técnicas vitícolas e de produção de vinhos. A introdução de variedades européias como a Malbec, Cabernet Sauvignon, Merlot e Chenin Blanc, melhorou substancialmente a qualidade do vinho argentino. Foram então os colonizadores italianos e espanhóis os que formaram a base da viticultura e da produção de vinhos na Argentina, outorgando à área a riqueza cultural que possui atualmente (ENCICLOPÉDIA DO VINHO, 2010).

A Argentina é o quinto maior produtor e o quinto maior país consumidor mundial de vinhos (16,80 L/per capita/ano) tendo ocupado a quarta posição na década de oitenta (OFFICE INTERNATIONAL DE LA VIGNE ET DU VIN, 2009). As 879 vinícolas de Mendoza produzem 70% do vinho argentino seguido de San Juan (22%), sendo que mais de 96% do vinho exportado são dessas duas regiões (FANZONE et al., 2010). A Argentina possui aproximadamente 210 mil hectares de vinhas plantadas, distribuídas

em várias regiões produtoras (Figura 4), sendo as mais importantes Mendoza, Salta, La Rioja, San Juan e Rio Negro. Ao todo, o país produz três milhões de caixas anuais e exporta 25% da sua produção (ACADEMIA DO VINHO, 2010). A Argentina possui todas as condições desejadas para a produção de vinhos com excelente qualidade sensorial: uma boa exposição solar, o contraste entre dias quentes e noites frescas, e antigos vinhedos de alta densidade (FIELDEN, 2001).

A província de Mendoza está localizada no centro oeste do país e faz divisa ao norte com San Juan, ao leste com a província de San Luis, ao sul com La Pampa e Neuquén e fronteira a oeste com o Chile. A maioria dos vinhedos usa métodos de irrigação que vão desde as tradicionais acequias (canais que levam a água do degelo), passado por diques ou o mais atual, a irrigação por gotejamento. O clima muito seco nas regiões de cultivo, os ventos fortes e as características dos solos resultam em uvas ótimas para a produção de vinhos, sendo que as principais uvas cultivadas são Malbec, Bonarda, Cabernet Sauvignon, Merlot, Syrah, Sangiovese, e Tempranillo, dentre os quais o Malbec é o mais conhecido internacionalmente por seus atributos sensoriais. Além disso, essa varietal corresponde a cerca de 30% do total de vinhos produzidos na Argentina.

Figura 5: Principais regiões produtoras de uvas e vinhos da Argentina. (Academia do vinho, 2010).

2.2.3 Vinhos chilenos

Segundo a Enciclopédia do Vinho (2010), as primeiras videiras plantadas no Chile foram trazidas em meados de 1550 pelos missionários da Espanha, que queriam produzir vinhos de mesa e para as missas. Essas variedades espanholas, particularmente Pais e Moscatel (produzido ainda hoje), produziram vinhos chilenos por vários séculos. Os produtores utilizavam técnicas primitivas (os vinhos eram adocicados e estabilizados, freqüentemente fervidos) para produzir vinhos rústicos. Com o início da importação das variedades dos grandes vinhos de Bordeaux, os chilenos descobriram que podiam produzir uma classe superior dos vinhos, e a era moderna de produção começou. A partir de 1990 houve um grande impulso à modernização, os investidores instalaram equipamentos de produção avançados, e trouxeram uma nova geração de

profissionais do vinho formados em universidades. O resultado de tanto investimento em tecnologia e pessoal especializado foi o aumento do índice de exportação, chegando em algumas vinícolas até 99% da produção. Como o mercado externo valoriza a qualidade dos vinhos, isso faz com que o produto chileno esteja presente nos Estados Unidos, Canadá, Inglaterra, Japão, Holanda, Dinamarca, Brasil, entre outros países (ENCICLOPÉDIA DO VINHO, 2010).

O Chile é o país mais tradicional, e segundo maior consumidor de vinho da América do Sul, com uma taxa de 16,11 L/per capita/ano (OFFICE INTERNATIONAL DE LA VIGNE ET DU VIN, 2009). Segundo Viotti (2010a), o Chile está entre os dez maiores produtores de vinho do mundo, com cerca de 9 milhões de hectolitros anuais, e em relação à exportação, o país posiciona-se em sexto lugar no volume de litros exportados. Com relação à qualidade do vinho, o Chile é o produtor sul-americano com maior qualidade, ganhando da Argentina e do Brasil. A raiz desta constatação está na necessidade de atender aos requisitos de qualidade exigidos pelos consumidores internacionais (VIOTTI, 2010).

O país possui muitas áreas de produção de vinhos (Figura 5) oriundos de *V. vinifera*, ao passo que vinhos de mesa não são produzidos. O Chile apresenta uniformidade de solo e clima, tornando desnecessária a criação de um complexo sistema de denominações de origem, como acontece na França, Itália ou Portugal. As principais regiões são o Vale de Maipo, Aconcagua, e Vale de San Antonio (ACADEMIA DO VINHO, 2010).

Figura 6: Principais regiões produtoras de uvas e vinhos do Chile. (Academia do vinho, 2010).

O Vale de Maipo é a única região vinícola do mundo com vinhedos nos limites urbanos de uma capital de 5,5 milhões de habitantes. O vale abriga o maior número de vinícolas do Chile, muitas delas com uma longa tradição vinícola e caves do século XIX. Os vinhedos atingem desde os sopés dos Andes, onde os mais tradicionais e apreciados Cabernets do país são produzidos, até o planalto central. Seu clima mediterrâneo é estável, com estações bem definidas e baixo risco de chuvas durante o período da colheita, o que garante condições ideais para o plantio de vinhedos e a produção de vinhos com propriedades sensoriais bem definidas e apreciadas pelos consumidores internacionais. As variedades tintas que predominam nesta região são Cabernet Sauvignon, Merlot, e Carmenère.

O Aconcágua, batizado com o nome do mais alto pico dos Andes (6958 m), forma o vale mais ao sul do Chico Norte. Situa-se 180 km ao norte de Santiago entre a Cordilheira dos Andes e o Oceano Pacífico. É irrigado pelas águas do degelo dos Andes e produz 50% dos vinhos chilenos para exportação. Essa região oferece

excelentes condições para o cultivo de uvas tintas, principalmente a Cabernet Sauvignon (cerca de 70% da produção chilena dessa uva se origina desta região) (ACADEMIA DO VINHO, 2010). O clima é estável, com alta insolação e baixo risco de geadas, condições que permitem a elaboração de vinhos de grande qualidade. As variedades tintas que predominam nessa região são Cabernet Sauvignon, Merlot, Syrah, e Carmenére.

Os vales litorâneos de San Antonio formam a região vinícola mais próxima do oceano. Os vinhedos estão localizados em áreas entre montanhas da área litorânea, parcialmente protegidos da influência do mar. Essas condições criam um microclima bastante peculiar, propiciando o cultivo de uvas de clima frio como a tinta Pinot Noir, com excelente tipicidade.

2.3 Vinho e o Paradoxo Francês

O vinho é uma bebida muito apreciada e desempenha papel importante na economia de diversos países do mundo como Itália, Espanha, França, China e Brasil. É uma matriz muito complexa, e entre os muitos componentes que atribuem qualidade e valor nutricional estão os flavonóides, fenólicos não-flavonóides, lantanídeos, cálcio, cromo, cobalto, potássio, selênio e zinco (GALGANO et al., 2008). É uma das mais importantes fontes de antioxidantes polifenólicos cientificamente aceita, e tem recebido atenção especial devido aos seus efeitos inibitórios contra a oxidação do colesterol LDL tanto em ensaios *in vitro* quanto *in vivo*. Como a oxidação dessa lipoproteína tem papel importante no desenvolvimento da doença cardiovascular aterosclerótica, o consumo moderado do vinho tinto é abordado no estudo da prevenção de eventos cardiovasculares (CACCETTA et al., 2000; TEDESCO et al., 2000; PIGNATELLI et al., 2000), além do potencial inibidor do desenvolvimento de certos tipos de câncer e doenças inflamatórias (BROWNSON et al., 2002; LUCERI et al., 2002).

Em 1992, Renaud e Logeril introduziram o 'Paradoxo Francês' para justificar a baixa taxa de mortalidade em relação à isquemia coronariana entre franceses, que consomem altas doses de gorduras saturadas em sua dieta regular. Os autores atribuíram esse efeito ao consumo regular de vinho tinto baseado nas descobertas do MONICA (sistema de monitoramento de doenças cardiovasculares), um projeto de

pesquisa organizado pela Organização das Nações Unidas. Esse projeto envolveu pesquisadores de 21 países e mais de 7 milhões de pessoas (idade entre 35 e 64 anos) por um período de 10 anos (1980 – 1990). Os organizadores descobriram que a França apresentava menor mortalidade por doenças cardiovasculares do que os Estados Unidos e Reino Unido, por exemplo. Os fatores de risco como alta pressão arterial, índice de massa corpórea, e o hábito de fumar eram equivalentes nesses países, sendo que o consumo de vinho tinto era o fator que diferenciava as três populações avaliadas.

2.4 Vinho tinto: composição de fenólicos

O interesse por parte dos produtores de vinhos no conteúdo de polifenóis das uvas é cada vez maior, já que esses compostos influenciam diretamente a cor, amargor, adstringência e corpo. A médio e longo prazo, esse interesse tende a ficar mais marcante em posse do conhecimento de que os fenólicos também apresentam diversas potenciais atividades biológicas como capacidade antioxidante, antiinflamatória, anti-aterosclerótica, efeitos cardioprotetores e anticancerígenos (FRESCO et al., 2006; SOLEAS et al., 2006).

Os compostos fenólicos encontrados em uvas e vinhos podem ser separados em dois grandes grupos em razão da similaridade de suas cadeias de átomos de carbono: não-flavonóides (fenóis simples ou ácidos) e flavonóides (FERNÁNDEZ-PACHÓN et al., 2006). Os flavonóides mais comuns nos vinhos, em ordem crescente de concentração, são os flavonóis (quercetina, caempferol e mircetina), flavan-3-óis (catequina, epicatequina e os taninos) e as antocianinas. Dentro da classe dos não-flavonóides estão os derivados dos ácidos cinâmicos e benzóicos, encontrados, freqüentemente, na forma de ésteres de ácido tartárico. Diversas pesquisas têm demonstrado atividade antioxidante, antiproliferativa, antifúngica, e antibacteriana de vinhos tintos e sua correlação com o conteúdo de compostos fenólicos (ARNOUS; MAKRIS; KEFALAS, 2002; FERNÁNDEZ-PACHÓN et al., 2006; RIVERO-PEREZ, MUNIZ & GONZALEZ-SANJOSE, 2008; LI et al., 2009; ALÉN-RUIZ et al., 2009).

Como já foi citado anteriormente, diversos fatores ambientais, agrônômicos, tecnológicos e genéticos, bem como o *terroir* típico de cada vinícola contribuem

fortemente para as diferenças observadas em propriedades físico-químicas, sensoriais, cor, atividade antioxidante, e principalmente em compostos fenólicos, como pode ser observado nas Tabelas 2 e 3.

Tabela 2: Conteúdo de fenólicos totais, dado em miligramas de ácido gálico-equivalente por litro (GAE/L), em vinhos tintos de diversas origens.

País	Fenólicos totais (mg GAE/L)	Referência
Croácia	2809 – 4989	Katalinic et al. (2004); Piljac et al. (2005)
República Tcheca	874 – 2262	Stratil et al. (2008)
França	1018 – 3545	Landroult et al. (2001)
Grécia	1217 – 3722	Arnous et al. (2002); Roussis et al. (2005)
Itália	3314 – 4177	Minussi et al. (2003)
Japão	1810 – 2151	Sato et al. (1996)
Polônia	2200 – 3200	Tarko et al. (2008)
Espanha	1010 – 3292	Sanchez-Moreno et al. (1999); Villano et al. (2006)
Eslovênia	1637 – 2717	Košmerl e Cigic (2008)
Estados Unidos	2011 – 4246	Yang et al. (2009)
China	1402 – 3130	Li et al. (2009)
Uruguai	1119 - 1826	González-Neves et al. (2010)
Argentina	1932 - 3507	Fanzone et al., (2010)
Brasil	1142 - 2574	Minussi et al. (2003); Freitas (2006)

Tabela 3: Quantidade de fenólicos, em mg/L, encontrados em diversos tipos de vinhos em diferentes localidades

Substância (mg/L)	Espanha ¹	Espanha ²	Grécia ³	Grécia ⁴	África do Sul ⁵	Eslováquia/Austria ⁶
ác. Gálico	15,11 a 27,21	7 a 14	NE	NE	20,6 a 36,7	38 a 150
ác. Caféico	3,74 a 7,74	5 a 15	NE	30 a 42	7,7 a 33,1	9 a 27
AC. Cumárico	0,22 a 2,77	2 a 12	NE	NE	5,7 a 8,3	1 a 6
Resveratrol	NE	NE	NE	3 a 6	0,7 a 2,1	NE
Quercetina	8,45 a 25,57	0,5 a 4	0,38 a 2,14	NE	4,9 a 15	NE
Rutina	NE	NE	0,28 a 25	3 a 10	NE	NE
Catequina	17,70 a 30,77	19 a 37	2 a 12	NE	31,8 a 57,5	NE
Epicatequina	9,24 a 14,87	2 a 20	15 a 114	30 a 41	17,1 a 37,5	NE
Miricetina	0,32 a 1,47	1 a 5	8 a 68	22 a 50	4 a 8	NE
Kaempferol	0,37 a 1,72	0 a 3	NE	NE	1 a 2,5	NE
ác. Ferrúlico	0,47 a 0,81	0	NE	NE	0,4 a 0,6	NE
ác. Vanílico	1,71 a 2,99	5 a 10	NE	0,1 a 1,5	3,6 a 5,9	NE

NOTA: ¹ Rodríguez-Delgado et al. (2002); ² García-Falcón et al. (2007); ³ Sakkiadi et al. (2001); ⁴ Proestos et al. (2005); ⁵ Villiers et al. (2005); ⁶ Stasko et al. (2008); NE= não especificado.

2.4.1 Fenólicos não-flavonóides

Os compostos não-flavonóides compreendem os ácidos benzóicos e cinâmicos (Figura 6), e outros derivados fenólicos como os estilbenos (resveratrol). Nas uvas, os

ácidos fenólicos são principalmente os ácidos hidroxicinâmicos que se encontram nos vacúolos da célula da película e da polpa, sob a forma de ésteres tartáricos. Os ácidos fenólicos se encontram distribuídos na casca e na polpa da uva, e seus teores diminuem com o amadurecimento, podendo ser utilizados para discriminação de variedades (MACHEIX et al., 1991). Nos vinhos, esses compostos fenólicos têm sido freqüentemente associados com o aroma e adstringência (BLANCO et al., 1998).

ACIDOS HIDROXICINÂMICOS	
	<i>p</i> -Cumárico	Posição do OH 4
	Caféico	3,4
	Ferúlico	4,3-OCH ₃

ACIDOS HIDROXIBENZÓICOS	
	Salicílico	Posição do OH 2
	Gálico	3,4,5
	Siríngico	4,3,5-OCH ₃

Figura 7: Principais ácidos fenólicos. (HOLLMAN, 2001).

2.4.2 Flavonóides

Os flavonóides são compostos fenólicos que se caracterizam por um esqueleto básico e comum C6-C3-C6. A estrutura base consiste em dois anéis aromáticos ligados por um anel pirano. Esta classe de compostos fenólicos pode-se dividir em famílias que se distinguem pelo grau de oxidação do anel pirano: flavonas, flavonóis, isoflavonas, flavanóis, antocianinas, proantocianidinas e flavononas (SCALBERT & WILLIAMSON, 2000) (Figura 7).

FLAVONAS	Posição do OH
Luteonina	5,7,3',4'
Apigenina	5,7,4'
Crisina	5,7

FLAVONÓIS	Posição do OH
Quercetina	3,5,7,3',4'
Kaempferol	3,5,7,4'
Miricetina	3,5,7,3',4',5'

FLAVANÓIS	Posição do OH
Catequina	3,5,7,3',4'
Epicatequina	3,5,7,3',4'

FLAVANONAS	Posição do OH
Naringenina	5,7,4'
Taxifolina	3,5,7,3',4'

ANTOCIANIDINAS	Posição do OH
Delfinidina	3,5,7,3',4',5'
Cianidina	3,5,7,3',4'
Pelargonidina	3,5,7,4'

Figura 8: Estruturas químicas de alguns grupos representativos dos flavonóides. (SCALBERT & WILLIAMSON, 2000).

Grande parte da estrutura e da cor dos vinhos deve-se a esta família de compostos que se encontram nas grainhas, na polpa e na película das uvas. De todos eles, as antocianinas, os flavanóis e as proantocianidinas (2-50 moléculas de flavonol com adição ou não de antocianinas) se destacam (PASTRANA-BONILLA et al., 2003). Os flavonóides vêm despertando um crescente interesse devido aos estudos que mostram uma relação inversa entre o seu consumo e o risco de doenças crônicas não-transmissíveis como o câncer e doenças cardiovasculares (MIDDLETON & KANDASWAMI, 1994; ANDERSON et al., 2000). Esta possível proteção pelos flavonóides é atribuída à sua ação como antioxidantes, devido a suas propriedades seqüestradoras de oxigênio singlete, quelantes de metais ou doadores de hidrogênio, sendo potentes inativadores de radicais livres (RICE-EVANS; MILLER; PAGANGA, 1996). São também antitrombóticos, através da redução da agregação plaquetária (FREEDMAN et al., 2001); moduladores da síntese de óxido nítrico pelo endotélio vascular, levando ao vaso-relaxamento (FREEDMAN et al., 2001); anti-mutagênicos, interrompendo vários estágios do processo carcinogênico (MELO et al., 2010).

2.4.2.1 Flavonóis

Flavonóis são uma das maiores subclasses de flavonóides e possuem um anel C, com dupla ligação na posição C2-C3 (Figura 7). Estão presentes principalmente na casca na forma de monoglicosídeos com um resíduo de açúcar ligado ao radical hidroxila (HERRMANN, 1976). Dentre os flavonóis isolados de vinhos tintos, com conhecida atividade biológica, estão a quercetina, miricetina, e caempferol, compostos que possuem atividade antioxidante e antiestamínica (STECHER et al., 2001). A quercetina está presente na casca da uva e é reconhecida como inibidora de carcinogênese *in vivo* (FLAMINI, 2003) e por sua atividade antioxidante *in vivo* (STECHER et al., 2001).

