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POLIFENÓIS EM SUCOS DE UVA: INVESTIGAÇÃO SOBRE A ESTABILIDADE DURANTE O PROCESSO E ARMAZENAMENTO

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aos meus pais, Marli e
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SUMÁRIO

RESUMO

Estudos epidemiológicos demonstraram que alimentos e bebidas contendo polifenóis exercem efeitos fisiológicos benéficos. Sucos de uvas são fontes importantes de flavonóides, principalmente flavan-3-óis em suas formas monoméricas de catequinas [(+)-catequina e (-)-epicatequina]. Os objetivos deste trabalho foram caracterizar e quantificar fenólicos totais (TP), monômeros de catequinas (CAT+EPI), atividade sequestrante de radicais livres (RSA) e específicos atributos sensoriais, durante o processo e armazenamento de sucos de uva comerciais. Sucos concentrados (68° Brix) dos cultivares Concord (CCJ) e Isabel (CIJ) foram amostrados durante o processo e após a concentração e armazenados a 5°C. Sucos de uva pasteurizados integrais (14 a 19° Brix) de mesmos cultivares (PCJ e PIJ) foram mantidos à temperatura ambiente e luz indireta por 10 meses. Ambas situações simularam as condições de armazenamento industriais/comerciais. TP foi determinado pelo método de Folin-Ciocalteu; CAT+EPI por CLAE com detecção por fluorescência e RSA em ensaio com DPPH. Para o teste sensorial, a análise descritiva quantitativa (ADQ) foi utilizada com a seguinte lista pré-determinada de atributos e descritores: cor, adstringência, gosto amargo, gosto doce e sabor característico de suco de uva. As principais substâncias presentes nos sucos foram investigadas por espectrometria de massa com ionização por "electrospray" e infusão direta (ESI-MS), ambos no modo íon negativo. Durante o processamento, CCJ demonstrou maior RSA e TP em relação a CIJ com certa variação em cada etapa. CCJ apresentou 50% maior teor de TP, enquanto que a RSA foi em média 25% superior. No armazenamento,

a preservação de TP variou entre 93% (CCJ) e 84% (PCJ) e a de RSA entre 87% (PIJ) e 85% (CCJ e PCJ). A retenção de (+)-catequina no período esteve entre 63% (PCJ) e 52% (PIJ) e a de (-)-epicatequina ente 32% (CCJ) e 15% (CIJ). Sucos concentrados mostraram maiores concentrações de catequinas e CCJ apresentou o maior teor de TP entre as amostras. Os conteúdos de TP e catequinas foram distintos entre os sucos, porém a RSA foi similar. PIJ obteve maior valor de RSA "por unidade de fenólico". Nos sucos concentrados, peonidina e peonidina-3-O-glucosídeo deram lugar a malvidina e dimetoxi-flavilium como substâncias significativas durante o processo; malvidina e piceatanol-O-glucosídeo decresceram após oito meses de armazenamento e dimetoxi-flavilium manteve-se estável. No aspecto sensorial, sucos concentrados sob refrigeração demonstraram maior intensidade em atributos desejáveis como cor, gosto doce e sabor característico após oito meses. CCJ apresentou maior estabilidade durante o armazenamento e seus atributos característicos foram adstringência, gosto amargo e intensidade de cor. CIJ foi caracterizado por gosto doce e sabor de suco de uva. As análises e respectivos resultados demonstraram que o processamento e as condições de armazenamento aplicados comercialmente mantiveram o a capacidade antioxidativa e a qualidade sensorial de sucos de uva concentrados.

ABSTRACT

Epidemiological studies have demonstrated that polyphenol-rich foods and beverages exert beneficial physiological effects. Grape juices are relevant sources of flavonoids, particularly flavan-3-ols, mostly in the monomeric forms of catechins [(+)-catechin and (-)-epicatechin]. The objectives of this work were to characterize and quantify phenols (TP), catechin monomers (CAT+EPI), radical scavenging activity (RSA) and specific sensory attributes during processing and storage of commercial grape juices. Concentrated Concord (CCJ) and Isabel (CIJ) grapes juices (68° Brix) were sampled during processing and after concentration and stored at 5°C. Pasteurized ready-to-drink juices (14 to 19° Brix) of the same grape cultivars (PCJ and PIJ) were kept at room temperature under indirect lighting for 10 months, both simulating industrial/commercial storage conditions. TP were determined using the Folin-Ciocalteu method; CAT+EPI by HPLC with fluorescence detection and RSA by the DPPH assay. For the sensory analysis, the quantitative descriptive analysis (QDA) was used with a pre-determined list of attributes: colour, astringency, bitterness, sweetness and characteristic grape juice flavour. The major components of juices were investigated by direct infusion and electrospray ionization mass spectrometry (ESI-MS) both in the negative ion mode. During processing, CCJ showed higher RSA and TP contents than CIJ with slight variations at each step. Although CCJ showed 50% higher TP contents, RSA was on average 25% higher. During storage, TP retention ranged from 93% (CCJ) to 84% (PCJ) and RSA from 87% (PIJ) to 85% (CCJ and PCJ). (+)-Catechin retention during storage ranged between 63% (PCJ) and 52% (PIJ) and (-)-epicatechin

between 32% (CCJ) and 15% (CIJ). Concentrated juices of both cultivars showed notably higher catechin amounts and CCJ presented the greatest phenolic contents. Despite the marked differences in TP and CAT+EPI contents, similar RSA values were observed for all the juices. PIJ yielded the highest RSA “per phenolic unit”, with the lowest TP content. In the concentrated juices, peonidin and peonidin-3-O-glucoside gave way to malvidin and dimethoxy-flavylium as significant components during processing; malvidin and piceatanol-O-glucoside decreased after 8-month storage whereas dimethoxy-flavylium was maintained. Regarding the sensory aspects, concentrated juices stored under refrigeration received higher ratings for desirable attributes such as colour, sweetness and flavour than juices aged in bottles at room temperature. CCJ was more stable during storage with sensory characteristics of astringency, bitterness and higher colour intensity while CIJ was characterized by sweetness and juice flavour. Thus, processing and storage conditions applied to commercial concentrated grape juices are capable of maintaining antioxidant capacity and sensory quality.

Capítulo 1- INTRODUÇÃO GERAL

Apresentação e Objetivos

O Brasil é um produtor e exportador de suco de uva concentrado, principalmente para os Estados Unidos da América, Japão e Canadá. Em 2006, o país produziu 87 milhões de litros, comercializando quase 10% no mercado externo ao longo dos últimos anos. Hoje ocupa o décimo lugar no mercado mundial e, segundo setor da Empresa Brasileira de Pesquisa Agropecuária (Embrapa Uva e Vinho), está apto a se tornar um dos primeiros, dadas as características dos cultivares que processa, principalmente Concord e Isabel, desejáveis no mercado a que se destina. Internamente, o consumo de suco de uva cresceu, passando de 0,15 litros *per capita* em 1995 para 0,56 litros em 2006, como também ocorreu com uvas de mesa e vinho.

O crescimento do consumo ocorre ao mesmo tempo em que o suco de uva passa a ser estudado como nutracêutico e ingrediente em produtos cosméticos. A característica benéfica do produto é a quantidade e variedade de compostos fenólicos presentes, como ocorre com o vinho. Os efeitos fisiológicos dos derivados da uva parecem decorrer da sua ação antioxidante. No entanto, a capacidade de seqüestrar radicais livres e/ou quelar metais os tornam instáveis ao processamento e armazenagem. Assim, este trabalho pretendeu investigar a estabilidade do suco de uva concentrado de cultivares típicas (Concord e Isabel) frente ao processo de produção e armazenagem. Para efeitos de comparação, sucos pasteurizados integrais foram analisados em suas condições próprias de produção e estocagem.