2.4.2.2 Flavanóis

Dentre os flavanóis (Figura 7) destacam-se os flavan-3-óis, que caracterizam-se por possuírem um anel heterocíclico saturado. Os carbonos C2 e C3 constituem o centro assimétrico da molécula. Os principais flavan-3-óis encontrados nos vinhos são a (+)-catequina e a (-)-epicatequina, que são epímeros no carbono C3. Nas películas das uvas, a (+)-catequina é usualmente o flavan-3-ol mais representativo e a (-)-epicatequina aparece em menor quantidade. Esses compostos são responsáveis pela adstringência, amargor e corpo dos vinhos (KENNEDY; SAUCIER; GLORIES, 2006).

2.4.2.3 Antocianinas

As antocianinas são pigmentos responsáveis pela coloração vermelha, azul e roxa de uma grande variedade de flores e muitas frutas escuras como a framboesa, amora, cereja e uva, sendo que nas últimas estão concentradas predominantemente na casca (PASTRANA-BONILLA et al., 2003). As antocianinas oriundas da uva são glicosiladas, e podem ser esterificadas por diferentes ácidos orgânicos (RIVERO-PEREZ et al., 2008). Os pigmentos antociânicos majoritários em uvas são malvidina-3-glicosídeo, petunidina-3-glicosídeo, cianidina-3-glicosídeo, delphinidina-3-glicosídeo, peonidina-3-glicosídeo (KELEBEK et al., 2006), sendo que seu teor depende da variedade, e principalmente das condições climáticas e agrícolas expostas na produção das uvas (HERRMANN, 1976). As diferentes antocianinas diferem apenas nos grupamentos ligados aos anéis nas posições 3' (R1), 4' (R2), 5' (R3), 3 (R4), 5 (R5), 6 (R6) e 7 (R7), que podem ser átomos de hidrogênio, hidroxilas ou metoxilas, como mostra a Figura 8.

Figura 9: Estrutura química das antocianinas encontradas em vinho tinto. (PASTRANA-BONILLA et al., 2003).

As antocianinas são de grande interesse nutricional, não apenas por seu consumo diário elevado (180 - 215 mg/dia nos Estados Unidos) em suplementos alimentares, mas também por sua potencial capacidade antioxidante, antineoplásica, antiinflamatória, anticarcinogênica, antiviral e antibacteriana (VAREED et al., 2006; PEDRESCHI & CISNEROS-ZEVALLOS, 2007). Evidências epidemiológicas, bem como estudos em modelos *in vivo* e *in vitro*, sugerem que o consumo crônico de antocianinas está associado com a proteção contra doenças coronarianas (GARCÍA-ALONSO et al., 2004; TOUFEKTSIAN et al., 2008), e diminuição da incidência e proliferação de células cancerígenas (KAMEI et al., 1995). Apresentam também efeitos positivos na indução de produção de insulina em células pancreáticas isoladas (JAYAPRAKASAM et al., 2005), supressão de respostas inflamatórias (TALL et al., 2004) e proteção de neurônios frente aos efeitos deletérios do álcool (GUO et al., 2007).

2.5 Metodologias para avaliação da atividade antioxidante

A capacidade antioxidante de vinhos tem sido demonstrada em sistemas biológicos *in vivo* e *in vitro*, sendo geralmente um atributo de bioatividade dos compostos polifenólicos, especialmente os flavonóides (VILLANO et al., 2006; LI et al., 2009; ALÉN-RUIZ et al., 2009). Segundo esses autores, o teor de fenólicos totais em vinhos está diretamente associando com a atividade antioxidante utilizando as metodologias de 2,2-difenil-1-picrilhidrazila (DPPH), 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) e capacidade de absorção do radical de oxigênio (ORAC).

Em decorrência da grande diversidade química existente entre compostos antioxidantes, muitas metodologias de avaliação *in vitro* da atividade antioxidante de amostras têm sido desenvolvidas, sendo que os mais utilizados são o ORAC, poder antioxidante de redução do ferro (FRAP), ensaios utilizando o radical DPPH, e inibição da peroxidação de lipídeos (OLIVEIRA et al., 2009). Esses métodos diferem entre si pelos princípios de reação e condições experimentais, pelas moléculas-alvo, e pelo modo de expressar os resultados. Uma vez que muitos mecanismos e reações múltiplas estão envolvidos, nenhum método irá refletir precisamente todos os antioxidantes em uma matriz complexa, como é o caso do vinho. Assim, como não há um procedimento universal para avaliar a atividade antioxidante do vinho tinto, é necessário utilizar diferentes ensaios que utilizem mecanismos e fundamentos diferenciados (LI et al., 2009; OLIVEIRA et al., 2009). Entretanto, pesquisas recentes sugerem elevada correlação entre os métodos ORAC e DPPH na avaliação da capacidade antioxidante de vinhos tintos e de compostos fenólicos, indicando a adequação e reprodutibilidade de tais metodologias (RIVERO-PEREZ et al., 2008).

2.5.1 Metodologia do 2,2-difenil-1-picrilhidrazila (DPPH)

Um dos métodos mais usados para verificar a capacidade antioxidante consiste em avaliar a atividade seqüestradora do radical 2,2-difenil-1-picril-hidrazila, de coloração púrpura, que absorve em um comprimento de onda de 517 nm. Por ação de

um antioxidante, o DPPH[•] é reduzido formando 2,2-difenilpicril-hidrazina (DPPH-H), de coloração amarela (Figura 9), com conseqüente decréscimo da absorbância (BRAND-WILLIAMS; CUVELIER; BERSET, 1995). A partir dos resultados obtidos, determina-se a porcentagem de atividade antioxidante (quantidade de DPPH[•] consumida pelo antioxidante) ou seqüestradora de radicais e/ou a porcentagem de DPPH[•] remanescente no meio reacional. O mecanismo de reação é baseado em transferência de elétrons, enquanto a abstração de átomo de hidrogênio é uma reação marginal, pois a mesma acontece lentamente em solventes que estabelecem fortes ligações de hidrogênio. O método é influenciado pelo solvente e pelo pH das reações, sendo considerado fácil e útil para análise de substâncias puras e amostras complexas como o vinho ou extratos vegetais (SOUSA et al., 2007).

Figura 10: Reação genérica entre o radical DPPH e o um antioxidante (AH), na formação do 2,2-difenilpicril-hidrazina (DPPH-H), de coloração amarela (adaptado de OLIVEIRA et al., 2009).

2.5.2 Metodologia da capacidade de absorção do radical de oxigênio (ORAC)

O método ORAC utiliza uma molécula alvo dos radicais livres de oxigênio, as ficobiliproteínas β -ficoeritinas ou R-ficoeritina, que são altamente fluorescentes e que contém um pigmento fotoreceptor. O fundamento do método consiste na medida do decréscimo da fluorescência das proteínas, como conseqüência da perda de sua conformidade ao sofrer um dano oxidativo por radicais peroxila, formando assim, compostos mais estáveis. O AAPH [2,2'-azobis (2-amidinopropano) dihidroclorato] é responsável pela geração de radicais peroxila (HUANG et al., 2002). As vantagens

desse método são: com o uso da fluorescência como medida do dano oxidativo, há uma menor interferência dos compostos coloridos na leitura, fator útil na análise de alimentos coloridos, como o vinho; utilização de temperatura de 37°C tanto para a formação de radicais peroxila quanto para a reação entre o radical, fluoresceína e antioxidantes; e a utilização de um valor de pH próximo ao encontrado em humanos (pH 7,1 – 7,4).

2.6 Análise sensorial de vinhos tintos

A análise sensorial no meio enológico é o método mais completo e recomendado para se avaliar a qualidade global de um vinho. Somente as análises químicas, mesmo descritivas dos constituintes, são insuficientes para tal caracterização (PEREIRA et al., 2008). Entretanto, devido à grande variabilidade de descritores que podem existir para diferentes tipos de vinhos, a análise sensorial torna-se uma tarefa de difícil condução. Assim, no presente estudo, o perfil aromático das amostras de vinhos tintos oriundas de diversas regiões foi avaliado utilizando os principais descritores encontrados em vinhos tintos (descritos nas fichas de avaliação comercial da *Wine Spirits Education Trust*- em anexo), como forma de caracterizar as variedades de uva e correlacionar com as análises químicas, valor comercial, cor instrumental e atividade antioxidante *in vitro*.

A composição aromática é um aspecto de relevância da qualidade dos vinhos, pois se este apresenta algum defeito, é pouco provável que seja degustado. Neste projeto de pesquisa foi traçado um perfil sensorial dos aromas mais pronunciados de vinhos Sul Americanos, no intuito de caracterizar as amostras e observar diferenças entre as regiões produtoras. Muitos pesquisadores têm estudado a composição aromática de vinhos oriundos de diversas origens e variedades de uvas, usando a análise descritiva com painel especialista em vinhos (CLIFF & DEVER, 1996; PARR et al., 2007; PRADO et al., 2007). Um estudo do perfil sensorial de 56 vinhos ‘Champagne’ mostrou que essa variedade apresentou 19 aromas diferentes e marcantes (VANNIER et al., 1999), enquanto que vinhos ‘Rieseling’ canadenses apresentaram marcante aroma de *apricot*, mel, e carvalho (NURGEL et al., 2004). Vinhos Merlot e Cabernet Sauvignon franceses apresentaram aroma floral, herbáceo, frutado ou com tons de

carne assada (PEYNAUD, 1980; ALLEN et al., 1994). Já vinhos Merlot e Cabernet Sauvignon australianos e californianos foram caracterizados por um aroma altamente frutado, verde, caramelo e terroso. A diferença entre a percepção de tais aromas foi detectada por cromatografia gasosa (CG), onde foi possível provar que os vinhos Merlot possuíam de 4 a 5 vezes mais etil-octanato do que as amostras de vinho Cabernet Sauvignon, mostrando assim, um aroma mais frutado que o Merlot (GURBUZ et al., 2006). Vinhos Cabernet Sauvignon brasileiros produzidos em áreas de alta altitude são caracterizados com aroma de pimentão, ao passo que os produzidos em baixas altitudes apresentam tons de frutas vermelhas e geléia (FALCÃO et al., 2007). Estudo realizado por Tao, Liu e Li (2009) mostrou que vinhos Cabernet Sauvignon chineses apresentam aroma de pimenta verde, fumaça, baunilha e canela. Assim, os estudos de análise sensorial de vinhos, especialmente os tintos, têm corroborado pesquisas anteriores no sentido que a altitude, clima, qualidade do solo, quantidade de chuvas e outros fatores climáticos, além da variedade de uva e tecnologia de fabricação, afetam substancialmente o grau de maturação das uvas, e, portanto a composição aromática e química dos vinhos.

2.7 Quimiometria na ciência e tecnologia do vinho

A quimiometria é uma ciência de natureza multidisciplinar, envolvendo estatística multivariada, modelagem matemática e informática, e é especialmente aplicada a dados químicos e bioquímicos. Suas principais divisões incluem a otimização das medições químicas e a extração do máximo de informações a partir de dados analíticos (KRUZLICOVA et al., 2009). As pesquisas que utilizam a quimiometria concentram-se nas áreas reconhecimento de padrões e calibração multivariada, bem como modelagem matemática para fins de controle de qualidade (BRERETON, 2007). Nesse sentido, métodos quimiométricos têm sido vastamente usados na ciência e na tecnologia de vinhos para resolver problemas relacionados à produção, caracterização química/sensorial, verificação de autenticidade e procedência de vinhos. Entre esses métodos destacam-se a análise por componentes principais (ACP) e a análise

hierárquica de agrupamentos (AHA), que são métodos exploratórios de dados experimentais (BRERETON, 2007).

2.7.1 Análise por componentes principais (ACP)

A análise por componentes principais consiste essencialmente em reescrever as coordenadas das amostras em outro sistema de eixos mais conveniente para a análise dos dados. Em outras palavras, as p-variáveis originais geram, através de suas combinações lineares, p-componentes principais, cuja principal característica, além da ortogonalidade, é que são obtidos em ordem decrescente de máxima variância, ou seja, a componente principal 1 detém mais informação estatística que a componente principal 2, que por sua vez tem mais informação estatística que a componente principal 3, e assim por diante (BARROS NETO et al., 2006). Esse método permite a redução da dimensão dos pontos representativos das amostras, pois, embora a informação estatística presente nas p-variáveis originais seja a mesma dos p-componentes principais, é comum obter em apenas 2 ou 3 das primeiras componentes principais mais que 70% desta informação. O gráfico da componente principal 1 *versus* a componente principal 2 fornece uma janela privilegiada estatisticamente para observação dos pontos no espaço p-dimensional. A análise de componentes principais também pode ser usada para julgar a importância das variáveis originais com maior peso (*loadings*) na combinação linear dos primeiros componentes principais, que são as mais importantes do ponto de vista estatístico (SASS-KISS et al., 2008).

2.7.2 Análise hierárquica de agrupamentos (AHA)

A análise hierárquica de agrupamentos (AHA) é um método de classificação preliminar que tem como objetivo estudar as características do conjunto de dados, na procura por grupos de amostras que apresentam valores semelhantes da resposta a ser avaliada (GEMPERLINE, 2006). A premissa inicial é que a distância entre as amostras no espaço dimensional, definida pelas variáveis de respostas, reflita a similaridade das propriedades entre essas amostras. Tipicamente nos métodos de

agrupamento, as amostras contidas em cada grupo (*cluster*) são consideradas similares. O objetivo principal da AHA é mostrar em um espaço bidimensional os agrupamentos formados de acordo com as variáveis selecionadas (KETTENRING, 2006). Dessa forma, os resultados podem ser expressos em dendogramas, tanto para variáveis quanto para as amostras, e permite a visualização de correlações entre esses fatores (GEMPERLINE, 2006).

Com o uso da AHA é possível coletar os diferentes dados de um conjunto de amostras (em diferentes unidades) e então separá-las dessa população em grupos, seguindo critérios geométricos de posicionamento. Entretanto, usando apenas a AHA não é possível identificar a razão pela qual as amostras foram separadas dessa forma. Para alcançar essa resposta, assim como para permitir a visualização dos grupos num espaço p-dimensional, é comum a aplicação de outras técnicas quimiométricas, como a ACP, quando se tratam de variáveis contínuas (BRERETON, 2007). Assim, tanto a AHA e ACP são técnicas complementares e que geram informações valiosas e de fácil interpretação quando muitos dados analíticos e amostras estão sendo analisados (KETTENRING, 2006).

2.7.3 Aplicações de métodos quimiométricos na ciência e tecnologia do vinho

Mesmo sabendo que o uso de métodos estatísticos multivariados está crescendo na área de ciência de alimentos, ainda é muito comum encontrar pesquisas nas quais os autores utilizam métodos estatísticos univariados como a ANOVA para verificar diferenças entre um grande número de amostras e determinar correlações lineares entre as variáveis de resposta (LEE & RENNAKER, 2007; LI et al., 2009). Embora esse método seja cientificamente aceito e de grande utilidade em outras aplicações, na área de ciência de vinho, em especial nas determinações de compostos fenólicos por cromatografia líquida de alta eficiência, atividade antioxidante, antibacteriana e antiproliferativa de um grande número de amostras, essa abordagem univariada não permite a visualização de tendências na matriz de dados. Além disso, não é possível verificar correlações entre todas as variáveis e similaridades entre as amostras

simultaneamente, o que torna a quimiometria uma ferramenta extremamente necessária em tal área.

Assim, métodos quimiométricos ou sensiométricos (ligados à análise sensorial) têm sido aplicados com sucesso para diferenciar vinhos jovens e envelhecidos baseados no teor de compostos fenólicos (MAKRIS; KALLITHRAKA; MAMALOS, 2006), caracterizar vinhos tintos em relação à sua composição química (SON et al., 2009), avaliar a origem geográfica de vinhos tintos de acordo com seu conteúdo de minerais (FABANI et al., 2010), avaliar os principais compostos aromáticos de vinho tinto (TAO; LIU; LI, 2009), monitorar o envelhecimento de vinho tinto em barris de carvalho baseado em propriedades sensoriais (PARRA et al., 2006), monitorar a autenticidade e qualidade sensorial de vinhos (ARVANITOYANNIS et al., 1999), para classificar vinhos brancos de diversas variedades usando o nariz eletrônico (COZZOLINO et al., 2005), para classificar vinhos Riesling de países europeus através de dados espectroscópicos (LIU et al., 2008), entre outras aplicações. Dessa forma, quando tais técnicas são utilizadas, gráficos bidimensionais são gerados a partir das variáveis de respostas selecionadas e, assim, os resultados podem ser mais bem interpretados e compreendidos.

Levando em consideração que métodos univariados não são inteiramente capazes de mostrar associações de diversas variáveis de respostas e amostras simultaneamente, o uso de métodos multivariados seria uma opção viável e cientificamente aceita para avaliar resultados oriundos de diversos tipos de análises. Neste sentido, neste projeto de pesquisa métodos quimiométricos exploratórios como a ACP e AHA foram aplicados para verificar a associação entre composição química, qualidade sensorial, preço, safra, atividade antioxidante e cor instrumental de vinhos Sul Americanos. A partir dessa informação foi possível verificar qual varietal possui maior potencial antioxidante aliado à qualidade sensorial bem como quais classes de compostos químicos correlacionaram significativamente com a atividade antioxidante *in vitro*.

3. OBJETIVOS

O objetivo deste estudo foi identificar associações entre a atividade antioxidante *in vitro* e fatores relacionados ao tipo de uva, região de produção, perfil sensorial, valor comercial, cor, safra, e concentração de compostos fenólicos de vinhos produzidos no Brasil, Chile e Argentina.

Através deste estudo pretendemos responder às seguintes questões:

- 1) A atividade antioxidante do vinho tinto Sul Americano está associada ao tipo de uva? À região produtora? Ao perfil sensorial? À cor? Ao valor comercial? À safra? Por exemplo, vinhos de custo mais elevado apresentam maior atividade antioxidante que vinhos de valor comercial menor?
- 2) Quais são os compostos fenólicos que caracterizam os vinhos Sul Americanos classificados com maior atividade antioxidante, preço e qualidade sensorial?
- 3) Se um indivíduo deseja reduzir o risco de desenvolvimento de DCNT através do consumo de vinho tinto, qual tipo de vinho e qual procedência poderia ser recomendado para esse indivíduo, levando em consideração o preço, qualidade sensorial e atividade antioxidante *in vitro*?

4. DESCRIÇÃO DOS CAPÍTULOS

CAPÍTULO 1: Assessing the association between phenolic compounds and the antioxidant activity of Brazilian red wines using chemometrics

O objetivo deste trabalho foi identificar associações entre a atividade antioxidante *in vitro* de vinhos tintos (n = 29) produzidos no Brasil e fatores relacionados ao tipo de uva, cor, pH, valor comercial e concentração de compostos fenólicos de vinhos tintos. Verificou-se que vinhos envelhecidos em barris de carvalho apresentaram maior atividade antioxidante do que vinhos que não passaram por esse processo. Assim, sugeriu-se que a atividade antioxidante de vinhos brasileiros está diretamente ligada aos flavonóides não-antociânicos, mais especificamente os compostos polimerizados como catequinas e proantocianidinas. Tal observação foi constatada também pela análise de agrupamento e por correlações lineares de Pearson.

O Capítulo 1 do presente estudo está publicado no periódico *LWT- Food Science and Technology*.

CAPÍTULO 2: Characterization of red wines from South America based on sensory properties and antioxidant activity

Neste capítulo, os vinhos Sul Americanos (n = 80) foram submetidos à análise sensorial e os dados foram correlacionados com valor comercial, cor instrumental, e a atividade antioxidante medida por ORAC e DPPH, no intuito de oferecer informações para os consumidores de vinho de quais variedades e sua procedência seriam mais indicadas para o consumo. Os resultados mostraram que apenas uma amostra não apresentou qualidade sensorial satisfatória, sendo que os vinhos chilenos e argentinos se destacaram por apresentar maior valor comercial, atividade antioxidante, intensidade de odor, qualidade sensorial global, índice de acidez e taninos, ao passo que os vinhos brasileiros apresentaram menores valores para os atributos sensoriais. Nesse sentido, os vinhos produzidos com uvas americanas apresentaram menores valores para todas

as variáveis estudadas. De forma geral, concluí-se que os vinhos oriundos de uvas Syrah, Malbec e Cabernet Sauvignon apresentaram maior capacidade antioxidante e melhores características sensoriais do que as demais varietais, sendo que esse resultado foi independente da safra e procedência, corroborando a hipótese de que mesmo que haja grandes diferenças em métodos de produção, clima, solo, procedência, essas diferenças jamais se sobrepõem ao perfil genético de cada varietal.

O Capítulo 2 do presente estudo está aceito para publicação no periódico *Journal of the Science of Food and Agriculture*.

CAPÍTULO 3: Phenolic composition of South American red wines classified according to their antioxidant activity, retail price and sensory quality

Neste capítulo, as 73 amostras de vinhos finos (*V. vinifera*) classificados, usando a análise hierárquica de agrupamentos, de acordo com a qualidade sensorial global, atividade antioxidante medida por ORAC e DPPH, e valor comercial foram caracterizados por sua composição fenólica. Com o uso de análise por componentes principais e análise hierárquica de agrupamentos foi possível identificar os vinhos com a melhor combinação de características sensoriais, preço e atividade antioxidante. As varietais mais favoráveis foram a Malbec, Cabernet Sauvignon e Syrah produzidos, principalmente, na Argentina e Chile, ao passo que Pinot Noir apresentou os menores valores de atividade antioxidante e qualidade sensorial. A atividade antioxidante das amostras se correlacionou positivamente com o conteúdo de fenólicos totais, flavonóides totais, ácido gálico, quercetina, catequina, ácido ferúlico, miricetina, caempferol, e rutina, sendo que apenas os conteúdos de miricetina, ácido gálico e quercetina foram diferentes entre os grupos. Nenhum composto fenólico se associou com as diferenças sensoriais entre os grupos de vinhos.

O Capítulo 3 do presente estudo está publicado no periódico *Food Chemistry*.

CAPÍTULO 1

ASSESSING THE ASSOCIATION BETWEEN PHENOLIC COMPOUNDS AND THE ANTIOXIDANT ACTIVITY OF BRAZILIAN RED WINES USING CHEMOMETRICS

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Abstract

The objective of this study was to evaluate the association among chemical parameters, the commercial value, and the antioxidant activity of Brazilian red wines using chemometric techniques. Twenty-nine samples from five different varieties were assessed. Samples were separated into three groups using hierarchical cluster analysis: cluster 1 presented the highest antioxidant activity towards DPPH (68.51% of inhibition) and ORAC (30,918.64 $\mu\text{mol Trolox Equivalents/L}$), followed by cluster 3 (DPPH = 59.36% of inhibition; ORAC = 25,255.02 $\mu\text{mol Trolox Equivalents/L}$) and then cluster 2 (DPPH = 46.67% of inhibition; ORAC = 19,395.74 $\mu\text{mol Trolox Equivalents/L}$). Although the correlation between the commercial value and the antioxidant activity on DPPH and ORAC was not statistically significant ($P = 0.13$ and $P = 0.06$, respectively), cluster 1 grouped the samples with higher commercial values. Cluster analysis applied to the variables suggested that non-anthocyanin flavonoids were the main phenolic class exerting antioxidant activity on Brazilian red wines.

Keywords: Principal Component Analysis, ORAC, DPPH, cluster analysis, red wine.

1. INTRODUCTION

The health benefits of moderate wine consumption are well documented, and have been associated with a diminished risk of cardiovascular and neurological diseases through a variety of mechanisms such as free radical scavenging, intracellular metal chelation, inhibition of transcription factors, and enzyme modulation ability (Singh et al., 2008). Antioxidant activity is currently considered to be one of the most significant characteristics of red wines and is associated with the content of polyphenols such as flavonoids, phenolic acids, stilbenes, coumarines, and lignoids. This antioxidant activity has been repeatedly demonstrated in numerous experimental models, both chemical and biological (Cimino, Sulfaro, Trombetta, Saija, & Tomaino, 2007; Radovanovic, Radovanovic, & Jovancicevic, 2009).

Anti-atherogenic effects of red wine are thought to be due at least in part to the antioxidant properties of polyphenolic compounds which act *in vivo* against reactive oxygen species. Phenolic compounds include two classes: non-flavonoids (i.e., hydroxycinnamates, hydroxybenzoates, and stilbenes), and flavonoids (i.e., flavonols, flavanols, and anthocyanins). Most phenolic compounds, especially those found in red wine, derive from the condensation of flavan-3-ol into oligomers (proanthocyanidins) and polymers (condensed tannins). Resveratrol, gallic acid and quercetin are thought to act against allergies, inflammation, hypertension, arthritis, and carcinogens (Soleas, Grass, Josephy, Goldberg, & Diamandis, 2002), which makes red wine a suitable option for the intake of health-promoter substances.

To date, there has been little published research on the chemical characteristics and antioxidant parameters of wines produced in Brazil (Pastore et al., 2003; Ishimoto, Ferrari, Bastos, & Torres, 2006; Stefenon et al., 2009) and most of this research presents a reduced quantity ($n < 20$) of samples. In addition, a large part of such this research does not correlate the commercial value and antioxidant activity of wine with the major classes of phenolic materials; rather, they only associate two parameters at a time, such as the total phenolic content and the antioxidant activity. The well-documented association between the moderate consumption of red wine and the reduction in the risk of cardiovascular diseases has increased the demand of information identifying which red wine variety (i.e., Pinot Noir, Carbernet Sauvignon and Malbec, among others) could better achieve this objective.

It is known that it is very usual to find researches applying univariate methods for determining relationships between total or individual phenolics with antioxidant properties of red wines (Lee & Rennaker, 2007; Li, Wang, Li, Li, & Wang, 2009). However, this one-dimensional approach fails to guarantee the determination of simultaneous correlations among all results. In order to overcome this problem, the interest in chemometric methods, which are multivariate by nature, has been recognized as a valuable tool in the wine science. Chemometric techniques have been used to assure red wine authenticity and quality (Arvanitoyannis, Katsota, Psarra, Soufleros, & Kallithraka, 1999), to classify their geographical origin based on chemical compounds (Liu, Wu, Fan, Li, & Li, 2006; Kallithraka, Mamalos, & Makris, 2007) and sensory properties (Kallithraka, Arvanitoyannis, Kefalas, El-Zajouli, Soufleros, & Psarra, 2001), among other applications.

The phenolic composition of red wines presents a high variation according to the environmental and climate conditions, soil type, grape variety, degree of ripeness, winemaking processes, and length of maceration process (Freitas, 2006; Lachman, Sulc, & Schilla, 2007). However, this variation will never be sufficient to mischaracterize the grape variety and its antioxidant activity. Based on this fact, the objective of this study was to evaluate the association among chemical parameters and the commercial value of Brazilian red wines and their antioxidant activity measured by *in vitro* methodologies by using chemometric techniques.

2. MATERIALS AND METHODS

2.1 Chemicals

The Folin–Ciocalteu reagent, the azo-initiator 2-2' azobis (2-methylpropionamedine dihydrochloride (AAPH), Trolox, 1,1-diphenyl-2-picrylhydrazyl (DPPH), catechin, and gallic acid were obtained from Sigma (St. Louis, MO, USA). The aqueous solutions were prepared using ultra-pure Milli-Q water. All other reagents used in the experiments were of analytical grade.

2.2 Sampling

A total of 26 Brazilian red wines of different vintages were studied, and were produced from *Vitis vinifera* grapes of the five most characteristic red grape varieties (Merlot, Malbec, Pinot Noir, Cabernet Sauvignon, Syrah) cultivated in important winemaking regions in Brazil. Three blended wines produced with *Vitis labrusca* grapes were also evaluated. Table 1 summarizes all of the 29 Brazilian red wine samples and

their commercial value, vintage, grape variety, and place of production. All of the wines were purchased from 3 different markets in São Paulo, SP, Brazil. Wines were brought to the laboratory, aliquoted into 2 mL eppendorfs, immediately immersed in liquid nitrogen and stored at -80°C until further analysis.

2.3 Measurement of pH

A sample of approximately 50 mL was separated from the bottle immediately after uncorking. The pH was then measured in triplicate in accordance with AOAC protocols (2005) using a pH meter (model pH 21, Hanna Instruments, Woonsocket, RI, USA) with a combined electrode and a temperature probe.

2.4 Determination of total phenolic compounds

Total phenol concentration in selected red wine samples was determined in triplicate according to the Folin–Ciocalteu colorimetric method (Singleton & Rossi, 1965). First, the wine was diluted 25 times in water, and 250 µL of this solution was then mixed with 250 µL of 2-fold-diluted Folin–Ciocalteu phenol reagent. Two milliliters of water were added and after 5 min, 250 µL of a 10 g/100g sodium carbonate solution was added to the mixture and shaken thoroughly. The mixture was allowed to stand for 60 min in the dark at 25°C. The blue color formed in the mixture was measured at a wavelength of 725 nm using a spectrophotometer (model mini 1240 UV-VIS, Shimadzu Corporation, Kyoto, Japan). A standard curve of gallic acid (ranging from 0 to 100 mg/L) was prepared and the results, determined from a regression equation (phenolic

concentration = $118.19 \times \text{absorbance}$; $R^2 = 0.9951$), were expressed as mg gallic acid equivalents per liter of wine (mg GAE/L).

2.5 Determination of monomeric anthocyanins

The pH differential method is based on the structural change of the anthocyanin chromophore between pH values of 1.0 and 4.5. Anthocyanins have a maximum absorbance at a wavelength of 510 nm at a pH of 1.0. The colored oxonium form predominates at a pH of 1.0 and the colorless hemiketal forms at a pH of 4.5. Measurements at 700 nm are performed to correct for haze (Lee, Durst, & Wrolstadt, 2005). Following this method, an aliquot of the wine (250 μL) was added to 2.25 mL of buffer (KCl, 0.025 mol/L) with a pH of 1.0. The pH was adjusted with HCl (0.20 mol equi/L). Another 250 μL of red wine was also placed into a 10 mL volumetric flask and 2.25 mL of the buffer ($\text{CH}_3\text{CO}_2\text{Na}$, 0.4 mol/L) with a pH of 4.5. The pH was adjusted with HCl (0.20 mol equi/L). Absorbance was measured in a spectrophotometer (model mini 1240 UV-VIS, Shimadzu Corporation, Kyoto, Japan) at 510 and 700 nm. Results were calculated using Equation 1 and were expressed as mg per liter (mg/L).

$$\text{Total monomeric anthocyanins (mg/L)} = [(A \times \text{MW} \times D \times 100)] / e \quad (1)$$

where $A = (A_{510} - A_{700})_{\text{pH}1.0} - (A_{510} - A_{700})_{\text{pH}4.5}$; e is cyanidin 3-glucoside molar absorbance (26,900); MW is the molecular weight for cyanidin-3-glucoside (449.2); and D is a dilution factor (10). The results in each assay were obtained from three replicates.

2.6 Determination of flavonoid content

The total flavonoid content of the red wines was determined using a modified colorimetric method (Jia, Tang, & Wu, 1999). Briefly, 250 μ L of 1:25 diluted wine was mixed with 2.00 mL of distilled water and subsequently with 120 μ L of a 0.5 mol/L sodium nitrite solution and was allowed to react for 5 min. A 10 g/100g aluminum chloride solution was added (120 μ L), the mixture was vortexed and was allowed to react for a further 5 min before 800 μ L of 1 mol/L sodium hydroxide was added. The absorbance of the mixture was immediately measured at a 510 nm wavelength against a prepared blank using a spectrophotometer (model mini 1240 UV-VIS, Shimadzu Corporation, Kyoto, Japan). The flavonoid content was determined by a standard curve of catechin (0 to 100 mg/L) and the results, determined from a regression equation (flavonoid concentration = $395.21 \times \text{absorbance}$; $R^2 = 0.9984$), were expressed as mg catechin equivalents per liter of wine (mg CTE/L). Data presented are the average of three measurements.

2.7 Other phenolic compounds

The content of non-flavonoid phenolic compounds (NFP; cinnamic and benzoic acids, stilbenes, lignans, lignins, and coummarins) was estimated by subtracting the total phenolics and total flavonoids, while the content of non-anthocyanin flavonoids (NAF; flavanols, isoflavonoids, flavonols, flavones, and flavonones) was obtained by subtracting the anthocyanins from the flavonoid content. The results are expressed in mg/L.

2.8 Antioxidant activity of red wines

2.8.1 Free radical scavenging capacity (DPPH)

The free radical scavenging capacity of the wine samples was analyzed using 1,1- diphenyl-2-picrilhydrazyl (DPPH) assay according to Brand-Williams, Cuvelier, & Berset (1995), with slight modifications. This assay is based on the ability of antioxidant to scavenge the DPPH cation radical. This method determines the hydrogen donating capacity of molecule and does not produce oxidative chain reactions or react with free radical intermediates.

Briefly, a 25 μ L aliquot of the red wine (diluted 25 times in water) was mixed with 900 μ L of methanol and 5.0 μ L of a methanolic DPPH solution (10.0 mmol/L). The reaction was allowed to take place in the dark for 30 min at 25°C and the absorbance at 517 nm was read using a spectrophotometer (model mini 1240 UV-VIS, Shimadzu Corporation, Kyoto, Japan). The antioxidant activity was calculated according to Equation (2):

$$\% \text{ scavenging activity} = [1 - (A_{517} \text{ sample} / A_{517} \text{ blank})] \times 100\% \quad (2)$$

2.8.2 Oxygen radical absorbance capacity assay (ORAC)

The ORAC assay was performed according to Huang, Ou, and Prior (2005). Following thermolysis at 37°C, the azo-initiator AAPH generates carbon-centered radicals, which then react with oxygen to give the reactive peroxy radical (ROO•). This peroxy radical can either react with the fluorescein probe or directly with the antioxidant. The antioxidant can also react with the fluoresceinyl radical, regenerating fluorescein and thereby preserving its fluorescence measured at 485 nm excitation and 525 nm

emission. This persists until the antioxidant is consumed, at which point the fluorescein is rapidly oxidized.

Briefly, red wine samples were diluted 900 times in 75 mmol/L phosphate buffer (pH 7.1), and 25 μ L of each solution were then transferred in triplicate to microtiter plates. Next, 150 μ L of 40 nmol/L fluorescein, diluted in 75 mmol/L phosphate buffer (pH 7.1), was added. The microplate also contained a blank (200 μ L of phosphate buffer), a control (25 μ L phosphate buffer + 150 μ L 40 nmol/L fluorescein), and a Trolox dilution series (150 μ L 40 nmol/L fluorescein + 25 μ L of the appropriate Trolox dilution). Trolox standard solutions were prepared at concentrations ranging from 6.25 to 100 μ mol/L. To maintain the temperature on the plate, 270 μ L water was added around the wells that would be read. After incubation (37°C, 30 min), 25 μ L AAPH (153 mmol/L in 75 mmol/L phosphate buffer, pH 7.1) was added to all of the wells with the exception of the blank, and the plate was shaken for 10 s at maximum intensity. The plate reader (Multi-Detection microplate reader; Synergy-BIOTEK, Winooski, VT, USA) was programmed to record the fluorescence every minute following the addition of AAPH for 60 min, and the area under the curve of the fluorescence decay was integrated using Gen5 software. The areas corresponding to the blank (200 μ L of solvent) and to the control (25 μ L solvent + 150 μ L 40 nmol/L fluorescein) were subtracted from the areas obtained for each compound concentration. Using the Trolox calibration curve ($\text{ORAC} = 273,475.58 \times \text{fluorescence} + 4,106,262.30$; $R^2 = 0.9807$), expressed as the net area vs. mmol/L concentration, the antioxidant activity of the red wines was expressed as μ mol Trolox Equivalents (TE)/L. The results in each assay were obtained for three replicates.

2.9 Instrumental color

A sample of approximately 50 mL was separated from the bottle after uncorking and color measurements were performed less than 4 min after opening the bottle. Instrumental color measurements were conducted by transmittance four times using a colorimeter (ColourQuest-XE, Hunter Assoc. Laboratory, Reston, VA, USA) with a D65 optical sensor, 0° geometry, and a 10° angle of vision. The CIE $L^*a^*b^*$ system was considered where two color coordinates, a^* and b^* , were measured. a^* has positive values for reddish colors and negative values for greenish colors. Conversely, b^* takes positive values for yellowish colors and negative values for bluish colors. The chroma (C^*) of a food is used to determine the degree of difference of a hue in comparison to a gray color with the same lightness. The higher the chroma value, the higher the intensity of the color perceived by human vision. Chroma was calculated by: $C^* = (a^{*2} + b^{*2})^{1/2}$. The hue angle (h^*) is the attribute which has been traditionally used to define colors. Hue angle was calculated by: $h^* = \tan^{-1} (b^*/a^*)$.