A tese a seguir é apresentada em forma de artigos, com objetivos específicos, e dividida em seis capítulos. O capítulo a seguir é uma atualização bibliográfica sobre polifenóis, com ênfase em produtos de uva e à atividade antioxidante. Os seguintes trazem os artigos das pesquisas desenvolvidas durante o programa tendo como foco a integridade química e sensorial do suco de uva concentrado. Ao final, a conclusão geral pretende unificar os achados demonstrados nas pesquisas e apresentar perspectivas futuras.

Capítulo 2: ARTIGO DE REVISÃO: "Características e Estabilidade de Polifenóis em Sucos de Uva"

A ser submetido à Revista de Nutrição (*Brazilian Journal of Nutrition*)

Características e Estabilidade de Polifenóis em Sucos de Uva

Resumo

Fenólicos estão relacionados à prevenção de enfermidades e morbidade por doenças resultantes do estresse oxidativo. Foi demonstrado que o suco de uva apresentou capacidade antioxidante na proteção do LDL em humanos. A revisão aborda a natureza química de polifenóis com ênfase naqueles presentes em suco de uva tinta. Trata das modificações que ocorrem durante o processo e armazenamento. O processamento e armazenamento em geral alteram o perfil fenólico de sucos de uva, sendo alguns grupos mais afetados. Em geral, o armazenamento preserva a capacidade antioxidante da bebida, que está mais relacionada ao teor de fenólicos totais que a certas classes específicas.

1-Natureza química de polifenóis e presença em alimentos

Polifenóis formam um grupo de mais de oito mil estruturas conhecidas, sendo que um pequeno número está presente na dieta humana em teores significativos. Os compostos fenólicos resultam do metabolismo secundário das plantas, atuando no mecanismo de defesa contra raios ultravioletas (BRAVO, 1998; SCALBERT & WILLIAMSON, 2000). Estão relacionados com a qualidade sensorial e o "status" oxidativo de alimentos que os contêm (SHI et al., 2003; KARAKAYA, 2004; MANACH et al., 2004). Do ponto de vista sensorial, proporcionam cor, gosto amargo e sensação adstringente em vinhos, sucos de uva e chás (LESSCHAEVE e NOBLE, 2005; VIDAL et al., 2004).

A estrutura básica de polifenóis consiste de um anel aromático com um ou mais grupos hidroxila e freqüentemente conjugada com açúcares. Ocorrem na natureza desde ácidos fenólicos até compostos polimerizados complexos como taninos. São substratos para várias enzimas em alimentos e altamente instáveis a luz e oxigênio. As principais fontes de polifenóis na dieta são frutas e bebidas derivadas de plantas: sucos, chás, café e vinho (SCALBERT et al., 2005). A diversidade estrutural dos compostos fenólicos dificulta a estimativa de seu teor nos alimentos e no cálculo da ingesta diária. Scalbert e Williamson (2000), citam estudo de Kühnau de 1976 que estimou a ingesta de polifenóis nos Estados Unidos em 1g/dia. É evidente que a ingesta deste compostos depende fundamentalmente dos hábitos culturais, preferências pessoais e aspectos sociais e econômicos.

Os polifenóis são comumente divididos em quatro grandes grupos: flavonóides, ácidos fenólicos, estilbenos e lignanas, sendo os flavonóides um dos mais freqüentes e abrangentes, representados na Figura 1.

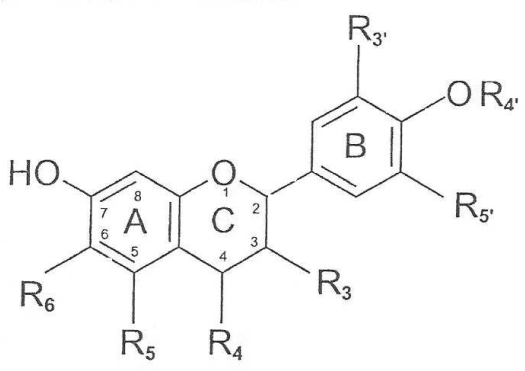


Figura 1. Estrutura básica de flavonóides. Fonte: Cook e Samman (1996).

A diversidade estrutural de flavonóides é explicada pelas reações que podem sofrer: hidroxilação, metilação, glicosilação e polimerização, entre outras. Também são agrupados em sub-classes, sendo as principais: flavonóis, flavonas, flavanonas, flavanóis, antocianinas e isoflavonas (Figura 2).

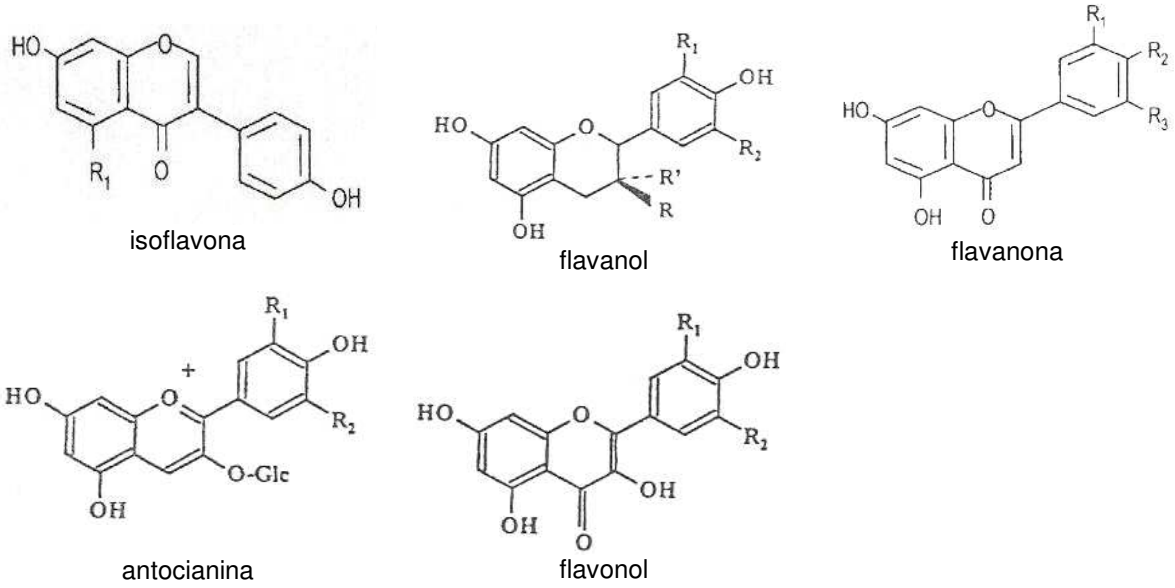
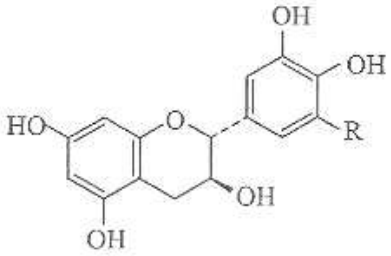


Figura 2. Exemplos dos principais tipos de flavonóides. Fonte: Cook e Samman (1996).