2.10 Statistical analysis and chemometrics application

Data were presented as means $\pm$ pooled standard deviations. Pearson products (r) were used to evaluate the strength of correlations among the parameters evaluated. Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA), implemented in the STATISTICA 7.1 software (Stat-Soft Inc., Tulsa, OK, USA), were the chemometric methods used to analyze the results.

2.10.1 Principal Component Analysis (PCA)

Principal component analysis (PCA) involves a mathematical procedure that identifies patterns in data, and expressing them in such a way as to highlight similarities and differences. Since patterns in results can be hard to find in a set of high dimension, where the luxury of graphical representation is not available, PCA is a powerful and suitable tool for analysing results. The other main advantage of PCA is that once you have found these patterns in the data, and you compress them by reducing the number of dimensions, without much loss of information (Shaw, 2003).

PCA was applied to separate the samples ($n = 29$) according to their values of chroma, hue angle, pH, antioxidant activity (ORAC and DPPH), non-anthocyanin flavonoids (NAF), non-flavonoid phenolics (NFP), commercial value, and monomeric anthocyanins. In the PCA, the results obtained for each parameter were adopted as columns and the wine samples were used as the rows. Analyses were based on correlations and variances were computed as $SS/(n-1)$. Eigenvalues higher than 1.0 were adopted to explain the projection of the assays on the factor-plane. The data were autoscaled before analysis.

2.10.2 Hierarchical Cluster Analysis (HCA)

The term Hierarchical Cluster Analysis (HCA) encompasses a number of different algorithms and methods for grouping samples of similar kind into respective categories. It is an exploratory data analysis tool which aims at sorting different samples into groups in a way that the degree of association between two samples is maximal if they belong to the same group and minimal otherwise (Shaw, 2003). In our study, HCA was used to look for association between phenolic compound classes and antioxidant activity.

A uniform hierarchical cluster analysis methodology was applied to the auto scaled data using a two-step approach. In the first step, HCA using Ward's method and Euclidean distances generated a dendrogram for samples. Next, the same procedure was applied to following the variables: chroma, hue angle, non-flavonoids phenolics, commercial value, non-anthocyanins flavonoids, pH, monomeric anthocyanins, DPPH, and ORAC. In order to compare the results within the three suggested clusters, Hartley's test was carried out to check for homogeneity of variances. One-way ANOVA and Tukey's HSD *pos hoc* tests were then applied to identify contrasts. For the variables that presented non-homogenous variances ($P < 0.05$), the non-parametric multiple comparison Kruscall-Wallis test was applied. P-values below 0.05 were considered to be significant.

3. RESULTS AND DISCUSSION

The content of the total phenolic compounds of the Brazilian red wines ranged from 1041.63 to 1958.78 mg GAE/L. The anthocyanins are solely found in their monomeric structure in wines, and the pH differential method has been demonstrated to be a simple, quick, and accurate means of measuring the total monomeric anthocyanin content (Lee, Durst, & Wrolstadt, 2005). In our study, the content of monomeric anthocyanins varied from 9.35 to 237.31 mg/L, while the flavonoid content varied from 520.36 to 1,794.91 mg CTE/L (Table 1). The pH values of the samples ranged from 3.47 to 3.99. Wines used in this study constitute a quite heterogeneous group, made from five different grape varieties, including 3 blended wines with diverse ages and, therefore, differences in phenolic composition and *in vitro* antioxidant activity were attained. As is well known, the quantities of phenolic materials vary considerably in different types of

wines, depending on the grape variety, environmental factors in the vineyard, the wine processing techniques, soil and atmospheric conditions during ripening, aging process, and berry maturation (Pérez-Magarin & González-Sanjosé, 2006; Freitas, 2006; Lachman, Sulc, & Schilla, 2007). The phenolic compounds present in red wine can be divided into two major classes based on the carbon skeleton: flavonoids and non-flavonoids. The former is composed by anthocyanidins (malvidin, delphinidin, petunidin, peonidin, and cyanidin), flavonols (quercetin, isorhamnetin, myrecetin, and kaempferol), flavanols (catechin, epicatechin, epicatechin 3-gallate, and gallo-catechin), flavones (luteolin, apigenin), and flavanones (naringenin). The main non-flavonoid phenolics are composed of cinnamic acids (caffeic, *p*-coumaric, and ferulic acids), benzoic acids (gallic, vanillic, and syringic acid), and stilbenes such as resveratrol (Cheynier, 2006).

Polymerization and condensation reactions between wine flavanols and anthocyanidins occur during aging in oak barrels, creating more stable and active compounds such as polymerized catechins and proanthocyanidins (Bartolomé, Gómez-Cordovés, & Monagas, 2006). Thus, it is no wonder that in our study aged wines exhibited a lower anthocyanin content than the younger wines. Similar results have also been reported by others (Freitas, 2006; Roussis et al., 2008). Once the anthocyanin content has decreased, the wine color changes considerably through time: the red-bluish color of young wines (determined by the a^* coordinate) changes to red–orange hues (determined by the b^* coordinate). Chroma values close to or higher than 50 correspond to vivid colors. In our study, young wines presented a higher content of monomeric anthocyanins and higher chroma values. On the other hand, the samples included in cluster 1 (aged wines) presented lower chroma values and hue angles. Another effect of aging on the chemical composition of red wines is the concentration of

the hydroxybenzoic acids (ethyl gallate, gallic, *p*-hydroxybenzoic, and ellagic acids) and their derivatives that increase with time (Cadahía, Simón, Sanz, Poveda, & Colio, 2009). However, in our study, no statistical differences were observed in the content of non-flavonoid phenolics among the suggested clusters.

The ORAC test showed that the antioxidant activity of the Brazilian red wines varied from 10,527.69 to 38,551.90 $\mu\text{mol TE/L}$, and Pinot Noir and blended wines generally presented the lowest values. The DPPH assay showed that the wine samples were able to inhibit between 33.27 and 76.39% of such free radicals. Chroma (C^*) values ranged from 39.35 to 80.61, and the average hue angle was 49.05. All of these findings are in accordance with the values reported by other researchers where red wine were evaluated (Pastore et al., 2003; Freitas, 2006; Ishimoto, Ferrari, Bastos, & Torres, 2006; Radovanovic, Radovanovic, & Jovancicevic, 2009; Stefenon et al., 2009).

The main contribution of our research was the identification of non-anthocyanin flavonoids as the key compounds responsible for the measured antioxidant activity of Brazilian red wines. Indeed, an important correlation between DPPH and ORAC assays and the content of non-anthocyanin flavonoids was found, corroborating the findings of Sánchez-Moreno, Larrauri, and Saura-Calixto (1999), Fernández-Pachón, Villano, García-Parrilla, and Troncoso (2004), and Cimino, Sulfaro, Trombetta, Saija, and Tomaino (2007). This activity may be due to the chemical structure of such compounds, which promote the reactions that imply the cession of electrons to the free radical due to the ability of the aromatic ring to support an unpaired electron. Monomeric anthocyanins showed weak and non-significant correlations with DPPH and ORAC, corroborating the results previously found by Giovanelli (2005) and Zafrilla et al. (2003). These authors

also found that there was no correlation between anthocyanins and antioxidant activity measured by ORAC and DPPH assays. On the other hand, Fernández-Pachón, Villano, García-Parrilla, and Troncoso (2004) studied the anti-radical ability of various polyphenol fractions in red wines (phenolic acids, anthocyanins and flavonols) and concluded that flavonols play no prominent role as antioxidants. Alén-Ruiz, García-Falcón, Pérez-Lamela, Martínez-Carballo, and Simal-Gándara (2009) and Roussis et al. (2008) found a significant correlation between total flavonols and anthocyanins with the scavenging capacity of DPPH of Spanish red wines, suggesting that these two flavonoid classes can substantially influence the antioxidant properties of red wines. Capitani, Carvalho, Rivelli, Berlanga, and Castro (2009) evaluated five different phenolics individually (carnosic acid, caffeic acid, rutin, quercetin, and resveratrol) using DPPH and ORAC assays and verified that the non-flavonoid phenolics presented a higher antioxidant activity on DPPH while flavonoids presented a similar activity to non-flavonoid phenolics using the ORAC methodology. Although these recent studies show substantial differences in the antioxidant activity of phenolic compounds, the results remain contradictory. The antioxidant activity depends on many factors other than the phenolic composition of the test material, including the concentration of the free radical, the time employed in the assay and the dilution factor of the sample. In addition, red wine is a complex matrix and contains large quantities of phenolic and non-phenolic compounds and thus antioxidant activity cannot be predicted by the content of a specific class or substance alone.

The bioactivity of polyphenolic compounds is due to two factors: the acidity of their phenolic hydroxyl groups and the resonance between the free electron pair on the phenolic oxygen and the benzene ring. This resonance increases electron delocalization

and confers a partial negative charge in the chemical structure of the phenolic compound and thus, a nucleophilic character (Cheynier, 2006). Polyphenols can scavenge stable free radicals by reduction via electron transfer or by hydrogen atom transfer from aromatic hydroxyl groups. The combination of these mechanisms could lead to synergistic, antagonistic or even additive antioxidant effects in wines. Furthermore, the effectiveness of antioxidants depends on factors such as the bond dissociation energy between oxygen and a phenolic hydrogen, pH, reduction potential, solubility, stereochemical structure, and delocalization of the antioxidant radicals (Cao, Chen, Sun, Guo, Song, & Tian 2007). These factors help to explain the differences in antioxidant activity found in our study.

Samples were separated along the first principal component (PC) by differences observed in chroma, hue angle, antioxidant activity (ORAC and DPPH assays), non-anthocyanin flavonoids (NAF), and pH. The second PC separated the samples on the basis of anthocyanins, hue angle, and chroma, explaining 58.22% of all of the variation in the data (Figure 1). The content of non-flavonoid phenolics (NFP) was projected on the third PC, while commercial value was represented on fourth PC. The first three PCs contributed to explain up to 73.42% of variability in the data set. The correlation of each original variable with the PCs is shown in Table 2 and the cluster analysis applied to the variables is shown in Figure 2.

Using the hierarchical cluster analysis applied to the samples, three clusters were suggested. Cluster 1 contained the samples with the highest non-anthocyanin flavonoid content, pH, antioxidant activity measured by DPPH and ORAC, and the lowest monomeric anthocyanin content, color intensity and hue angle. Samples included in cluster 2 presented the lowest antioxidant activity, and non-anthocyanin flavonoid

content, and the highest monomeric anthocyanin content, while the samples in cluster 3 presented the highest commercial value, chroma, hue angle, and intermediate antioxidant activity (Table 3). With respect to the antioxidant activity, clusters could be ordered by the following sequence: $1 > 3 > 2$. It is noteworthy that the blended wines, which presented the lowest antioxidant activity, were included in the second cluster, whereas Syrah wines, which showed the highest antioxidant activity, were included in cluster 1, and Cabernet Sauvignon wines were presented in a higher proportion in cluster 3.

Table 3 shows that aged wines (clusters 1 and 3; mean vintage year: 2006) presented a higher antioxidant activity in comparison to young wines (cluster 2; mean vintage year: 2008). Data in the literature are contradictory: while some researchers suggest that antioxidant activity is not correlated with wine age (Zafrilla et al., 2003; Giovanelli, 2005), others (Echeverry, Ferreira, Reyes-Parada, Abin-Carriquiry, Blasina, & González-Neves, 2005; Roussis et al., 2008; Alén-Ruiz, García-Falcón, Pérez-Lamela, Martínez-Carballo, & Simal-Gándara, 2009) indicated that aged wines exhibit a higher antioxidant activity than young wines. Our findings corroborate the hypothesis that the polymerized forms of polyphenols such as catechins and proanthocyanidins in aged wines are those most strongly contributing to inhibition activity on DPPH and AAPH radicals. Changes in the phenolic composition during aging involve both enzymatic and chemical processes. The former is restricted to the early stages of wine-making, whereas the latter rapidly becomes prevalent as the enzymes become inactivated, and continues throughout aging (Cheynier, 2006). The evolution of such compounds in wine during controlled storage is complex because of the wide variety of factors involved such as grape variety, oak wood kind, and the length of time that the wine is kept in barrels.

Throughout aging in oak barrels, diverse bioactive polyphenolic compounds such as ellagitannins are extracted from wood by ethanol. These compounds play an important role in the antioxidant power of wines due to their ability to consume high quantities of oxygen, regulating the oxidation reactions. Thus, accompanying the chemical reactions during the aging process, the *in vitro* antioxidant activity also changes considerably. Aging processes also increase the commercial value of red wines (Table 3). One of our objectives was to check the correlation of the commercial value of Brazilian red wines with the *in vitro* antioxidant activity. This correlation was statistically non-significant ($P > 0.05$) and a potential tendency was observed: cluster 2, which presented the lowest antioxidant activity, included wine samples with lowest mean commercial value (US\$ 8.64), while clusters 1 and 3, which presented the highest antioxidant properties, averaged US\$ 16.53 and US\$ 25.78, respectively.

4. CONCLUSIONS

Chemometric methods were successfully used to show that the antioxidant activity of Brazilian red wines measured by DPPH and ORAC is highly influenced by the content of non-anthocyanin flavonoids. The correlation between commercial value and antioxidant activity measured by ORAC and DPPH was non-significant; however, there was a tendency for Brazilian wines with a higher commercial value to have higher antioxidant properties. In this regard, more samples should be evaluated in order to confirm this hypothesis. Furthermore, the phenolic compounds present in each wine should be isolated in order to correlate with the observed antioxidant activity.

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Table 1

Phenolic composition, vintage, commercial value, pH, color attributes, and antioxidant activity of the red wine samples.

Grape variety	Code	Commercial value (US\$)	Vintage	C*	h*	DPPH (% reduction)	ORAC (μmol TE/L)	pH	Anthocyanins (mg/L)	Total phenolics (mg GAE/L)	Flavonoids (mg CTE/L)
Pinot Noir	P1	28.54	2008	66.92	39.12	38.62	12,721.63	3.57	72.14	1204.29	629.04
Pinot Noir	P3	13.69	2008	57.55	35.54	50.44	15,416.10	3.78	132.51	1234.07	885.93
Pinot Noir	P5	6.86	2009	64.64	32.95	47.84	10,527.69	3.64	174.17	1356.26	1040.72
Pinot Noir	P8	13.97	2009	53.31	32.30	49.06	13,352.10	3.80	237.71	1153.12	803.59
Pinot Noir	P9	20.71	2008	80.61	42.49	53.71	17,353.85	3.79	40.24	1379.93	1060.48
Malbec	Ma8	16.49	2006	62.16	34.47	53.96	30,505.16	3.62	227.02	1646.45	1347.01
Malbec	Ma7	17.57	2006	61.94	31.95	72.70	34,881.33	3.75	73.14	1958.78	1794.91
Syrah	S4	17.09	2005	47.30	28.26	75.90	38,551.90	3.99	42.92	1914.49	1422.76
Syrah	S9	8.43	2007	43.14	25.95	64.75	29,801.65	3.83	94.27	1786.20	1356.89
Syrah	S2	16.71	2006	56.58	30.72	66.29	28,018.42	3.84	83.41	1753.36	1251.50
Cabernet Sauvignon	C9	39.14	2004	65.84	23.69	71.94	35,745.36	3.80	45.75	1787.72	1129.64
Cabernet Sauvignon	C5	27.43	2006	65.56	31.48	52.96	20,737.42	3.81	61.62	1260.04	520.36
Cabernet Sauvignon	C7	31.43	2002	65.48	38.94	64.53	31,162.16	3.75	9.35	1402.08	1024.25
Cabernet Sauvignon	C20	16.57	2007	39.35	22.62	72.45	31,589.09	3.84	27.14	1690.74	1455.69
Cabernet Sauvignon	C49	13.71	2004	57.60	36.14	64.17	27,406.04	3.71	36.90	1893.87	882.64
Cabernet Sauvignon	C1	34.86	2004	47.21	27.19	63.27	34,805.16	3.80	21.21	1269.96	1050.60
Cabernet Sauvignon	C34	6.86	2004	61.95	37.81	64.44	26,993.06	3.74	70.39	1712.12	991.32
Cabernet Sauvignon	C62	5.57	2007	58.55	35.49	76.39	22,858.35	3.78	132.42	1699.14	1241.62
Cabernet Sauvignon	C16	10.80	2007	51.76	31.32	61.13	29,747.93	3.76	187.97	1620.48	905.69
Cabernet Sauvignon	C88	46.86	2004	57.38	32.38	67.78	35,050.44	3.63	183.94	1839.65	1360.18
Merlot	Mt38	7.97	2007	49.76	29.52	52.33	26,267.35	3.74	217.09	1598.34	1060.48
Merlot	Mt55	15.40	2007	43.90	26.42	56.35	26,843.20	3.86	83.41	1769.40	1287.73
Merlot	Mt70	14.83	2006	40.68	22.81	33.27	35,393.83	3.63	80.82	1695.32	1027.55
Merlot	Mt94	41.14	2005	57.27	31.20	66.04	24,114.29	3.69	57.94	1737.32	1136.23
Merlot	Mt12	27.43	2005	70.17	37.69	51.57	16,294.62	3.69	59.39	1318.07	642.22
Merlot	Mt15	9.57	2006	79.61	40.11	62.59	24,976.26	3.70	36.32	1844.23	1587.43
Blended	B3	2.57	2009	66.96	38.97	41.01	15,476.27	3.65	144.61	1041.63	652.10
Blended	B6	3.94	2009	65.86	40.95	41.07	11,591.12	3.62	122.32	1122.58	566.47
Blended	B9	3.09	2009	63.61	36.45	43.88	16,789.24	3.47	176.01	1208.87	964.97
Pooled standard deviation				10.45	5.57	11.76	8380.76	0.10	66.86	278.42	311.93

Table 2

Correlation between the original variables and the first four principal components.

Original variables	Factor 1	Factor 2	Factor 3	Factor 4
Chroma (C*)	0.58	0.75	−0.11	0.01
Hue angle (h*)	0.73	0.55	−0.15	0.18
Non-flavonoid phenolics (NFP)	0.06	−0.05	0.89	0.24
Non-anthocyanin flavonoids (NAF)	−0.76	0.21	−0.51	−0.07
DPPH ●	−0.79	0.33	−0.13	0.17
ORAC	−0.87	−0.02	0.06	−0.21
pH	−0.71	−0.00	0.05	0.54
Monomeric anthocyanins	0.44	−0.62	−0.51	−0.20
Commercial value	−0.36	0.43	0.43	−0.61
Cumulative variance (%)	40.66	58.22	73.42	83.15

Table 3

Statistical comparison among the three suggested clusters.

Variables	Cluster 1 (n = 8)	Cluster 2 (n = 9)	Cluster 3 (n = 12)	PSD ^a	p-value ^b	p-value ^c
pH	3.83 ^a	3.68 ^b	3.71 ^b	0.10	0.54	<0.01
Commercial value (US\$)	16.53 ^b	8.64 ^b	25.78 ^a	12.00	0.03	<0.01
ORAC (μmol TE/L)	30,918.64 ^a	19,395.74 ^b	25,255.02 ^{a,b}	8,380.56	0.36	0.01
DPPH (% reduction)	68.51 ^a	46.67 ^b	59.36 ^a	11.76	0.72	<0.01
Non-anthocyanin flavonoids (mg/L)	1287.97 ^a	714.92 ^b	950.82 ^b	327.44	0.24	<0.01
Non-flavonoid phenolics (mg/L)	372.55	458.13	559.58	196.05	0.41	0.10
Chroma	49.75 ^b	57.13 ^{a,b}	65.88 ^a	10.46	0.92	<0.01
Hue angle	28.58 ^b	33.42 ^{a,b}	35.46 ^a	5.57	0.71	0.02
Monomeric anthocyanins (mg/L)	69.74 ^b	163.69 ^a	75.08 ^b	66.86	0.33	<0.01
Average vintage year	2006	2008	2006	NA	NA	NA
Grape variety in each cluster				p-value ^d		
Pinot Noir	0	3	2	0.25		
Cabernet Sauvignon	3	1	6	<0.01		
Syrah	3	0	0	0.05		
Merlot	1	2	3	0.61		
Malbec	1	0	1	0.61		
Blended	0	3	0	0.05		

^a PSD = Pooled standard deviation^b Probability values obtained by Hartley test (F max) for homogeneity of variances^c Probability values obtained by one-way ANOVA or Kruskal–Wallis test^d Probability values obtained by χ^2 test. NA = not applicable; Different letters in the same line represent statistical different results ($P < 0.05$).

Figure. 1. A scatter plot of PC 1 vs. PC 2 of the main sources of variability between the wine samples.

See Table 1 for the definition of sample abbreviations.

Figure 2. Cluster analysis for variables and some significant ($P < 0.05$) Pearson correlations (r) calculated for the results.

CAPÍTULO 2

CHARACTERIZATION OF RED WINES FROM SOUTH AMERICA BASED ON SENSORY PROPERTIES AND ANTIOXIDANT ACTIVITY

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ABSTRACT

BACKGROUND: It is widely accepted that red wines constitute one of the most important sources of dietary polyphenolic antioxidants. However, it is still not known how some variables such as variety, vintage, country of origin, and retail price are associated to the antioxidant activity and sensory profile of South American red wines. In this regard, 80 samples produced in Brazil, Chile, and Argentina were assessed in relation to their sensory properties, color, and *in vitro* antioxidant activity, and results were subjected to multivariate statistical techniques.

RESULTS: Samples were grouped in clusters, characterized by high, intermediate and low *in vitro* antioxidant activity, sensory properties and prices. It was possible to observe that wines with high antioxidant activity were associated to high retail prices and overall perception of sensory quality.

CONCLUSION: South American wines produced from *Vitis vinifera* such as Syrah, Malbec and Cabernet Sauvignon had higher *in vitro* antioxidant activity and also higher

sensory quality than wines produced from *Vitis labrusca*. This result was independent of vintage (2002-2010), corroborating the idea that the same grape varietal, even when produced in different years, displays similar sensory characteristics and antioxidant activity.

Keywords: red wine, antioxidants, consumers, sensometrics, price.

1. Introduction

It is widely accepted that wines constitute one of the most important sources of dietary polyphenolic antioxidants¹. Among beverages, red wine has been reported to be more protective effect on oxidative stress than other alcoholic beverages, suggesting a possible role of red wine polyphenols in the reduction of risk of diseases related to oxidative stress such as cancer, cardiovascular, and neurodegenerative diseases². It is known that red wines have a higher antioxidant activity measured by numerous *in vitro* assays such as ORAC, FRAP, ABTS, and DPPH compared to white/rosé wines or purple grape juices^{3,4}. Indeed, many of these health effects have been attributed to the antioxidant activity of the phenolic compounds (hydroxycinnamates, hydroxybenzoates, stilbenes, flavonols, flavanols, and anthocyanins) present in red wines, which act against reactive oxygen species^{1,5}. Moreover, the phenolic compounds present in red wines also act by reducing the inflammatory process⁶.

Multivariate statistical techniques coupled with chemical, physicochemical, and sensory evaluations have been successfully applied to accomplish several objectives, including the differentiation of young and aged wines based on principal polyphenolic constituents⁷, the characterization of red wine varieties in relation to their chemical properties⁸, the verification of the geographical origin of wines by means of evaluating

wine's mineral content⁹, the evaluation of the main chemical compounds responsible for red wine aroma¹⁰, the monitoring of red wine aging in oak barrels based on sensory attributes¹¹, and the assessment of the correlation between the antioxidant activity of red wines with phenolic compounds¹², among other applications.

The well-documented association between the moderate consumption of red wine and the reduction in the risk of degenerative diseases has increased the demand of information identifying which red wine variety (i.e., Merlot, Carbernet Sauvignon and Malbec, blended, among others) could better achieve this objective. Consumers do not have access to data regarding antioxidant activity of red wines, but they do have access to their retail prices and some sensory properties; thus, it would be interesting to investigate the correlation among these parameters in order to offer useful information for consumers who are interested in red wine antioxidant activity besides sensory aspects. For example, by using these multidimensional correlations it would be possible to investigate the association among antioxidant activity, sensory properties and retail price. This kind of information could contribute to consumers choose which wine variety, country of origin, and retail price would be more suitable to increase the uptake of antioxidants. In order to accomplish this objective, multivariate statistical tools such as Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) can be employed to point out similarites and correlations among parameters and samples^{8,12}. Based on this fact, the objective of this study was to evaluate the association among retail price, sensory parameters, and color of 80 samples of Brazilian, Chilean, and Argentinean red wines and their *in vitro* antioxidant activity by using multivariate statistical techniques.

2. Materials and methods

2.1 Chemicals

Fluorescein (dihydroxyspiro[isobenzofuran-1[3H],9'[9H]-xanthen]-3-one), 2-2'-azobis 2-methylpropionamedine dihydrochloride (AAPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Sigma Chemicals Co. (St. Louis, MO, USA). The aqueous solutions were prepared by using ultra-pure Milli-Q water (Millipore, São Paulo, Brazil). All other reagents used in the experiments were of analytical grade.

2.2 Red wines

A total of 72 red wines produced in Brazil (n = 20), Chile (n = 28), and Argentina (n = 24) made from the five most characteristic *Vitis vinifera* red grape varieties (Merlot, Malbec, Pinot Noir, Cabernet Sauvignon, and Syrah) were studied. A total number of 7 blended wines produced with *Vitis labrusca* grapes (Concord, Niagara, and Izabel varieties) and 1 blended wine produced with *Vitis vinifera* (30% Syrah + 70% Cabernet Sauvignon) were also evaluated, totalling 80 wine samples. Table 1 presents the samples grouped by country and grape variety and their commercial value, vintage (average by group), and place of production. All of the wines were purchased from 3 different importers in São Paulo, SP, Brazil. Wines were brought to the laboratory, aliquoted into 2 mL eppendorfs, immediately immersed in liquid nitrogen and stored at -80°C until further analysis.

2.3 Instrumental color and *in vitro* antioxidant activity

For assessing the wine color, a sample of approximately 50 mL was separated from the bottle after uncorking and color measurements were performed less than 4 min after opening the bottle. Instrumental color measurement was conducted four times using a spectrophotometer (Model D25L-2, Hunter Assoc. Laboratory, Reston, VA, USA) with a D65 optical sensor and 10° angle of vision. The CIE $L^*a^*b^*$ system was considered, where two color coordinates, a^* and b^* , as well as lightness, L^* , were measured. Chroma (C^*), which measures the color intensity perceived by human vision was calculated by $C^* = (a^{*2} + b^{*2})^{1/2}$.

Free-radical-scavenging activity towards DPPH radical was determined in triplicate by the method proposed by Brand-Williams, Cuvelier, & Berset¹³ with slight modifications. Briefly, a 25 μ L aliquot of red wine (diluted 25 times in water) was mixed with 900 μ L of methanol and 5.0 μ L of a methanolic DPPH solution (10.0 mmol/L). The reaction was allowed to take place in the dark for 30 min at 25 °C, and absorbance at $\lambda = 517$ nm was read using a spectrophotometer (model mini 1240 UV-VIS, Shimadzu Corporation, Kyoto, Japan). The scavenging activity was calculated as % scavenging activity = $[1 - (A_{517} \text{ sample} / A_{517} \text{ blank})] \times 100$.

The oxygen radical absorbance capacity (ORAC) assay was conducted to measure the peroxy-radical-scavenging activity of each wine by following a method previously reported by Prior et al.¹⁴. Briefly, wine samples were diluted accordingly (1:900) in 75 mmol/L phosphate buffer (pH 7.1). Trolox standard solutions were prepared at a concentration ranging from 6.25 to 100 μ mol/L. The fluorescence decay, at an excitation wavelenght of 545 nm and an emission wavelenght of 572 nm, was followed using a plate reader (Multi-Detection microplate reader; Synergy-BIOTEK,

Winooski, VT, USA) every minute following addition of AAPH (153 mmol/L in 75 mmol/L phosphate buffer, pH 7.1) for 60 min, and the area under the curve of the fluorescence decay was integrated using Gen5 software. The antioxidant activity of the red wines was carried out three times and results were expressed as μmol Trolox Equivalents per liter ($\mu\text{mol TE/L}$).

2.4 Sensory evaluation

Seven professional oenologists/sommeliers (3 men and 4 women, aged 24-46 years) were selected to evaluate the wine samples. All of them had more than five years experience in evaluating all types of wine using the same protocol applied in this study. The 80 samples were assessed in groups of 8, where one group was evaluated per day. Samples were coded with random 3-digit numbers and served monadically. In order to reduce carry-over effects, a 4-min break was taken between samples, during which the panelists were required to eat a piece of bread and rinse the mouth thoroughly with spring water. To balance out any order effects that may occur, the order of presentation was randomized for each subject, and wines were evaluated using a completely randomized design¹⁵. The bottles were opened roughly 30 min before tasting, and no information about which type of red wine or its country of origin was provided to the panelists. Panelists were seated in separate booths and there was a uniform source of lighting, absence of noise and distracting stimuli in the laboratory. Panelists were presented with 50 ml samples at 17 °C, served in crystal tulip-shaped glasses, and they were asked to swirl/smell each sample for about 15 s and to start to rate the intensity of each attribute.

The following parameters were analyzed: color intensity (1= water-white, 4= medium, 7= dark), intensity of odors (1= low, 3= medium, 5= pronounced), degree of development (1= young, 3= completely evolved, 5= oxidised), acidity level (1= low, 3= medium, 5= high), tannin level (1= low, 3= medium, 5= high), overall perceived quality (1= faulty, 3= acceptable, 6= outstanding), and approximate retail price (no scale was given). For color intensity evaluation, samples were put against a white background under natural light. The intensity of each sensory parameter was measured by the structured scales applied to levels 3 and 4 from the Wine & Spirits Education Trust¹⁶. The sensory evaluation protocol was approved by the University of São Paulo Research Ethics Committee and written consent was given by all wine experts.

2.5 Statistical assessment

2.5.1 Univariate analysis

Data were presented as mean $\pm$ pooled standard deviation. A bivariate correlation matrix of the data was produced to measure the association between the variables, displayed in Pearson's correlation coefficient (r). The significance (p -value) of such correlations was also provided.

2.5.2 Multivariate statistical techniques

Hierarchical Cluster Analysis (HCA) was performed on autoscaled data, and sample similarities were calculated on the basis of the squared Euclidean distance¹¹. The Ward hierarchical agglomerative method was used to establish clusters. The following variables were used to HCA: retail price, C^* , DPPH, vintage, ORAC, intensity

of color, intensity of odors, degree of development, acidity level, tannin level, overall perception of sensory quality, and approximate retail price given by the taste panel. A dendrogram for samples and one for variables were built. In order to compare the results within the four suggested clusters, Hartley's test was applied to check for homogeneity of variances, while one-way ANOVA and Tukey's HSD *pos hoc* tests were then performed to identify contrasts. For the variables that presented non-homogenous variances ($p < 0.05$), the non-parametric multiple comparison Kruscall-Wallis test was used. P-values below 0.05 were considered to be significant.

Principal Component Analysis (PCA) was applied to the data set to separate the samples according to the same response variables used in HCA. For this purpose, the results obtained for each parameter were adopted as columns (12 variables) and the wine samples ($n = 80$) as rows, totalling 960 results. Eigenvalues higher than 1.0 were adopted to explain the projection of the samples on the factor-plane. Autoscaling was used as a pretreatment of the results to equalize the statistical importance of all the variables¹¹. For all statistical procedures, the Statistica 9.0 software (Stat-Soft, Tulsa, OK, USA) was employed.

3. Results

All the 80 wines evaluated in this study constitute a quite heterogeneous group, made from five different grape varieties, including 8 blended wines (7 *V. labrusca* and 1 *V. vinifera*), with diverse ages, made with distinct technological procedures, produced in different growing areas. Therefore, differences on color, sensory properties, and *in vitro* antioxidant activity were encountered. The results (Table 1) showed that the inhibition of DPPH ranged from 41.99 to 66.70%, while the ORAC results varied from 13,285 to

35,114 $\mu\text{mol TE/L}$. The redness within the wine varieties, measured by the a^* coordinate, ranged from 39.17 to 52.60, while the chromaticity ranged from 43.14 to 65.48.

In regard to the sensory evaluation, a total of 18 samples (22.50%) garnered scores between 'acceptable' to 'good' and 61 out of 80 samples were comprehended between 'good' and 'outstanding'. A total of 38 samples (47.50%) presented a medium to medium-high level of intensity of odors. Forty-three wines (53.75%) showed a medium-low to medium level of acidity.

Retail price was correlated significantly ($p < 0.01$) to tannin level ($r = 0.45$), degree of development ($r = 0.38$), intensity of color ($r = 0.38$), intensity of odors ($r = 0.47$), overall perception of sensory quality ($r = 0.45$), and retail price given by panelists ($r = 0.56$). With exception of degree of development, all the other parameters were correlated ($p < 0.01$) to the antioxidant activity. Wine vintage did not correlate to any evaluated parameter. It is known that high values of correlation coefficients (r) are desirable when physicochemical and sensory attributes are analyzed. However, when very different parameters such as sensory attributes and antioxidant assays are correlated to retail price (dependable on many economical factors and therefore changes dramatically from one importer to another), it is expected that the correlation coefficients do not reach valued above 0.8.

Using HCA applied to the 12 variables, associations among the sensory properties, instrumental color, price and *in vitro* antioxidant activity could be observed (Figure 1), while for the samples, four clusters were suggested (Figure 2). Cluster 1 contained the samples with the highest retail price, antioxidant activity measured by

DPPH and ORAC methods, intensity of color, overall perception of sensory quality, intensity odors, acidity level, tannin level, and the lowest redness and chroma. Hence, this cluster included the high-quality wines (Table 2). On the other hand, the 7 blended wines made from *Vitis labrusca* grapes, which were included in cluster 4, garnered the lowest means for sensory attributes and antioxidant activity, being therefore the cluster with the low-quality wines. Red wines included in clusters 2 and 3 presented intermediate values for the response variables. Cabernet Sauvignon samples were distributed in all clusters; however a total of 60.87% of the samples were in cluster 2, whereas the Syrah and Malbec samples were included in the first and second clusters. A total of 5 out of 9 Merlot were included in the cluster with intermediate-high quality.

The PCA showed that the first principal component (PC) (eigenvalue 5.66) explained 47.15% of the variation and was associated with retail price, antioxidant activity, intensity of color and odors, acidity level, overall perception of sensory quality, tannin level, and retail price given by the taste panel (Figure 3a). The second PC (eigenvalue 1.69) explained another 14.07% of the variation and was associated with retail price, chroma, and degree of development. Figure 3b shows the projection of variables on the factor plane.

4. Discussion

From the direct comparison between samples included in cluster 1 and cluster 4, it is possible to observe that wines with high antioxidant activity were associated to high retail prices and overall perception of sensory quality. Although there is an expectative about this association, no study has reported this information based on chemical and sensory parameters. Price was directly related to degree of development of wines ($r =$

0.38; $p < 0.01$) and intensity of color observed by the taste panel ($r = 0.38$; $p < 0.01$). It is known that wines that are not subjected to aging have a lower retail price compared to the ones that undergo aging in oak barrels. This process is very costly for wineries due to the high cost of oak casks, to the lengthy periods that wine must stay in them, to logistics, and winery operation problems that aging wine in barrels implies¹⁷. Polymerization and condensation reactions between wine flavanols and anthocyanidins are responsible for these chemical and chromatic changes¹⁸. Wine color plays a significant role in the perception of quality, once it is related to the degree of development, content of monomeric anthocyanins, and acceptability by consumers¹⁹.

The tannin level assessed by the taste panel, which is directly related to the phenolic compounds present in red wines, and the intensity of color perceived by the taste panel, which is derived from the pigments in the berry skin, were both significantly correlated to the antioxidant activity of the samples. Different researchers have attributed the high antioxidant activity of red wines to the total phenol content^{1,12,20} and to the red-purple pigments, namely anthocyanins, present in wines²¹.