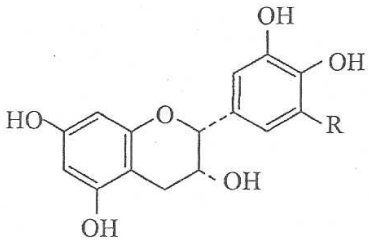
2- Polifenóis em produtos da uva

Os polifenóis representam o terceiro maior constituinte de uvas e derivados, depois de carboidratos e ácidos orgânicos. Do total passível de extração, 10% ou menos está na polpa, 60-70% está na semente e de 28-35% está na casca. Produtos de uvas preparados na presença de casca e sementes são fontes

relevantes de ácidos fenólicos e flavonóides, particularmente os do tipo flavan-3-óis. Deste grupo são mais abundantes as catequinas e procianidinas oligoméricas (Figuras 3 e 4), presentes na camada mais externa da semente de uvas, e as antocianinas, abundantes nas cascas de uvas tintas (YILMAZ & TOLEDO, 2004).

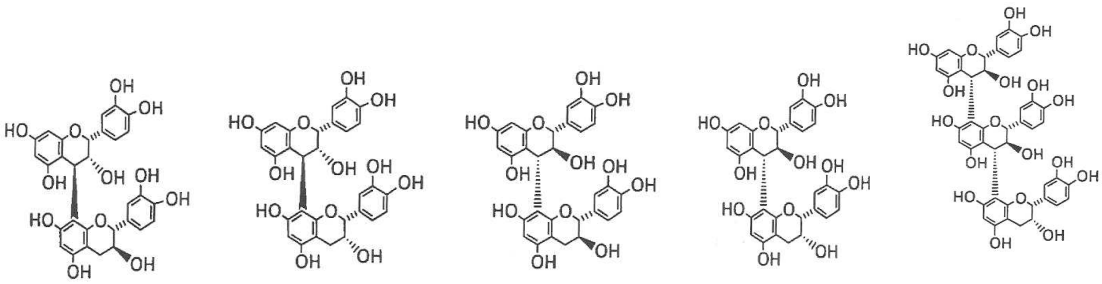


(+) catequina



(-) epicatequina

Figura 3: Estrutura básica de catequinas. Fonte: Shi et al. (2003).



B1 B2 B3 B4 trímero

Figura 4: Procianidinas: dímero B1; dímero B2; dímero B3; dímero B4; trímero. Fonte: Tsang et al. (2005).

A concentração e composição de fenólicos em sucos de uva e vinho dependem do tempo de extração, do contato com casca e semente e de fatores

agro-geográficos como cultivar, clima, solo, exposição à luz e grau de maturação (SHI et. al., 2003). A presença de flavonóides nestas bebidas também decorre da degradação de polímeros no processo térmico. Foi demonstrado que proantocianidinas (taninos condensados) da casca da uva, quando submetidos à hidrólise, resultaram nos monômeros (+)-catequina e (-)-epicatequinas (SOUQUET et al., 1996). Por serem sensíveis a oxigênio, luz, pH, e relativamente menos sensível a temperatura, a quantidade de fenólicos em sucos de uva comerciais deve variar conforme as condições de processo e armazenamento.

Ocorrem discrepâncias em estudos que investigaram teores e perfis fenólicos de sucos de uva e vinhos. Diferentes métodos de detecção e quantificação contribuíram para as divergências. Landbo & Meyer (2001) encontraram 53% de flavan-3-óis, 31% de ácidos fenólicos e 16% de antocianinas em sucos de uva tinta, similar aos achados de Goldberg et al. (2000) e Bermúdez-Soto e Tomás-Barberán (2004). Entretanto, Frankel et al.(1998) detectaram 57% de antocianinas, 29% de ácidos fenólicos e 7% de flavan-3-ols em sucos comerciais de uva Concord. Quanto ao teor de fenólicos totais em produtos da uva, ocorre grande variação conforme o método de quantificação e obtenção de amostras (comerciais ou processadas em laboratório). Os valores ficam entre zero e 8.800 mg de ácido gálico equivalente/L (GAE) em sucos de uva e vinhos (FULEKI e RICARDO-DA-SILVA, 2003 e LANDBO e MEYER, 2001). Em estudo com sucos de uva comerciais brasileiros (integrais e reconstituídos), o teor de fenólicos totais variou entre 270 mg/L e 2.410 mg/L (MALACRIDA e MOTTA, 2005). Vinhos parecem conter maior teor de monômeros de catequinas. Arts et al. (2000) encontraram maiores teores de catequinas em vinhos tintos (entre 27,5 e

95,5 mg/L) que em sucos de uva tinta (8,0 e 9,1 mg/L) consumidos na Holanda. Gürbüz et al. (2007) encontraram teores de catequinas e epicatequinas de 5 a 10 vezes maiores em vinhos que em mosto de uva antes da fermentação.

3. Efeitos fisiológicos

Há evidências de que polifenóis são biodisponíveis e podem exercer funções durante os processos metabólicos naturais humanos e animais (MANACH et al., 2005; WILLIAMSON e MANACH, 2005). Dezessete estudos epidemiológicos sobre o consumo de flavonóides e risco de doença arterial coronariana e infarto já haviam sido publicados até 2005, dos quais sete identificaram efeitos protetores atribuíveis a flavonóis, flavonas e catequinas (ARTS e HOLLMAN, 2005).

Arts e colaboradores demonstraram em dois estudos a redução de morbidade em populações que ingerem flavonóides. O *Zutphen Elderly Study* (ARTS et al., 2001a), indicou que, após 10 anos, sob maior ingesta de catequinas, menor foi a mortalidade por doenças crônicas em homens de 65 a 84 anos. Foi observada relação inversa entre ingesta de catequinas e infartos do miocárdio nesta população. Em outro estudo prospectivo, Arts et al. (2001b), observaram uma relação inversa entre consumo de maçã e vinho e morte por acidente de coronárias em mulheres americanas menopausadas.

Outros efeitos fisiológicos atribuídos aos polifenóis são o aumento da resistência do DNA à oxidação, proteção ao trato gastrointestinal contra radicais livres resultantes da digestão e regulação da síntese de glutationa, principal

antioxidante endógeno (COLLINS, 2005; HALLIWELL et al., 2005; MOSKAUG et al., 2005).

3.1 Ação antioxidante

A presença de radicais livres é crítica para a manutenção de funções fisiológicas normais e para a estabilidade química e sensorial de alimentos (BELITZ e GROSCH, 2001). O efeito do antioxidante ocorre pela inativação de radicais livres por meio de complexação de íons metálicos ou conversão de hidroperóxidos a produtos incapazes de propagar a reação. Os flavonóides podem atuar em qualquer destes estágios, bloqueando a ação deletéria destas substâncias. (BLACHE et al., 2002; KIMURA et al., 2002). A capacidade inibitória de peroxidação lipídica *in vitro*, por exemplo, é aumentada por certas características estruturais dos flavonóides: número de grupos hidroxila, presença de grupo hidroxila na posição 3 do anel C, hidroxilas em posições padronizadas C-5, C-7, C-3´e C-4´ e ausência de açúcar conjugado ou grupos metoxila (COOK E SAMMAN, 1996).

Os estudos laboratoriais com animais evidenciaram a ação antioxidante dos flavonóides e já permitiram considerar os cinco resultados a seguir (DISILVESTRO, 2001; NIJVELDT et al., 2001):

1- Redução de produtos de reações oxidativas;
2- Aumento da resistência ao estresse oxidativo;
3- Elevação da concentração de antioxidantes endógenos ou prevenção de sua depleção;
4- Diminuição da oxidação lipoprotéica em estudos em ratos *ex vivo;*

5- Elevação da capacidade antioxidante no soro ou plasma determinada em *ex vivo*.