The use of wine taster experts in the evaluation of sensory perception of quality of wines can be criticized. However, it reflects the real conditions in which wine prices are defined by the market. In this research, the overall perception of wine quality was closely related to intensity of odors (Figure 1). Indeed, wine odor is the first characteristic after appearance that is assessed by a trained panelist, and its quality depends on various factors such as grape variety, maturation and aging, winemaking procedures, management practices, and yeast, among others²². All these factors influence directly the occurrence and content of alcohols, aldehydes, esters, acids, monoterpenes, and other minor components that are responsible for both odor and aroma perceptions when

smelling/swirling the wine²³. Obviously, the perception of acidity/tannin/alcohol levels, intensity of flavor, aroma profile, and body also play an important and decisive role in the overall perception of quality of each wine. In our study, they also complemented the classification of wines.

Blended wines made of *Vitis labrusca* (mixture of Concord, Niagara, and Isabel varieties) are widely consumed in Brazil during regional parties and for confection of many different types of recipes; they are sold in 3-L containers and have a lower retail price in comparison to *V. vinifera* wines. Data show that around 77% of the total grape production is directed to make wines from *V. labrusca*, whereas only 13% of red wines is made of *V. vinifera* grapes. In Argentina, the blended wines are basically made of *V. vinifera* grapes, and in Chile these wines can not be produced, thus the availability *V. labrusca* blended wines in the commerce scarce. In this study, blended wines (*V. labrusca*) garnered the lowest scores for all sensory attributes and the lowest antioxidant activity. Woraratphoka, Intarapichet, & Indrapichate²⁴ verified that *V. labrusca* wines had the lowest antioxidant measured by DPPH and FRAP assays as well as the lowest content of total phenol and flavonoid materials. Likewise, Hermosín-Gutiérrez and Nixdorf²⁵ compared the antioxidant activity of Brazilian *V. labrusca* (Isabel) and *V. vinifera* red wines using the DPPH assay and verified that Isabel wines presented the lowest values of inhibition of DPPH. Rizzon, Miele, and Meneguzzo²⁶ evaluated the suitability of Isabel variety to produce wine and verified that besides the wine presented a satisfactory chemical profile, the final product had no sensory acceptability as measured by a consumer panel by having garnered low scores for balance and softness.

From Table 2, it is possible to observe that the Brazilian wines garnered, in general, the lowest mean scores for the sensory attributes. Only one sample was included in the cluster with high quality, while the great part of samples was included in cluster with intermediate-low quality. It is obvious that Argentina and Chile are the main red wine producers in South-America and possess the wines with superior sensory quality than the Brazilian ones¹⁶.

Another aspect that needs to be pointed out here is the division of grape varieties within clusters. The HCA did not include grape variety as variables; however, cluster 1 and 2 grouped a great number of Malbec, Cabernet Sauvignon, and Syrah wines, while cluster 3 contained all *V. labrusca* wines, and Pinot Noir wines made in Brazil predominated in cluster 4. It is known that many factors such as environmental and climate conditions, soil type, grape variety, degree of ripeness, winemaking processes, and length of maceration process may contribute to great differences in sensory properties, color, and antioxidant properties of wine varieties²⁷. In the present work, a large set of different samples was used to corroborate the fact that besides there are many environmental, agricultural, and technological factors that directly influence wine properties, these aspects will never be sufficient to cause wine typicality loss.

Sensory aspects are partially due to the presence of phenolic compounds in the chemical composition of red wines. The same compounds are responsible for the antioxidant activity. Thus, it is demanding to investigate if there is a direct relationship between the retail price (associated with the sensory quality) and the antioxidant activity. Checking this direct relationship is required because consumers have no access to the chemical, antioxidant activity, or even sensory characteristics of the wines, but they do have access to their retail prices. This correlation (price x antioxidant activity) was

sparse, although significant, for both ORAC ($r = 0.28$; $p = 0.01$) and DPPH ($r = 0.24$; $p = 0.03$) assays. However, when the retail price given by panelists was associated to ORAC and DPPH a higher correlation was attained ($r = 0.49$ and $r = 0.43$, respectively; $p < 0.01$). The retail price was in line with the proximate retail price given by the taste panel ($r = 0.56$; $p < 0.01$). Our results are in accordance with those obtained by Granato, Katayama, and Castro¹² who evaluated 29 Brazilian red wines (Merlot, Syrah, Cabernet Sauvignon, Pinot Noir, *Vitis labrusca* wines, and Malbec) and found that there is a sparse correlation between wine retail price and antioxidant activity (ORAC and DPPH). In the same way, Faustino, Sobrattee, Edel, and Pierce²⁸ studied the relationship between the antioxidant activity and retail price of Merlot wines from Chile, United States and Canada and concluded that if a consumer is interested in purchasing a Merlot wine with high antioxidant activity, it would appear to be erroneous to use the price of the wine as an indirect indicator of antioxidant capacity. It is important to mention that besides there is information about the association between retail price and wine quality of red wines made in different countries, no other information about association between antioxidant activity and retail price was found in the literature.

5. Conclusions

South American wines produced from *Vitis vinifera* such as Syrah, Malbec and Cabernet Sauvignon had higher *in vitro* antioxidant activity and also higher sensory quality than wines produced from *Vitis labrusca*. This result was independent of vintage (2002-2010), corroborating the idea that the same grape varietal, even when produced in different years, displays similar sensory characteristics and antioxidant activity. The results support the fact that wines with lowest sensory quality and price are associated

with lowest antioxidant capacity, but the major part of samples grouped in the intermediate-high quality cluster had higher antioxidant capacity and better sensory properties, but not higher price. Therefore, price does not seem to be the most suitable factor to discriminate wines according to their quality and antioxidant activity.

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Table 1. Red samples data grouped by country, variety, vintage, retail price, color properties, sensory attributes, and antioxidant activity.

Commercial information			Antioxidant activity			Sensory evaluation					Color			
Country	Variety	Vintage	Retail price (US\$) ^a	DPPH ^b	ORAC ^c	Intensity of color	Intensity of odor	Degree of development	Acidity level	Tannin level	Overall perception of quality	Retail price (US\$) - sensory panel	a *	C *
Sensory scale	NA	NA	NA	NA	NA	1= Water-white; 7= dark	1= Low; 5= pronounced	1= Young; 5= oxidised	1= Low; 5= high	1= Low; 5= high	1= Faulty; 6= outstanding	NA	NA	NA
Brazil	Pinot Noir (n=5)	2008	16.75	47.93	13,874	3.06	2.77	1.55	2.94	2.26	2.97	25.20	52.60	64.61
	Syrah (n=1)	2006	8.43	64.75	29,801	4.71	3.00	1.29	3.00	3.14	3.71	32.14	39.17	43.14
	Cabernet Sauvignon (n=9)	2005	24.71	66.44	29,594	4.16	2.90	1.56	2.81	2.75	3.38	31.13	47.95	57.66
	Merlot (n=5)	2006	15.04	51.22	25,955	4.00	2.86	1.86	2.83	2.63	2.89	26.72	48.12	56.82
	Blended (V. labrusca; n=3)	2009	3.20	41.99	13,285	2.05	2.66	1.05	1.67	1.14	2.48	8.02	52.22	65.48
Argentina	Pinot Noir (n=5)	2007	24.67	55.35	20,161	3.40	2.81	1.49	2.43	2.17	3.26	21.96	46.22	54.37
	Malbec (n=9)	2007	25.19	58.24	26,524	3.33	2.80	1.50	2.54	2.19	3.86	22.60	42.99	47.65
	Syrah (n=4)	2007	29.78	64.12	28,966	2.93	2.76	1.34	2.21	1.83	3.93	17.53	40.21	51.24
	Cabernet Sauvignon (n=5)	2007	25.57	67.71	29,849	2.93	2.76	1.34	2.21	1.83	3.89	17.53	38.73	42.14
	Merlot (n=1)	2002	13.66	53.47	32,147	2.57	2.86	1.29	3.00	2.29	3.86	37.50	47.29	57.44
Chile	Blended (V. labrusca; n=4)	2006	7.32	45.35	18,017	3.00	2.61	1.50	2.25	2.04	2.97	22.46	43.75	50.25
	Blended (V. vinifera; n=1)	2007	9.14	46.13	20,124	3.43	2.00	1.00	2.29	2.00	2.86	16.64	31.59	33.04
	Pinot Noir (n=7)	2007	34.48	49.44	23,777	3.35	3.02	1.55	3.14	2.84	3.67	38.93	49.81	62.44
	Syrah (n=7)	2007	29.94	61.48	31,470	4.61	3.45	1.47	3.12	3.14	4.02	44.44	41.05	45.59
	Cabernet Sauvignon (n=9)	2007	16.67	55.25	33,412	4.02	3.13	1.32	2.91	3.06	3.68	33.06	43.03	48.16
	Merlot (n=3)	2007	28.27	63.00	33,708	4.14	3.09	1.62	3.24	3.48	3.71	40.24	47.02	54.45
	Malbec (n=2)	2007	31.10	66.70	35,114	4.50	3.36	1.15	2.93	3.50	4.29	41.29	41.43	45.03

Note: ^aThe retail price corresponds to a bottle of 750 mL; ^bExpressed as % of scavenging activity; ^cExpressed as $\mu\text{mol TE/L}$; NA= not applicable.

Table 2. Univariate statistical comparison among the four suggested clusters.

Variables	Cluster 1: High quality wines (n = 15)	Cluster 2: Intermediate-high quality wines (n = 41)	Cluster 3: Intermediate- low quality wines (n = 17)	Cluster 4: Low quality wines (n = 7)	PSD ^a	p-value ^b	p-value ^c
Retail price - sensory panel (US\$/750 mL)	45.03 ^a	36.60 ^b	27.03 ^c	11.50 ^d	11.02	0.29	< 0.01
Retail price (US\$/750 mL)	37.18 ^a	20.57 ^b	21.20 ^b	4.99 ^c	14.57	< 0.01	< 0.01
ORAC (μmol TE/L)	35,064 ^a	28,338 ^b	18,967 ^c	15,504 ^c	8,761	0.31	< 0.01
DPPH (% reduction)	65.35 ^a	56.72 ^b	54.87 ^b	43.43 ^c	9.76	0.09	< 0.01
Intensity of color	5.03 ^a	4.05 ^b	3.37 ^c	2.45 ^d	0.94	0.08	< 0.01
Acidity level	3.10 ^a	3.02 ^a	2.85 ^a	1.83 ^b	0.44	0.64	< 0.01
Overall sensory quality	4.08 ^a	3.74 ^b	2.97 ^c	2.47 ^d	0.61	0.20	< 0.01
Tannin level	3.37 ^a	2.98 ^b	2.47 ^c	1.49 ^d	0.60	0.77	< 0.01
Degree of development	1.59 ^{ab}	1.38 ^{bc}	1.83 ^a	1.18 ^c	0.37	0.01	< 0.01
Intensity of odors	3.48 ^a	3.08 ^b	2.81 ^c	2.37 ^d	0.43	0.34	< 0.01
Chroma (C*)	43.75 ^c	51.68 ^b	62.62 ^a	53.82 ^{abc}	10.86	0.12	< 0.01
Redness (a* coordinate)	40.25 ^b	44.09 ^{ab}	51.43 ^a	45.49 ^{ab}	7.10	0.24	< 0.01
Vintage (average)	2007	2007	2006	2007	NA	NA	NA
Grape variety in each cluster							
Pinot Noir	0	7	10	0			
Cabernet Sauvignon	5	14	4	0			
Syrah	5	7	0	0			
Merlot	1	5	3	0			
Malbec	4	7	0	0			
<i>Vitis labrusca</i>	0	1	0	7			
Number of samples in each cluster							
Brazil (n = 23)	1	7	12	3			
Argentina (n = 29)	7	15	3	4			
Chile (n = 28)	7	19	2	0			

^aPSD = Pooled standard deviation; ^bProbability values obtained by Hartley test (F max) for homogeneity of variances; ^cProbability values obtained by one-way ANOVA or

Kruskal-wallis test; NA = not applicable; Different letters in the same line represent statistical different results (P < 0.05).

Figure 1: Hierarchical Cluster Analysis applied to the variables followed by the Pearson coefficient and its respective p-value.

Figure 2: Hierarchical Cluster Analysis (HCA) applied to the red wines samples according to the 12 response variables presented in Figure 1.

Figure 3: A scatter plot (a) for the main sources of variations among the red wines: PC1 *versus* PC2. The first letter beginning with C = Cabernet Sauvignon, P = Pinot Noir, Mt = Merlot, S = Syrah, Ma = Malbec, B = Blended; the second letter beginning with A = Argentina, B = Brazil, C = Chile; (b) Projection of variables on the factor plane (1x2).

CAPÍTULO 3

PHENOLIC COMPOSITION OF SOUTH AMERICAN RED WINES CLASSIFIED ACCORDING TO THEIR ANTIOXIDANT ACTIVITY, RETAIL PRICE AND SENSORY QUALITY

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ABSTRACT

In this study, 73 South American red wines (*Vitis vinifera*) from 5 varietals were classified based on sensory quality, retail price and antioxidant activity and characterized in relation to their phenolic composition. ORAC and DPPH assays were assessed to determine the antioxidant activity, and sensory analysis was conducted by seven professional tasters using the Wine & Spirits Education Trust's structured scales. The use of multivariate statistical techniques allowed the identification of wines with the best combination of sensory characteristics, price and antioxidant activity. The most favourable varieties were Malbec, Cabernet Sauvignon, and Syrah produced in Chile and Argentina. Conversely, Pinot Noir wines displayed the lowest sensory characteristics and antioxidant activity. These results suggest that the volatile compounds may be the main substances responsible for differentiating red wines on the basis of sensory evaluation.

Keywords: wine, phenolic composition, flavonoids, antioxidants, chemometrics, cluster analysis.

1. Introduction

Studies have shown that the phenolic contents of red wine may explain the French paradox; that is, the ability to consume a high-fat diet while maintaining a low incidence of atherosclerosis and other related coronary diseases in populations that drink red wine daily (Renaud & Lorgeril, 1992). There is some evidence that certain age-related diseases occur because of the oxidation of cell components caused by free radicals, and antioxidants protect the body by scavenging these reactive species (Zbarsky, Datla, Parkar, Rai, Aruoma, & Dexter, 2005; Zhang et al., 2006). Free radicals take an electron from neighboring molecules/atoms to become stable; however, this process generates other free radicals. This chain reaction is thought to contribute to lipid peroxidation, DNA damage, and protein degradation during oxidative-stress events (Clarkson & Thompson, 2000; Shahidi, 2009). The cells respond to the oxidation promoted by the reactive species by increasing the expression and activity of endogenous antioxidant enzymes, namely catalase, glutathione peroxidase, glutathione reductase, and superoxide dismutase. However, this response may not be enough to scavenge and buffer the reactive species. Hence, exogenous antioxidant compounds should be included in the diet (de Zwart, Meerman, Cammandeur & Vermeulen, 1999). In this regard, the phenolic materials in red wines represent a suitable source of this exogenous protection.

A well-balanced characterization of the antioxidant capacity and chemical composition of wines is therefore necessary to determine their health effects. For example, Lotito, Renart, and Fraga (2002) assessed the association between antioxidant activity,

measured using the ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) method, and the content of total phenolic compounds, (+)-catechin, and gallic acid of Argentina Cabernet Sauvignon and Malbec red wines and observed high correlations ($r > 0.83$, $p < 0.05$) between *in vitro* antioxidant activity and the phenolic compounds concentration. In the same way, Que, Mao, and Pan (2006) studied the effect of some phenolic compounds on the free radical scavenging activity measured by the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay and verified that vanillic acid, *p*-coumaric acid, and quercetin contributed minimally to the antioxidant activity of wines. In a previous study, we observed that both the total phenolic compounds and total flavonoids, especially non-anthocyanin flavonoids, were the main substances responsible for *in vitro* antioxidant activity in Brazilian red wines, as measured by ORAC (oxygen radical absorbance capacity) and DPPH assays (Granato, Katayama & Castro, 2010).

The phenolic compounds present in red wine can be divided into two major classes, based on their carbon skeletons: flavonoids and non-flavonoids. Flavonoids include anthocyanidins (malvidin, delphinidin, petunidin, peonidin, and cyanidin), flavonols (quercetin, rutin, myricetin, and kaempferol), flavanols (catechin, epicatechin, epicatechin 3-gallate, and galocatechin), flavones (luteolin, apigenin), and flavanones (naringenin). The main non-flavonoid phenolics include cinnamic acids (caffeic, *p*-coumaric, and ferulic acids), benzoic acids (gallic, vanillic, and syringic acids), and stilbenes (resveratrol) (Cheynier, 2006). These compounds are primarily responsible for the health benefits associated with moderate red wine consumption. The quantities of these phenolic compounds vary considerably in different types of wines depending on the grape variety, environmental factors in the vineyard, the wine processing techniques, soil and atmospheric conditions during ripening, the aging process, and berry maturation

(Lachman, Sulc, & Schilla, 2007). Therefore, each type of grape presents distinct biological activity, chemical composition, and sensory appeal.

It is not known whether the same phenolic compounds involved in the sensory quality, and consequently the retail price, of red wines are responsible for the wines' antioxidant effects. Considering that these two aspects (sensory quality and health benefit) contribute to the consumer appeal of red wines, this study aimed to characterize the phenolic composition of 73 *V. vinifera* red wines from South America classified according to their antioxidant activity, retail price, and sensory quality.

2. Material and Methods

2.1 Chemicals

Folin-Ciocalteu reagent, 2-2' azobis 2-methylpropionamedine dihydrochloride (AAPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), 3',6'-dihydroxyspiro[isobenzofuran-1[3H],9'[9H]-xanthen]-3-one (fluorescein), and chemical HPLC-grade standards (purity $\geq 95\%$) of *trans*-resveratrol, gallic acid, caffeic acid, *p*-coumaric acid, (+)-catechin, (-)-epicatechin, vanillic acid, ferulic acid, kaempferol, quercetin, rutin, and myricetin were obtained from Sigma (St. Louis, MO, USA). Methanol, acetonitrile, and acetic acid were of HPLC grade, while the other reagents used in the experiments were of analytical grade. The aqueous solutions were prepared using ultra-pure Milli-Q water (Millipore, São Paulo, SP, Brazil).

2.2 Red wine samples

A total of 73 red wines produced in Brazil (n = 20), Chile (n = 28), and Argentina (n = 25) with the five most characteristic *Vitis vinifera* red grape varieties (Merlot, Malbec, Pinot Noir, Cabernet Sauvignon, and Syrah) were studied. Table 1 presents the samples according to country and grape variety, including their commercial value and vintage. The wines were purchased from 3 different importers in São Paulo, SP, Brazil. Wines were brought to the laboratory, aliquoted into 2-mL eppendorfs, immediately immersed in liquid nitrogen and stored at -80 °C for further analysis.