3.2 Suco de uva e ação fisiológica

Efeitos fisiológicos atribuídos ao consumo de vinho estão vastamente investigados (BURNS et al., 2000; KAMPA et al., 2000; DUBICK e OMAYE, 2001; DONOVAN et al., 2002). O suco de uva como alimento desprovido de teor alcoólico e efeito fisiológico similar demonstra atividades interessantes, tais como: manutenção da função endotelial e conseqüente diminuição da agregação plaquetária (OSMAN et al., 1998; KEEVIL et al., 2000; FREEDMAN et al., 2001), aumento da capacidade antioxidante (SAITO et al., 1998; DÁVALOS et al., 2004), proteção contra oxidação de LDLs (FRANKEL et al., 1998; LANDBO e MEYER, 2001).

O´Byrne et al. (2002) compararam a capacidade antioxidante do suco de uva Concord com a de α-tocoferol em 32 adultos saudáveis, mostrando que o consumo de 10mL do suco/kg/dia mostrou o mesmo efeito que 400 UI de α-tocoferol na proteção do LDL. Stein et al. (1999) observaram que o consumo de suco de uva tinta por 14 dias foi associado ao aumento da dilatação da artéria braquial em 15 adultos com doença arterial coronariana. Nesse estudo, a susceptibilidade do LDL a oxidação em *ex vivo* foi reduzida, sugerindo efeito antioxidante.

Não há consenso quanto aos tipos de fenólicos de vinhos e sucos de uva mais efetivos e seus mecanismos de ação. *In vitro*, a atividade antioxidante de

vinhos se mostrou superior àquela do suco de uva e a capacidade de seqüestrar radicais livres correlacionou-se melhor com teor de fenólicos totais do que com específicos grupos fenólicos (SÁNCHEZ-MORENO et al., 1999; VINSON et al. 1999). Para De Beer et al. (2003), flavanóis tiveram maior ação antioxidante que antocianinas em vinhos. Em suco de uva Concord a atividade antioxidante esteve distribuída entre vários grupos fenólicos, sem predominância de grupo específico (FRANKEL et al., 1998). Em modelo animal, suco de uva tinta demonstrou maior eficácia na redução da aterosclerose e parâmetros lipídicos que vinho tinto com ou sem álcool (VINSON et al., 2001).

4. Estabilidade de sucos de uvas ao processamento e armazenamento

Não há amplo consenso sobre o comportamento de fenólicos de sucos de uva durante o processamento e armazenamento. Gonzáles-Manzano et al. (2004); Freitas et al. (2000), Goldberg et al. (1998) e Monagas et al. (2003), mostraram que a composição qualitativa e quantitativa de flavan-3-óis varia com a temperatura e duração do processo de maceração das uvas e do contato com cascas e sementes.

Em um dos primeiros estudos sobre a estabilidade de fenólicos durante o processo e armazenamento, Spanos e Wrolstad (1990) verificaram redução de monômeros e oligômeros de catequinas e ácidos fenólicos após a concentração de sucos de uva brancas, chegando a zero após nove meses de estocagem a temperatura ambiente. O teor de fenólicos totais medidos pelo método de Folin-Ciocalteu, contudo, manteve-se constante. Este ensaio colorimétrico, freqüente

em quantificação de fenólicos totais em produtos de uva, é sensível a polifenóis polimerizados e os quantifica, mesmo que a natureza química seja desconhecida (SINGLETON e ROSSI, 1965).

Fuleki e Ricardo-da-Silva (2003) investigaram a influência das condições de processamento sobre teor de catequinas em sucos de uvas tintas e concluíram que o tipo de maceração produz o maior impacto, seguido pelo tipo de cultivar e safra. Os autores demonstraram que a maceração da fruta a quente extrai (até dez vezes) maior quantidade de compostos do que a frio. Musingo et al. (2001), observaram que o cultivar pode causar o maior impacto sobre o comportamento de fenólicos de sucos e vinhos armazenados. Pinelo et al. (2005) mostraram em ensaio-modelo que o aquecimento de extratos de uva provoca polimerização de catequinas e conseqüente aumento da atividade antioxidante.

É conhecido que durante o armazenamento, as antocianinas sofrem polimerização e alteram a cor e adstringência em vinhos. Foi relatada redução no teor de antocianinas e antocianidinas monoméricas em sucos e vinhos tintos em até um sexto da concentração inicial após 60 dias, com pouca diferença entre os dois produtos (TALCOTT e LEE, 2002) e em até um décimo do valor inicial em vinhos após sete meses de envelhecimento (ZAFRILLA, et al., 2003). Flavanóis, por sua vez, estão envolvidos em interações com proteínas, resultando na formação de espuma, participam em reações de condensação com outros flavanóis e em reações de escurecimento enzimático (MONAGAS et al., 2006). De fato, a oxidação enzimática pela polifenoloxidase se inicia com o rompimento da integridade celular da fruta, causando escurecimento durante o processamento e armazenamento, afetando a qualidade (CHEYNIER, 2005). No entanto, verificou-

se que a capacidade antioxidante de sucos de uva sofre pouca alteração durante o armazenamento (LEE e TALCOTT, 2002).

Modificações sensoriais do vinho durante o envelhecimento são perceptíveis e estão relacionadas a alterações de fenólicos, principalmente polimerização, dando origem a taninos com aumento da sensação de adstringência (PREYS et al., 2006). Em ensaio-modelo, Es-Safi et al. (2003) mostraram que durante o armazenamento de bebidas de frutas ricas em catequinas e antocianinas ocorre polimerização destes compostos e conseqüente estabilização da cor. Neste estudo, (+)-catequinas também demonstraram reagir com ácidos orgânicos originando compostos escuros que ajudam a estabilização.

5- Conclusão

Sucos de uva são alimentos ricos em polifenóis que exercem efeito protetor à saúde quando do consumo regular. Do ponto de vista químico e sensorial são alimentos com perfil complexo, cujo comportamento durante o processamento e armazenagem depende de vários fatores e requer maior elucidação. Os mecanismos de ação *in vivo* ainda não estão demonstrados, o que traz à luz a necessidade de ensaios em animais e clínicos que apóiem os achados *in vitro*.

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Capítulo 3: ARTIGO DE PESQUISA: **"Processing and Storage of Concentrated Grape Juices: Radical Scavenging Activity and Major Active Polar Components"**

Submetido ao *Journal of Agriculture and Food Chemistry*

Processing and Storage of Concentrated Grape Juices: Radical Scavenging Activity and Major Active Polar Components

Abstract:

This work characterized and quantified total phenolic (TP) and radical scavenging activity (RSA) of concentrated grape juices during processing and storage. TP were determined using the Folin-Ciocalteu method and RSA by the DPPH assay. The main components of juices were investigated by direct infusion electrospray ionization mass spectrometry (ESI-MS) both in the negative ion mode. Concord juice (CJ) demonstrated higher RSA and TP contents than Isabel juice (IJ) with some differences at each processing step. During storage, TP and RSA retentions were 90% and 77% in CJ and 81% and 86% in IJ, respectively. Peonidin and peonidin-3-O-glucoside gave place to malvidin and dimethoxy-flavylium as significant components during processing; malvidin and piceatanol-O-glucoside decreased after 8-months storage. Processing and storage conditions investigated were effective in preserving total phenolics and antioxidative status of grape juices.