2.3 Instrumental colour, total phenolics, flavonoids, and monomeric anthocyanins

To assess the wines' colour, a sample of approximately 50 mL was separated from each bottle after, and colour measurements were performed less than 4 min after the bottle was opened. Instrumental colour measurement was conducted four times by transmittance using a spectrophotometer (Model D25L-2, Hunter Assoc. Laboratory, Reston, VA, USA) with a D65 optical sensor and 10-degree angle of vision. The CIEL*a*b* system was utilized, in which two colour coordinates, redness (*a**) and yellowness (*b**) were measured, along with lightness (*L**) and chroma (*C**).

The total phenolic compound content of the red wines was determined in triplicate, using the Folin-Ciocalteu method (Singleton, & Rossi, 1965). The absorbance was measured using a spectrophotometer (Model Mini 1240 UV-Vis, Shimadzu Corporation, Kyoto, Japan) at the wavelength of 725 nm. The total phenolic content was determined by a standard curve of gallic acid (0 to 200 mg/L), and the results were expressed as mg of gallic acid equivalents per litre (mg GAE/L).

The total flavonoid content of the red wines was determined in triplicate, using the modified colourimetric method outlined by Jia, Tang, and Wu (1999). The absorbance

was measured with a spectrophotometer (Model Mini 1240 UV-Vis, Shimadzu Corporation, Kyoto, Japan) at the wavelength of 510 nm. The flavonoid content was determined by a standard curve of catechin (0 to 100 mg/L) and the results were expressed as mg catechin equivalents per litre (mg CTE/L).

The monomeric anthocyanin content was determined using the pH differential method (Lee, Durst, & Wrolstadt, 2005). Following this method, an aliquot of the red wine (250 μ L) was added to 2.25 mL of pH 1.0 buffer (KCl, 0.025 mol/L). Another 250 μ L of red wine were also added to 2.25 mL of pH 4.5 buffer (CH₃CO₂Na, 0.40 mol/L). Absorbance was measured in a spectrophotometer (Model Mini 1240 UV-Vis, Shimadzu Corporation, Kyoto, Japan) at λ = 510 nm and λ = 700 nm. Results were calculated using Equation 1 and expressed as mg per litre (mg/L).

$$\text{Total monomeric anthocyanins (mg/L)} = [(A \times \text{MW} \times D \times 100)]/e \quad (1)$$

whereby: $A = (A_{510} - A_{700})_{\text{pH}1.0} - (A_{510} - A_{700})_{\text{pH}4.5}$, e is cyanidin 3-glucoside molar absorbance (26,900), MW is the molecular weight for cyanidin-3-glucoside (449.2), and D is a dilution factor (10). The results in every assay were obtained from three replicates.

2.4 Measurement of *in vitro* antioxidant activity

Free radical-scavenging activity towards the 1,1-diphenyl-2-picrylhydrazyl radical was determined in triplicate using the method previously proposed by Brand-Williams, Cuvelier, and Berset (1995), with slight modifications. Briefly, a 25- μ L aliquot of red wine (diluted 25 times in water) was mixed with 900 μ L of methanol and 5.0 μ L of a methanolic DPPH solution (10.0 mmol/L). The mixture was left to react in the dark for 30

min at 25 °C, and then absorbance at a wavelength of 517 nm was read using a spectrophotometer (Model Mini 1240 UV-Vis, Shimadzu Corporation, Kyoto, Japan). The antioxidant activity towards the DPPH radical was calculated using Equation 2:

$$\% \text{ scavenging activity} = [1 - (A_{517} \text{ sample} / A_{517} \text{ blank})] \times 100 \quad (2)$$

The oxygen radical absorbance capacity (ORAC) assay was conducted to measure the peroxy radical-scavenging activity of each wine by following a method previously reported by Prior et al. (2003). Briefly, the samples were diluted (1:900) in 75 mmol/L phosphate buffer (pH 7.1). Trolox standard solutions were prepared at concentrations ranging from 6.25 to 100 µmol/L. The plate reader (Multi-Detection microplate reader; Synergy-BIOTEK, Winooski, VT, USA) was programmed to record the fluorescence every minute after the addition of AAPH (153 mmol/L in 75 mmol/L phosphate buffer, pH 7.1) for 60 min, and the area under the curve of the fluorescence decay was integrated using Gen5 software. Each red wine's antioxidant activity was measured three times, and results are expressed as mmol Trolox equivalents per litre (mmol TE/L).

2.5 Sensory evaluation

Seven professional wine tasters (3 men and 4 women, aged 24 to 46 years) were selected to evaluate the wine samples. The bottles were opened roughly 30 min before tasting, and no information about the type of red wine or its country of origin was provided to the panelists. The 73 samples were assessed in groups of 8, and one group was evaluated per day. Samples were coded with random 3-digit numbers and served monadically. To balance out any possible order effects, the order of presentation was randomized for each taster, and the wines were evaluated using a completely

randomized design (Macfie, Bratchell, Greenhoff, & Vallis, 1989). To reduce carry-over effects, a 4-min break was provided between samples, during which the panelists were required to eat a piece of bread and rinse their mouths thoroughly with spring water. Panelists were presented with 50 mL samples at 17 °C, which were served in crystal tulip-shaped glasses. Assessors were seated in separate booths, each with a uniform source of lighting and free from the noise and distracting stimuli of the laboratory. The panelists swirled and smelled each sample for about 15 s, then began to rate the intensity of each attribute.

The following parameters were analyzed: overall perception of quality (1= faulty, 3= acceptable, 6= outstanding), body (1= light, 3= medium, 5= full), alcohol level (1= low, 3= medium, 5= high), flavor length (1= short, 3= medium , 5= long), flavor intensity (1= low, 3= medium, 5= pronounced), and approximate wine age (no scale). The intensity of each sensory parameter was measured with the structured scales applied to Levels 3 and 4 of the Wine & Spirits Education Trust (2009), a worldwide wine school that prepares professionals to taste all types of wines and spirits. These scales are use by professionals worldwide to evaluate wine quality. Thus, the sensory results obtained by the WSET method seems to be the closest approach to the consumer's perception. All panelists were fully trained and had more than five years of experience in evaluating all types of wine using these scales, which means that the sensory evaluation of red wines with the WSET scales was routine for all the assessors.

2.6 Quantification of individual phenolic compounds

For high-performance liquid chromatography coupled with a diode array and fluorescence detection (HPLC-DAD-FL), Agilent Technologies 1200 series equipment

containing a quaternary pump, a 20- μ L injection loop, and a UV detector was used. The HPLC was controlled by a PC running HP Chem Station Software system. Stock solutions of all standards were prepared in methanol/water, and the calibration curves were obtained from triplicate injections of at least five concentrations. For all standard curves, correlation coefficients (r) were above 0.990. For the HPLC analysis, polyphenols were identified by comparing their retention times with those of pure standards.

Flavanols (catechin, epicatechin), hydroxybenzoic acids (gallic acid and vanillic acid), and hydroxycinnamic acids (caffeic acid, *p*-coumaric acid, and ferulic acid) were measured in triplicate with a Luna Phenomenex C18 column and a guard column kept at 30 °C with 200 x 4.6 mm, i.d. 5- μ m particle size. The mobile phases consisted of acetonitrile, acetic acid, and water, where the gradient elution conditions were as follows: 0 min (5% acetic acid: 15% methanol; 80% water), 5 min (5% acetic acid: 20% methanol; 75% water), and 40 min (5% acetic acid: 45% methanol; 50% water). The flow rate was 0.2 mL min⁻¹, and the injection volume was 20 μ L (López, Martínez, Del Valle, Orte, & Miró, 2001). Before analysis, wines were filtered with a 0.45 μ m filter with a PTFE membrane (Millipore, São Paulo, Brazil). The programmable variable wavelength UV-Vis detector system allows detecting at different wavelengths; so λ = 271 nm for gallic acid, λ = 279 nm for (+)-catechin and (-)-epicatechin, λ = 296 nm for vanillic and ferulic acids, λ = 308 nm for *p*-coumaric acid, and λ = 325 nm for caffeic acid.

The *trans*-resveratrol and flavonol (rutin, quercetin, myricetin, and kaempferol) contents were measured in triplicate using a Luna Phenomenex C18 column and guard column at 28 °C with 250 x 4.6 mm, i.d. 5- μ m particle size. The mobile phases consisted of A

(water–acetonitrile–acetic acid, 67:32:1 v/v/v) and B (water–acetic acid, 99:1 v/v). The gradient elution conditions were as follows: 0 min (20% A + 80% B); 4 min (30% A + 70% B); 8 min (40% A + 60% B); 12 min (65% A + 35% B); 16 min (80% A + 20% B); 20 min (95% A + 5% B); 21.8 min (97% A + 3% B); 24 min (100% A) and 60 min (100% A). The flow rate was 0.8 mL min⁻¹ and the injection volume was 20 µL (Quirós, Lage-Yusty, & López-Hernández, 2009). Before analysis, wines were filtered with a 0.45-µm filter with a PTFE membrane (Millipore, São Paulo, Brazil). The programmable variable wavelength UV–Vis detector system allows detection at different wavelengths; so $\lambda = 360$ nm was set to rutin and kaempferol and $\lambda = 373$ nm was set to myricetin and quercetin. The fluorescence detector was set at $\lambda_{em} = 392$ nm and $\lambda_{ex} = 300$ nm for *trans*-resveratrol.

2.7 Statistical assessment

Data were presented as mean $\pm$ pooled standard deviation. A bivariate linear correlation matrix of the data, displayed in Pearson's correlation coefficient (r), was produced to measure the association between the response variables, and the significance (p -value) of such correlations was also provided. Retail price, antioxidant activity measured by ORAC and DPPH, and overall sensory perception of quality were used to classify the set of red wines using hierarchical cluster analysis (HCA) (Figure 1). For this purpose, the values were autoscaled, and sample similarities were calculated based on the Euclidean distance and the Ward hierarchical agglomerative method. To characterize the red wines in each of the four suggested clusters, Hartley's or Levene's test was applied to check for homogeneity of variances, and one-way ANOVA and Tukey's HSD *pos hoc*

tests were then conducted to identify contrasts among clusters. For the variables that presented non-homogenous variances ($p < 0.05$), the equivalent to ANOVA non-parametric test was used. P-values below 0.05 were considered significant. Statistica 9.0 software (Stat-Soft, Tulsa, OK, USA) was used for all statistical procedures.

3. Results

The results (Table 1) showed that the inhibition of DPPH ranged from 47.93 to 66.70%, while the ORAC results varied from 13.87 to 35.11 mmol TE/L. The redness of the wine varieties, measured by the a^* coordinate, ranged from 39.17 to 52.60, while the colour intensity (C^*) ranged from 43.14 to 64.61. The phenolic compound contents varied within grape varieties and also within countries, as observed in Table 2: *trans*-resveratrol (1.56 to 4.30 mg/L), quercetin (5.18 to 21.81 mg/L), rutin (0.83 to 4.19), gallic acid (13.88 to 69.87 mg/L), caffeic acid (2.74 to 4.95 mg/L), epicatechin (19.75 to 44.53 mg/L), catechin (59.15 to 149.14 mg/L), myricetin (13.03 to 46.69 mg/L), ferulic acid (0.55 to 1.45 mg/L), *p*-coumaric acid (4.40 to 10.73 mg/L), vanillic acid (0.00 to 1.15 mg/L), and kaempferol (0.00 to 1.86 mg/L). All these results are in accordance with previous studies in which red wines from diverse grape varieties and countries were evaluated (Brenna, & Pagliarini, 2001; Bartolomé, Gómez-Cordovés, & Monagas, 2006). In the sensory evaluation, only one sample presented an unsatisfactory quality, with a mean for overall sensory quality ranging from “poor” to “acceptable”. A total of 11 samples (15%) garnered scores between “acceptable” and “good”, 49 samples (67%) scored between “good” and “very good”, while 12 wines (16%) were considered “very good” to “outstanding”, showing the considerable sensory potential of South American red wines. In general, the Chilean and Argentinean wines presented higher means ($p <$

0.05) for the sensory attributes, and the Chilean samples presented a higher ORAC value ($p > 0.05$) compared with Brazilian wines (Table 1).

The results of this research disclosed significant ($p < 0.01$) correlations between antioxidant activity, measured by ORAC and DPPH assays, and spectrophotometrically measured total phenolic compounds ($r = 0.61$; $r = 0.59$, respectively) and total flavonoids ($r = 0.51$; $r = 0.67$, respectively). The phenolic compounds that displayed significant ($p < 0.05$) correlations with either the ORAC or DPPH assays were quercetin, rutin, myricetin, gallic acid, catechin, ferulic acid, and kaempferol. Conversely, the correlations between antioxidant capacity and the levels of *trans*-resveratrol, *p*-coumaric acid, epicatechin, total monomeric anthocyanins, caffeic acid, vanillic acid, and total non-flavonoid phenolics were sparse and non-significant ($p > 0.05$).

The results of Pearson's correlation analysis showed a significant ($p < 0.01$) association between retail price and sensory quality ($r = 0.37$), ORAC and DPPH ($r = 0.53$), and ORAC and sensory quality ($r = 0.53$). Using retail price, ORAC, DPPH, and sensory quality to classify the 73 red wines, four clusters were suggested (Table 3): Wines in Cluster 2 presented the best combination of sensory quality, antioxidant activity, and retail price. This cluster was characterized by the Cabernet Sauvignon, Syrah, and Malbec made in Argentina and Chile. Samples in Clusters 1 and 4 displayed similar ($p > 0.05$) antioxidant activity, but the former was more expensive and the latter presented a lower sensory quality. Cluster 3 included the samples with lower antioxidant activity and sensory quality. The data from Table 3 suggested that the antioxidant activity was determined by the total content of phenolic compounds and flavonoids.

4. Discussion

A significant variance in phenolic composition, colour, and antioxidant activity among grape varieties and even within countries was observed (Tables 1 and 2). Recent studies have disclosed that the phenolic composition and antioxidant activity of red wines can be strongly affected both quantitatively and qualitatively by the grape varietal, environmental factors, grape ripeness, pressing regimen, the extent and temperature of maceration, the temperature of fermentation, the use of enzymes, the type of oak used during aging and the extent to which the wine was aged (García-Falcón, Pérez-Lamela, Martínez-Carballo, & Simal-Gándara, 2007; Alén-Ruiz, García-Falcón, Pérez-Lamela, Martínez-Carballo, & Simal-Gándara, 2009).

In this study, we found that among all 12 phenolic compounds evaluated, gallic acid, myricetin, and quercetin were the compounds responsible for differences in the antioxidant activity among clusters, corroborating the results reported in previous studies (Arnous, Makris, & Kefalas, 2001; Brenna & Pagliarini, 2001; Lotito, Renart, & Fraga, 2002; Cimino, Sulfaro, Trombetta, Saija, & Tomaino, 2007; Di Majo, La Guardia, Giammanco, La Neve, & Giammanco, 2008; Alén-Ruiz, García-Falcón, Pérez-Lamela, Martínez-Carballo, & Simal-Gándara, 2009). As mentioned before, the total content of monomeric anthocyanins did not significantly correlate to any antioxidant activity assay, corroborating the findings of Granato, Katayama, and Castro (2010) and Giovanelli (2005). However, it is important to note that when individual anthocyanins and proanthocyanidins (dimers, trimers and polymers) are quantified, a significant correlation between these compounds and the antioxidant activity is attained (Salaha, Kallithraka, Marmaras, Koussissi, & Tzourou, 2008). Therefore, it is possible to assume that quercetin, gallic acid, and myricetin, along with other phenolics compounds such as proanthocyanidins, contribute significantly to the *in vitro* antioxidant activity of red wines.

The antioxidant activity of phenolic compounds, especially flavonoids, is due on one hand to the number and acidity of their phenolic hydroxyl groups, and on the other hand to the resonance between the free electron pair on the phenolic oxygen and the benzene ring, which increases electron delocalization and confers a partial negative charge and thus a nucleophilic character upon the substitution position adjacent to the hydroxyl group (Cheyner, 2006). The A-ring shared by all wine flavonoids possesses two nucleophilic sites, in the C8 and C6 positions, due to the hydroxyl groups' activation of its phloroglucinol (1,3,5-trihydroxy)-type structure (Mira, Silva, Santos, Caroço, & Justino, 2002).

Quercetin and (+)-catechin (Figure 2) have 5 hydroxyl groups in the same positions, but quercetin also contains the 2,3-double bond in the C ring and the 4-oxo function (Cheyner, 2006). This structure enhances quercetin's total antioxidant activity towards free radicals by allowing electron delocalization across the molecule. In our study, both (+)-catechin ($r = 0.33$, $p < 0.01$) and quercetin ($r = 0.37$, $p < 0.01$) correlated with the antioxidant activity measured by ORAC, but only the quercetin content was significantly different among clusters. These results imply that the 2,3-double bond in the C-ring and the 4-oxo function may be responsible for the higher antioxidant activity of flavonols compared with flavan-3-ols.

Another observation was that the flavonols kaempferol (4 –OH groups) and myricetin (6 –OH groups) (Figure 2) correlated ($p < 0.01$) to ORAC ($r = 0.37$, $r = 0.32$, respectively), and both contain the 2,3-double bond in the C-ring and the 4-oxo function. However, only the myricetin content differed ($p < 0.05$) among clusters. This result supports the fact that the number of hydroxyl groups influences the antioxidant activity of flavonoids. In our study, neither rutin nor monomeric anthocyanins, which are glycosylated

flavonoids, influenced the antioxidant activity among clusters, which suggests that the glycosylation remarkably decreases the nucleophilic power, and thus the antioxidant activity, of flavonoids compared with their respective aglycones.

The antioxidant activity of phenolic acids (hydroxybenzoic and hydroxycinnamic acids) basically depends on the number of hydroxyl groups in the molecule (Rice-Evans, Miller, & Paganga, 1996). The monohydroxy benzoic acids, such as vanillic acid, show weak antioxidant activity due to the low reactivity of the hydroxyl radical (Cheyner, 2006). On the other hand, trihydroxy benzoic acids, such as gallic acid (Figure 2), have a strong antioxidant activity because of the nucleophilic power of their three available hydroxyl groups, which have a considerable reducing capacity. In our study, *p*-coumaric acid (1 – OH group) and caffeic acid (2–OH groups) did not correlate with the antioxidant activity measured by either ORAC or DPPH, but ferulic acid (1 –OH group and 1 –OCH₃) contents correlated with ORAC ($r = 0.30$, $p = 0.01$). Ferulic acid is, indeed, more effective at scavenging free radicals than *p*-coumaric acid because the electron-donating methoxy group increases the stabilization of free radicals through electron delocalization after hydrogen donation by the hydroxyl group (Rice-Evans, Miller, & Paganga, 1996). Thus, the antioxidant activity of hydroxybenzoic acids depends on the number of hydroxyl groups in the molecule, whereas for hydroxycinnamic acids, the presence of methoxy groups seemed to positively influence the antioxidant activity in red wines.