1- Introduction:

Phenolic-rich foods have received increasing interest due to recent findings on their association with disease prevention (ARTS & HOLLMAN, 2005; KNEKT et al., 2002; SESSO et al., 2003). Studies have identified polyphenol dietary sources as being mainly fruits, fruit juices and beverages such as wine, tea, coffee

(BEECHER, 2003; BRAVO, 1998; SCALBERT, JOHNSON & SALTMARSH, 2005). Average daily intake has been difficult to estimate for reasons mostly related to diversity in polyphenol structures and variation in content in certain foodstuffs influenced by cultivar and manufacturing processes (SCALBERT & WILLIAMSON, 2000). Some of the new compounds formed during processing and storage of fruit beverages are overlooked in most studies addressing food composition, although they may show particular properties different from their precursors (CHEYNIER, 2005). Concentrated grape juice is a phenolic-rich beverage, which undergoes heat treatments (pasteurization and concentration) followed by storage. Commercial grape juices are prepared from specific blends of concentrates after reconstitution, with or without the addition of other ingredients such as sucrose, natural flavours and sweeteners.

The efficacy of natural antioxidants is related to the protection of the food itself against oxidative damage and also to their continued action in animal fluids and tissues (SANCHEZ-MORENO, LARRAURI & SAURA-CALIXTO, 1999). Several assays have been used to measure free radical scavenging capacity and the International Organisation of Vine and Wine has recommended the $DPPH^{\bullet}$ assay as a rapid and precise method for grape products. The $DPPH^{\bullet}$ assay is based on the scavenging ability of the antioxidant under test to the stabilization of the radical. Moreover, the results are highly reproducible and comparable to other free radical scavenging methods such as ABTS (GIL et al. 2000).

Previous studies have earlier examined the antioxidant properties of wine (DE BEER, JOUBERT, GELDERBLOM & MANLEY, 2003; MUÑOZ-ESPADA, WOOD, BORDELON, WATKINS, 2004; KATALINIC, et al.; 2004). The antioxidant

capacity of fruit juices is not always the same as that of fresh fruits and its measurement in a wide range of food matrices raises discrepancies due to differences in plant cultivars (KARAKAYA, EL & TAS, 2001). Such inconsistencies reveal that certain properties of phenolic-rich products are influenced by polyphenolic composition, which is affected by vintage, grape cultivar, production techniques and aging.

Electrospray ionization mass spectrometry (ESI-MS) with direct infusion has appeared as a new analytical alternative, offering a fast and robust technique for several beverages such as tea (Bastos et al., 2007), whisky (MOLLER, CATHARINO & EBERLIN, 2005), wine (CATHARINO et al., 2006) and cachaça (de SOUZA et al., 2007). ESI has therefore greatly expanded the applicability of mass spectrometry to a variety of new classes of molecules with thermal instability, high polarity and mass (MOLLER, CATHARINO & EBERLIN, 2007b). ESI-MS has also been proven to be a powerful technique for the structural characterization of essential oils, fatty acids, organic acids and pigments in foods (CATHARINO, et al. 2005; MOLLER, et al., 2007a; MOLLER et al., 2007b). ESI, with direct sample introduction, is also a convenient technique for the direct introduction of grape juices into a mass spectrometer, as most molecules bearing acidic or basic sites will be detected and tandem MS/MS with collision-induced dissociation (CID) of ionized molecules can be used for structural elucidation studies. Thus, this paper characterizes and quantifies total phenolic contents and radical scavenging of concentrated grape juices during processing and storage.

2- Materials and methods

2.1 Samples and preparations

Concentrated grape juice samples of Concord and Isabel (known as "Isabella" in North-America) cultivars were received in February and March of 2006. These cultivars belong to the *Vitis labrusca* species and are the cultivars most used for grape juice production in Brazil, with variations in their soluble solids contents: Concord between 14 and 16°Brix and Isabel between 15 and 19°Brix. Concentrated grape juices were provided by a national producer from Rio Grande do Sul - Brazil. Samples of both cultivars were also obtained at each step of the industrial process, which consists of hot pressing of the grapes and pasteurization of the must (80°C, 30 sec) followed by filtration and concentration of juice to 68°Brix (98°C, 5 sec). Concentrated juices were stored at 5°C in the dark simulating industrial storage conditions. Every 30 days two samples of each grape cultivar were removed and stored at -18°C for subsequent analysis, with maximum aging time of 10 months. Prior to analysis, concentrated juices were reconstituted to 17° Brix by mixing 1mL juice with 3.85 mL of deionised water.

2.2 Determination of total phenols

Total phenols were measured by the Folin-Ciocalteu assay (SINGLETON & ROSSI, 1965) using gallic acid (Sigma-Aldrich, St Louis, MO, USA) for the standard curve and the results being expressed in mg gallic acid equivalents/L (GAE). Floating particles were removed from the juices by centrifugation and the

juice diluted 1:100 with deionized water. The readings (in duplicate) were taken at 760 nm using a Beckman spectrometer.

2.3 Determination of radical scavenging activity

The DPPH (1,1-diphenyl-2-picrylhydrazil) (Sigma-Aldrich, Steinheim, BW, Germany) assay was used based on the methods of BRAND-WILLIAMS, CUVELIER & BERSET (1995), as modified by KIM et al. (2002). The spectrophotometric test is based on the reaction of a potential antioxidant with the DPPH free radical:

$DPPH^{\bullet} + AH \rightarrow DPPH\text{-}H + A^{\bullet}$

In the radical form, $DPPH^{\bullet}$ presents a maximum absorption at 517 nm, but upon reduction by a radical scavenger, a pale-yellow non-radical form is produced. Methanolic solutions of DPPH (100 μm) were prepared daily using 80% methanol. Samples aliquots of 0.1 mL were added to 3.9 mL of fresh DPPH methanolic solution and the mixtures kept in the dark for 30 minutes at room temperature (25ºC). The absorbance was measured with a Beckman spectrometer before the addition of samples and 30 minutes after, in the presence of juice. A standard curve of the synthetic antioxidant Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) (Sigma-Aldrich, St. Louis, MO, USA) was built for concentrations from 0.08-1.28 mM/L. Analyses were carried out in duplicate and the results expressed in mM Trolox equivalent (mM TE).

2.4 Electrospray mass spectrometry fingerprinting

Grape juices were diluted in a solution containing 50% (v/v) chromatographic grade methanol (Tedia, Fairfield, OH, USA), 50% (v/v) deionized water and 0.5% of ammonium hydroxide (Merck, Darmstadt, Germany). Electrospray mass spectra (ESI –MS) fingerprints of juices were acquired and accumulated over 60 sec and the spectra scanned in the range between 250 and 600 m/z to investigate process and between 250 and 900 m/z for to compares cultivars, all in the negative ion mode using a Micromass-Waters Q-TOF mass spectrometer (Manchester, England). Capillary and cone voltages were set to -3000 V and -40 V, respectively, with a de-solvation temperature of 100 ℃. In general, ESI-MS was performed by direct infusion with a flow rate of 10 µl min-1 using a syringe pump (Harvard Apparatus). Structural analysis of single ions in the mass spectra of grape juices was performed by ESI-MS/MS. The ion with the mass to charge ratio of interest was mass-selected and submitted to 15-45 eV collisions with argon in the collision quadrupole. The collision gas pressure was optimized to produce extensive fragmentation of the ion under investigation.

2.5 Statistical analysis

The *t* test was applied to compare total phenols and radical scavenging activity averages between cultivars. To verify the relationships between parameters, Pearson correlation coefficients were calculated. Data analyses were conducted using Excel 97 (Microsoft Corporation, Washington, USA).

3- Results and discussion

Radical scavenging activity (RSA) and total phenol (TP) contents found during processing of concentrated grape juice are shown in Figure 1.

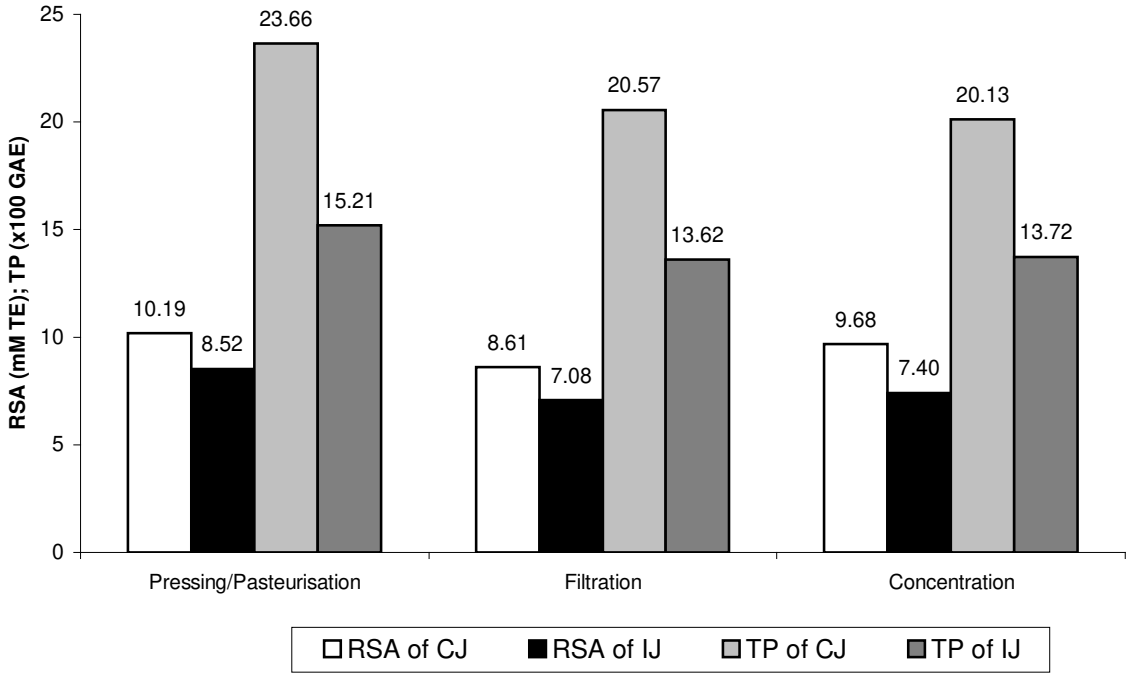


Figure 1. Radical scavenging activity (RSA) and total phenol contents (TP) found during processing of Concord (CJ) and Isabel (IJ) grape juices. Variation between duplicates was less than 5%.

Concord juice (CJ) and Isabel juice (IJ) demonstrated diverse amounts of TP and RSA. A stable behaviour throughout processing was observed for both parameters and juices with some variation after heat treatment (pasteurization). Although CJ showed *ca* 50% higher contents of TP, its RSA was on average only 25% higher. Such disparities could be attributed to different phenolic composition, which would yield different radical scavenging activities. Yildirin et al. (2005)

reported variations in total phenols and antioxidant activity during the steps of wine making. Figure 2 shows evolution of parameters during storage.

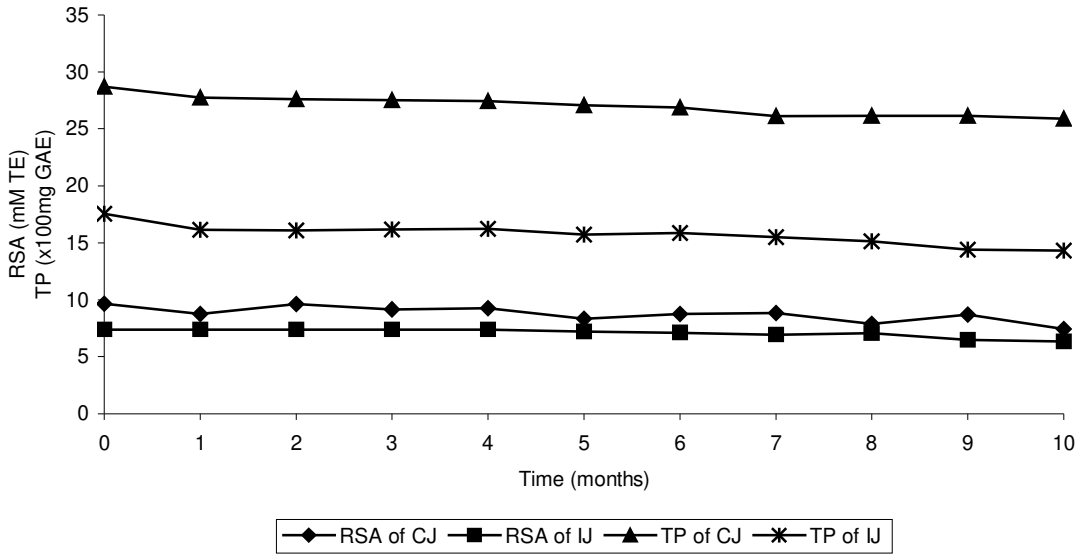


Figure 2. Radical scavenging activity (RSA) and total phenols content (TP) of Concord (CJ) and Isabel (IJ) grape juices at 68° Brix stored and 5° C. Variations between duplicates were less than 5%.

TP contents and RSA of CJ and IJ demonstrated fairly stable behaviour during aging. TP contents varied from 2872.9 to 2587.6 GAE in CJ and from 1756.8 to 1428.9 GAE in IJ. RSA went from 9.68 to 7.45 mM TE in CJ and from 7.40 to 6.33 mM TE in IJ. On average, Concord grape juice presented higher TP contents and RSA during 10 months of storage ($p<0.001$) and a positive and significant correlation was found between TP and RSA for Concord juice ($r=0.78$, $p=0.005$) and for Isabel juice ($r=0.88$, $p<0.001$). TP retention percentage was 90% and 81% for CJ and IJ, respectively, while RSA retention was 77% and 86% for CJ

and IJ, respectively. IJ showed higher RSA retention in spite of a higher TP loss. Pérez-Vicente et al. (2004) observed similar behaviour regarding TP contents of pomegranate juice: a 2% loss during process and *ca* 20% decrease after 5 months aging. However, contrary to our findings, the authors found no correlation between TP and RSA, which increased 10% after heat treatment and 30% after the storage period. In the present study, in order to investigate the contribution of a "phenolic unit" to the RSA of both juices, the mean TP:RSA ratio was calculated. The value was 0.0045 for IJ and 0.0032 for CJ, indicating that specific phenolic compounds or synergy among them were relevant for greater radical scavenging power "per unit" in IJ.