Most of the above-mentioned studies evaluate the antioxidant activity and phenolic composition of red wines and support their conclusions with a Pearson linear correlation, meaning that higher concentrations of these compounds in wine samples suggested higher antioxidant activity. In our study, our observations were supported by both linear

correlations and the analysis of variance (one-way ANOVA) among the four clusters. Although correlation studies are extremely useful, they do not imply a cause-effect relationship between the variables, and it is possible that other covariants are contributing to the response. In contrast, a one-factor ANOVA applied to the response variables within clusters yields a very specific evaluation of the variable's impact on the response. Using this method, our study demonstrated that among all the 12 phenolic compounds evaluated, gallic acid, myricetin, and quercetin influenced more remarkably on the antioxidant activity of wines. However, the antioxidant activity of these red wines is also highly influenced by other phenolic compounds such as monomeric anthocyanins and proanthocyanidins. Moreover, it is important to point out that the *in vitro* antioxidant activity of wines depends on many factors in addition to the phenolic composition, including the free radical concentration, the time employed in the assay, the dilution factor of the sample, the bond dissociation energy between oxygen and a phenolic hydrogen, pH, reduction potential, solubility, stereochemical structure, and delocalization of the antioxidant radicals (Cao, Chen, Sun, Guo, Song, & Tian 2007). In addition, red wine is a complex matrix that contains large quantities of organic materials (phenolics and non-phenolics), inorganic materials (minerals), and enzymes that affect directly the biological activity of the wine. Thus, although we identified the three compounds with the greatest contribution to the antioxidant activity, their concentration is not enough to predict the antioxidant value of red wines.

Table 3 shows that none of the phenolic compounds evaluated in this study could be associated with the sensory difference among clusters. This result indicates that other compounds, especially the volatile ones, may be primarily responsible for sensory differences among wines. In this regard, Cejudo-Bastante, Hermosín-Gutiérrez, and

Pérez-Coello (2011) studied the phenolic composition and sensory attributes of Merlot wines from Spain and verified that the phenolics (caffeic, ferulic, and *p*-coumaric acids, flavonols, and monomeric anthocyanins) in wines that underwent micro-oxygenation and aging in an American oak barrel for 25 days did not change significantly ($p > 0.05$). However, the authors noticed that the concentration of aldehydes, alcohols, terpenes, isoprenoids, and benzenic compounds increased significantly ($p < 0.05$), along with the odour and aromatic qualities of these wines. Similarly, Sáenz-Navajas, Campo, Fernández-Zurbano, Valentin, and Ferreira (2010) studied the effect of polyphenols and volatile compounds on the sensory properties of Chardonnay and Tempranillo wines and found that polyphenols are responsible for astringency and bitterness in wines, but had no significant impact on odour, and that taste and astringency are primarily driven by non-volatile molecules in these wines, while global odour intensity depends on the volatile compounds. In a recent study conducted by our group (unpublished data), we verified that the intensity of odors and the overall perception of sensory quality of red wines from South America could be adroitly predicted without the panelists swirling the samples, corroborating the fact that wine odor plays an important and decisive role in wine quality.

5. Conclusions

With the use of multivariate statistical techniques, it was possible to conclude that the red wines in Cluster 2 presented the best combination of sensory characteristics, price and antioxidant activity. The main wines in this cluster were Malbec, Cabernet Sauvignon, and Syrah produced in Chile and Argentina. None of the phenolic compounds evaluated in this study could be associated with the sensory difference

among clusters, however we believe that other compounds, probably the volatiles, may be the main substances that differentiate red wines during sensory evaluation.

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Table 1

Red wine sample data grouped by country, variety, vintage, retail price, colour properties, sensory attributes, and antioxidant activity.

Commercial information				Antioxidant activity			Sensory evaluation					Instrumental colour	
Country	Variety	Vintage	Retail Price (US\$) ^a	DPPH ^b	ORAC ^c	Body	Alcohol level	Flavour length	Flavour intensity	Wine age	Overall quality	a*	c*
Sensory scale	NA	NA	NA	NA	NA	1 = Light, 5 = Full	1 = Low; 5 = High	1 = Short, 5 = Long	1 = Low; 5 = Pronounced	years	1 = Faulty; 6 = Outstanding	NA	NA
Brazil	Pinot Noir (n = 5)	2008	16.75	47.93	13.87	2.51	2.91	2.37	2.43	2.57	2.97	52.60	64.61
	Syrah (n = 1)	2006	8.43	64.75	29.80	3.14	3.29	3.14	3.29	2.36	3.71	39.17	43.14
	Cabernet Sauvignon (n = 9)	2005	24.71	66.44	29.59	2.78	3.32	2.75	2.89	2.79	3.38	47.95	57.66
	Merlot (n = 5)	2006	15.04	51.22	25.95	2.63	3.14	2.17	2.46	3.00	2.89	48.12	56.82
Argentina	Pinot Noir (n = 5)	2007	24.67	55.35	20.16	2.46	3.29	2.69	2.80	2.89	3.26	46.22	54.37
	Malbec (n = 9)	2007	25.19	58.24	26.52	3.27	3.60	3.00	3.27	2.55	3.86	42.99	47.65
	Syrah (n = 4)	2007	29.78	64.12	28.97	3.21	3.50	3.25	3.25	2.70	3.93	40.21	51.24
	Cabernet Sauvignon (n = 6)	2007	23.50	62.18	28.79	3.21	3.50	3.21	3.21	2.83	4.05	39.75	44.06
Chile	Merlot (n = 1)	2002	13.66	53.47	32.15	3.27	3.14	2.86	2.71	2.29	3.86	47.29	57.44
	Pinot Noir (n = 7)	2007	34.48	49.44	23.78	2.94	3.51	3.06	3.19	2.78	3.67	49.81	62.44
	Syrah (n = 7)	2007	29.94	61.48	31.47	3.23	3.49	3.06	3.25	2.69	4.02	41.05	45.59
	Cabernet Sauvignon (n = 9)	2007	16.67	55.25	33.41	3.06	3.33	2.84	3.09	2.43	3.68	43.03b	48.16
	Merlot (n = 3)	2007	28.27	63.00	33.71	3.14	3.71	3.05	3.00	2.68	3.71	47.02	54.45
	Malbec (n = 2)	2007	31.10	66.70	35.11	3.50	3.57	3.36	3.21	2.29	4.29	41.43	45.03

NA = not applicable.

^a The retail price corresponds to a bottle of 750 mL.^b Expressed as % of scavenging activity.^c Expressed as mmol TE/L.**Table 2**

Red wine sample data grouped by country, variety, and chemical composition.

Country	Variety	Chemical composition (mg/l)											
		Cinnamic acids			Flavan-3-ols		Benzoic acids		Flavonols			Stilbene	
		p-coumaric acid	Caffeic acid	Ferulic acid	Catechin	Epicatechin	Gallic acid	Vanillic acid	Kaempferol	Myricetin	Quercetin	Rutin	Trans-reveratrol
Brazil	Pinot Noir (n = 5)	6.17	3.80	0.80	59.15	28.40	13.88	0.26	0.25	15.20	7.87	0.83	3.82
	Syrah (n = 1)	6.97	5.42	1.14	121.17	36.39	52.94	0.50	1.05	18.02	11.73	2.64	2.83
	Cabernet Sauvignon (n = 9)	8.02	4.62	0.84	76.46	25.64	37.92	0.16	0.26	28.51	9.08	2.95	3.34
	Merlot (n = 5)	10.73	3.61	0.95	64.39	25.88	32.48	0.92	0.30	42.17	12.24	2.51	3.55
	Mean	8.17 ^a	4.20 ^{ab}	0.87	71.35 ^b	27.93 ^b	31.29 ^b	0.39	0.31 ^b	28.07	9.70 ^b	2.30	3.49 ^a
	SD	2.47	1.25	0.29	38.73	12.17	17.93	0.51	0.47	13.82	5.05	1.26	1.13
Argentina	Pinot Noir (n = 5)	4.40	4.61	0.65	123.02	33.99	27.64	0.70	0.30	15.63	5.18	1.61	2.90
	Malbec (n = 9)	6.91	3.45	0.89	88.62	19.75	41.75	0.66	0.16	34.48	8.40	2.40	2.36
	Syrah (n = 4)	5.58	4.21	1.44	83.86	22.73	55.33	0.29	0.72	40.53	13.84	2.62	3.41
	Cabernet Sauvignon (n = 5)	4.81	2.74	0.99	116.18	24.03	62.71	0.50	0.60	35.87	12.83	3.09	1.56
	Merlot (n = 1)	7.56	3.03	0.55	85.57	20.12	63.24	1.15	0.00	23.36	8.73	2.51	2.66
	Mean	5.69 ^b	3.63 ^b	0.95	101.83 ^{ab}	24.09 ^b	46.13 ^a	0.57	0.38 ^b	32.01	9.69 ^b	2.44	2.45 ^b
Chile	SD	1.89	1.44	0.50	32.26	8.06	20.49	0.41	0.43	11.91	4.55	1.24	1.02
	Pinot Noir (n = 7)	4.97	4.93	1.27	93.23	41.72	41.96	0.30	0.40	13.03	5.18	1.53	4.30
	Syrah (n = 7)	6.88	4.91	1.02	86.94	39.54	44.01	0.42	1.22	32.13	18.52	2.74	3.14
	Cabernet Sauvignon (n = 9)	4.98	4.95	0.97	125.61	29.65	51.06	0.71	1.14	26.67	13.66	3.67	2.10
	Merlot (n = 3)	7.51	4.87	0.99	149.14	30.69	69.87	0.83	1.45	31.40	19.21	2.47	4.21
	Malbec (n = 2)	7.21	4.30	1.45	67.84	44.53	33.49	0.00	1.86	46.69	21.81	4.19	2.07
	Mean	5.88 ^b	4.88 ^a	1.09	106.24 ^a	37.13 ^a	47.78 ^a	0.49	1.06 ^a	25.56	15.08 ^a	2.81	3.14 ^{ab}
	SD	1.81	1.60	0.30	63.60	10.29	17.30	0.61	0.71	13.59	5.97	1.30	1.59
p (one-way ANOVA)		<0.01	0.01	0.13	0.04	<0.01	<0.01	0.52	<0.01	0.31	<0.01	0.35	0.02

Different letters in the same column represent statistical different results according to the Tukey's HSD test ($p < 0.05$).

Table 3

Chemical, sensory, colorimetric, and antioxidant activity characterisation of samples included in the selected clusters.

Response variables	Cluster 1 (n = 17)	Cluster 2 (n = 25)	Cluster 3 (n = 18)	Cluster 4 (n = 13)	PSD ^a	p-Value ^b	p-Value ^c
<i>Antioxidant activity</i>							
ORAC (mmol TE/L)	29.06 ^a	31.93 ^a	18.85 ^b	29.45 ^a	8.42	0.59	<0.01
DPPH (% scavenging activity)	61.25 ^a	61.92 ^a	47.93 ^b	60.51 ^a	9.03	0.11	<0.01
<i>Commercial information</i>							
Retail price (US\$/750 mL)	43.99 ^a	19.89 ^b	17.25 ^b	15.87 ^b	14.14	0.07	<0.01
Vintage	2006	2006	2007	2006	NA	NA	NA
<i>Instrumental colour</i>							
Colour intensity (c*)	51.91 ^{ab}	47.34 ^b	57.88 ^a	56.31 ^{ab}	10.79	0.53	<0.01
Redness (a* coordinate)	45.11 ^{ab}	41.16 ^b	48.51 ^a	47.47 ^a	7.11	0.95	<0.01
<i>Phenolic composition (mg/L)</i>							
p-Coumaric acid	6.61	5.89	6.41	7.36	2.28	0.49	0.31
Catechin	80.38	106.57	85.54	105.94	49.80	0.26	0.25
Epicatechin	28.53	29.88	29.97	33.01	11.76	0.83	0.78
Gallic acid	47.45 ^a	49.74 ^a	28.59 ^b	42.50 ^{ab}	19.68	0.38	<0.01
Caffeic acid	4.30	4.08	4.31	4.52	1.53	0.60	0.86
Ferulic acid	1.15	0.98	0.82	0.98	0.38	0.17	0.09
Vanillic acid	0.23	0.53	0.60	0.61	0.52	0.07	0.12
Myricetin	34.87 ^a	31.49 ^a	19.53 ^b	28.77 ^{ab}	13.14	0.84	<0.01
Kaempferol	0.77	0.83	0.40	0.34	0.66	0.05	0.05
Rutin	3.09	2.51	2.01	2.84	1.27	0.29	0.09
Quercetin	14.25 ^a	13.65 ^a	8.46 ^b	9.45 ^{ab}	11.76	0.14	<0.01
Trans-resveratrol	3.39	2.58	3.26	2.93	1.34	0.85	0.21
Monomeric anthocyanins	67.00	79.18	101.54	86.05	55.67	0.38	0.32
Total flavonoids	1087.89 ^a	1102.24 ^a	867.93 ^b	1083.93 ^a	230.22	0.07	<0.01
Total phenolics	1562.81 ^a	1490.32 ^a	1275.68 ^b	1578.13 ^a	241.69	0.12	<0.01
<i>Sensory properties</i>							
Alcohol level (1 = Low; 5 = High)	3.52 ^a	3.46 ^{ab}	3.23 ^b	3.32 ^{ab}	0.33	0.77	<0.01
Body (1 = Light; 5 = Full)	3.21 ^a	3.11 ^a	2.76 ^b	2.73 ^b	0.39	0.11	<0.01
Flavour length (1 = Short; 5 = Long)	3.22 ^a	3.10 ^a	2.65 ^b	2.34 ^b	0.47	0.07	<0.01
Wine age (years)	2.92	2.81	2.64	2.53	0.50	0.10	0.13
Overall sensory quality (1 = Faulty; 6 = Outstanding)	3.88 ^a	4.02 ^a	3.28 ^b	3.04 ^b	0.53	0.13	<0.01
Flavour intensity (1 = Low; 5 = Pronounced)	3.27 ^a	3.27 ^a	2.70 ^b	2.67 ^b	0.43	0.02	<0.01
<i>Grape variety in each cluster</i>							
Pinot Noir (n = 17)	3	2	11	1			
Cabernet Sauvignon (24)	5	10	3	6			
Malbec (n = 11)	3	6	2	0			
Merlot (n = 9)	1	2	2	4			
Syrah (n = 12)	5	5	0	2			
<i>Number of samples in each cluster</i>							
Brazil (n = 20)	3	3	7	7			
Chile (n = 28)	6	11	6	5			
Argentina (n = 25)	8	11	5	1			

NA = not applicable; different letters in the same line represent statistical different results ($p < 0.05$).^a PSD = pooled standard deviation.^b Probability values obtained by Hartley test ($F_{\max}$) for homogeneity of variances.^c Probability values obtained by one-way ANOVA.

Fig. 1. Hierarchical cluster analysis (HCA) applied to the variables and the respective correlation coefficients and *p*-values.

Fig. 2. Some phenolic compounds measured in south american red wines: (a) gallic acid, (b) quercetin, (c) myricetin, (d) ferulic acid, (e) (+)-catechin, (f) kaempferol, (g) *p*-coumaric acid, and (h) caffeic acid.

8. CONCLUSÕES FINAIS

A caracterização físico-química das amostras de vinho tinto brasileiro ($n = 29$) mostrou que a atividade antioxidante medida por ORAC e DPPH está diretamente correlacionada com o conteúdo de flavonóides não-antociânicos, mais especificamente os compostos polimerizados, como as proantocianidinas. Outra observação constatada foi que o valor comercial do vinho não está correlacionado significativamente ($p > 0.05$) com a capacidade antioxidante das amostras, apesar do fato que os vinhos mais caros terem apresentado maiores valores de ORAC e DPPH.

Ao avaliar a associação entre características sensoriais, químicos, colorimétricos e valor comercial de vinhos fabricados no Brasil, Chile e Argentina ($n = 80$), verificou-se que os vinhos chilenos e argentinos se destacaram por apresentar maiores preços, atividade antioxidante, intensidade de odor, qualidade sensorial global, índice de acidez e taninos, ao passo que os vinhos brasileiros apresentaram menores valores para os atributos sensoriais. Por outro lado, os vinhos brasileiros mostraram ter a menor qualidade sensorial comparado com os chilenos e argentinos. Os vinhos produzidos com uvas americanas (*V. labrusca*) apresentaram menores valores para todas as variáveis estudadas. De forma geral, conclui-se que os vinhos oriundos de uvas Syrah, Malbec e Cabernet Sauvignon apresentaram maior capacidade antioxidante e melhores características sensoriais comparadas com as demais varietais, sendo que esse resultado foi independente da safra e procedência.

Os dados experimentais indicaram que os vinhos com menor atividade antioxidante estão associados com menor valor comercial e qualidade sensorial, mas a maior parte das amostras inseridas no grupo de vinhos com 'qualidade intermediária-alta' apresentaram maior atividade antioxidante e melhores características sensoriais, mas não apresentaram maiores valores comerciais. Portanto, o valor comercial não parece ser o melhor fator para discriminar vinhos tintos em relação sua qualidade sensorial e atividade antioxidante.

Para os vinhos oriundos de *V. vinifera* ($n=73$), a atividade antioxidante *in vitro* correlacionou-se positivamente com o conteúdo de fenólicos totais, flavonóides totais, ácido gálico, quercetina, catequina, ácido ferúlico, miricetina, caempferol, e rutina,

sendo que apenas os conteúdos de miricetina, ácido gálico e quercetina foram diferentes entre os grupos. Observou-se que os compostos fenólicos avaliados neste estudo não estão associados às diferenças sensoriais entre os grupos de vinhos, indicando que os compostos aromáticos sejam, provavelmente, os responsáveis em diferenciar tais amostras em relação à análise sensorial.

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ANEXOS