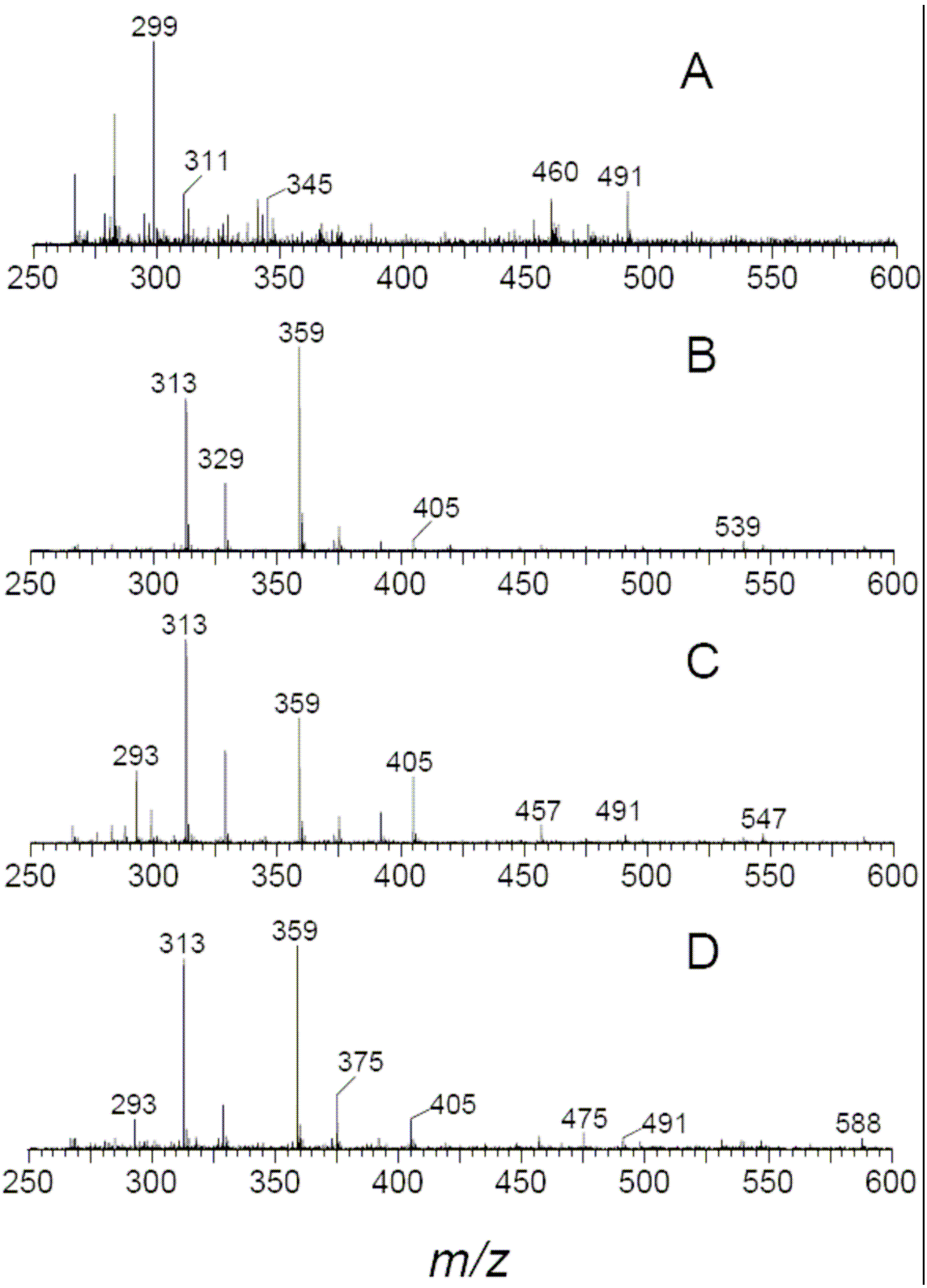
Figure 3 shows ESI(-)-MS fingerprints revealing compositional changes during processing of Concord grape juice. Considering that Isabel juice demonstrated equivalent behaviour, fingerprints of this cultivar will not be shown. Note that these spectra display characteristic profiles of polyphenols (and eventually hexose). The ESI(-)-MS fingerprints of juice samples showed characteristic distributions mainly of the following compounds: dimethoxy-flavylium (DF), malvidin (M), dimer of the hexose (H) and piceatanol-O-glucoside (PG) detected as the deprotonated molecules of *m/z* 313, 329, 359 and 405, respectively. Prior to heat treatment (Figure 3A), characteristic compounds such as peonidin (P), caffeoyltartaric acid (CA) and peonidin-3-O-glucoside (P3G) identified as the marker ions of *m/z* 299, 311 and 461 respectively were detected. After pasteurization (Figure 3B) juice was characterized by the predominance of three significant ions of *m/z* 313 (DF), *m/z* 329 (M) and *m/z* 359 (H), in a ratio of ca. 4:2:5. The ESI-MS fingerprint of juice after concentration (Figure 3C) was

characterized by the predominance of five significant major ions of *m/z* 293 (unknown), *m/z* 313 (DF), *m/z* 329 (M), *m/z* 359 (H) and *m/z* 405 (PG), in a ratio of ca 4:10:5:6:3. Figure 3D shows that after an 8-month storage period in concentrated condition under refrigeration, fingerprint of grape juice was characterized by four major marker ions of *m/z* 313 (DF), *m/z* 359 (H) in a ratio of ca 9:10. Earlier, Catharino et. al. (2006) had identified ions of *m/z* 313 (DF), *m/z* 329 (M), *m/z* 359 (H) as diagnostic ions for must of six varieties of red grapes. The present results revealed that peonidin and peonidin-3-O-glucoside gave place to malvidin and dimethoxy-flavylium as significant components during processing. Malvidin and piceatanol-O-glucoside underwent reduction during the 8-month storage, whereas dimetoxy-flavilium compounds remained stable. These findings confirm that glucosides and highly hydroxilated anthocynins are less stable under oxidative and thermal conditions than the methylated forms (von ELBE & SHWARTZ, 1996; TALCOTT & LEE, 2002). In bottled wines, Muñoz-Espada et al. (2004), observed the presence of several anthocyanin aglycons in higher amounts than glucosides.

The ESI-MS fingerprints of CJ and IJ (Figure 4) show similarities and some important differences between Isabel and Concord juices after concentration. IJ (Figure 4A) is characterized by significant ions of *m/z m/z* 313 (DF), *m/z* 329 (M), *m/z* 359 (H) in a ratio of ca. 9:3:10. CJ (Figure 4B) also produces a quite characteristic spectrum showing the predominance of five ions of *m/z* 293 (unknown), *m/z* 313 (DF), *m/z* 329 (M), *m/z* 359 (H), and *m/z* 405 (PG) in a ratio of ca 4:10:5:6:3. Concord juice presented higher proportion of malvidin and lower of

hexose dimers than Isabel, which is in agreement with the intense colour of CJ and greater sweetness of IJ.

Figure 3: ESI(-)-MS fingerprints of Concord grape juice: (A) at pressing; (B) after pasteurization and filtration; (C) after concentration; (D) after 8-month storage.


A
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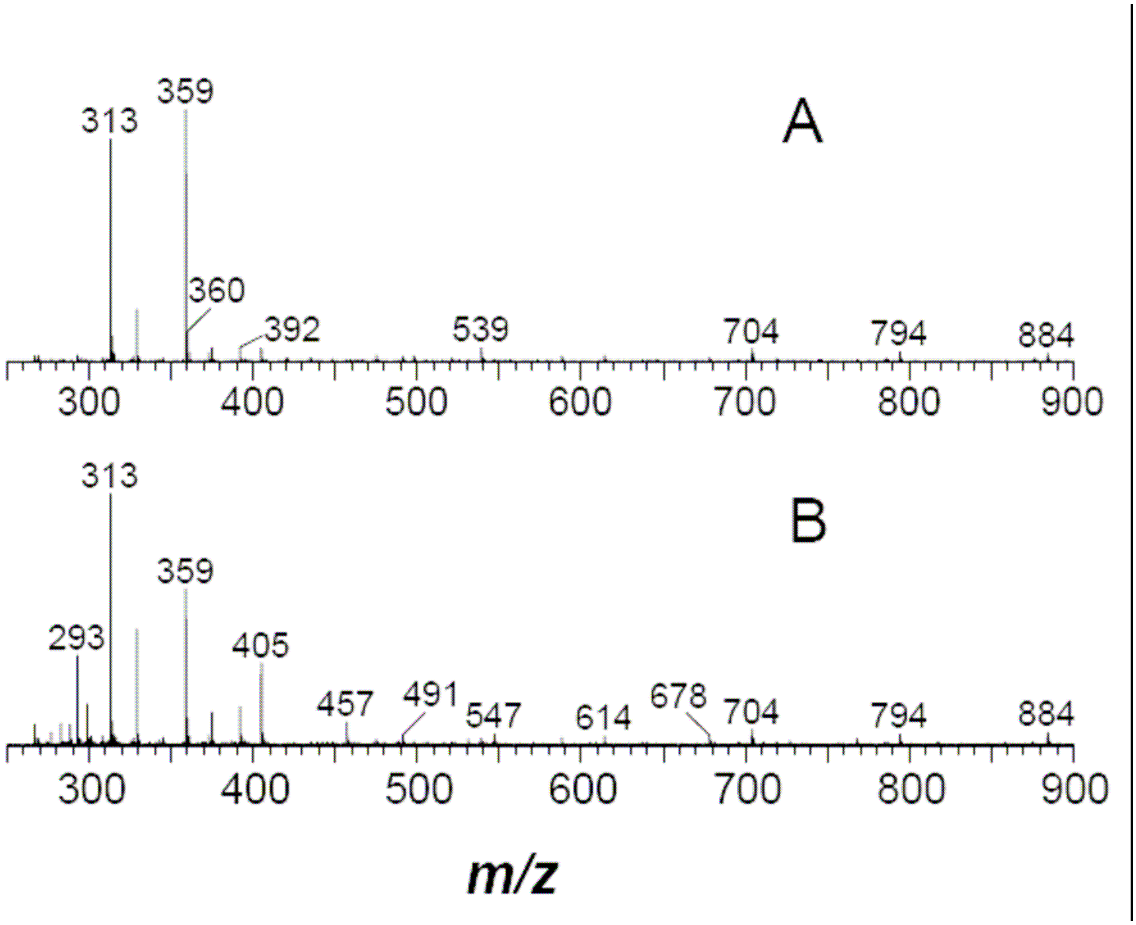

Figure 4: ESI-MS fingerprints of (A) Isabel and (B) Concord grape juices after concentration.

4- Conclusion

The grape juices investigated revealed similar TP and RSA values, as also reported for red wine (2036 GAE and 6-12 TE, respectively) and green tea infusion (1029 GAE and 8 TE, respectively). Phenolic composition of Isabel grape juice presented greater contribution to the antioxidant activity, indicating that particular alterations occur during processing and storage, and specific compositions should be taken into account when assessing the intake and antioxidative properties of

fruit beverages. The MS technique provided information about component structures, particularly the bioactive compounds. Processing and storage conditions of grape juices were shown to be effective in preserving juice quality with respect to phenolic compounds and oxidative status.

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Capítulo 4: **ARTIGO DE PESQUISA: "Storage of concentrated and ready-to-drink grape juices and impact on (+)-catechin and (-)-epicatechin"**

Submetido ao *International Journal of Food Science and Technology*

Storage of concentrated and ready-to-drink grape juices and impact on (+)-catechin and (-)-epicatechin

Abstract:

Commercial concentrated Concord (CCJ) and Isabel (CIJ) grapes juices were stored under refrigeration while pasteurized ready-to-drink juices of the same grape cultivars (PCJ and PIJ) were kept at room temperature under indirect light for 10 months, simulating industrial/commercial storage conditions. The preservation of (+)-catechin during storage ranged between 63% (PCJ) and 52% (PIJ); (-)-epicatechin retention was of 32% (CCJ) and 15% (CIJ). Total phenols retention ranged from 93% (CCJ) to 84% (PCJ) and radical scavenging activity from 87% (PIJ) to 85% (CCJ and PCJ). Concentrated juices showed greater amounts of catechin and CCJ greater phenolic contents. Despite great differences in phenolic and catechins contents in juices, scavenging activities were similar. PIJ yielded the highest radical scavenging activity during storage per phenolic unit. Processing and storage impacted catechins and not total phenolics and radical scavenging activity during the 10 months aging.

1- Introduction:

Epidemiologic studies have revealed that phenolic-rich diets reduce mortality from degenerative diseases caused by oxidative stress (SCALBERT et al. 2005). Amongst the various classes of phenolic compounds, flavan-3-ols exert physiological properties that may be the source of health benefits from wine

consumption (GÜRBÜRZ et al., 2007). Dietary intervention studies support flavan-3-ol-rich foods and beverages as being beneficial to cardiovascular health (KEEN et al. 2005). In grape juices, flavan-3-ols are mostly found in the monomeric forms of catechins [(+)-catechin and (-)-epicatechin] with large differences among cultivars (AUW et al., 1999; LEE & JAROWISKY, 1987; JAROWISKY & LEE, 1987; SPANOS & WROLSTAD, 1990).

Catechins are amongst the three polyphenols most well-absorbed by humans, after gallic acid and isoflavones (MANACH et al, 2005). In human intervention studies, catechins have been associated with increased plasma antioxidant activity, increased plasma ascorbate concentrations, increased resistance to LDL oxidation and decreased plasma lipid peroxide and malondialdehyde concentrations (LOTITO & FRAGA, 1997; KAMPA, et al., 2000; KIMURA, et al., 2002; WILLIAMSON & MANACH, 2005). In an epidemiological study, ARTS et al. (2001a,b) demonstrated a positive association between catechin consumption and reduced mortality by chronic diseases. The principal property of polyphenols including catechins is the antioxidant capacity, associated with product quality and health benefits.

Polyphenolic compounds are highly unstable and react with other substances and amongst themselves during food processing and storage. In wines, phenolics undergo changes during aging resulting in known and unknown phenolic species (CHEYNIER, 2005). Such modifications are overlooked in most studies concerning food composition. In 2000, Arts et al. noted that epidemiological research required further studies on catechins changes during process or aging. The design of the present study contemplates commercial products under

industrial conditions. Thus, the objectives of this work were to verify the impact of storage on total phenols, catechin monomers and antioxidant activity of commercial grape juices.

2- Materials and methods

2.1 Samples and preparations

Samples of concentrated and pasteurized ready-to-drink grape juices of Concord and Isabel cultivars (*Vitis labrusca* species) were received in February and March of 2006. Concentrated juices were supplied by a manufacturer after a process which consisted of pressing with simultaneous pasteurization followed by concentration to 68°Brix. Concentrated juices were stored at 5°C in the dark. Pasteurized grape juices (soluble solids contents ranging from 14 and 19° Brix) were obtained from another manufacturer after grape pressing, pasteurization and bottling. Juices were stored in their own transparent green glass bottles under indirect lighting at room temperature (20-25°C). Both storage situations simulated typical conditions in an industry/warehouse. Every 30 days two samples from each cultivar were taken from their specific storage conditions and placed in a freezer at -18°C in the dark until analysed, up to maximum aging time of 10 months. Prior to analysis concentrated juices were reconstituted to 17°Brix.

2.2 Determination of total phenols

Total phenols were measured by the Folin-Ciocalteu assay (SINGLETON & ROSSI, 1965) using gallic acid (Sigma-Aldrich, St Louis, MO, USA) for the standard curve, and the results were expressed in mg gallic acid equivalents/L

(GAE). Floating particles were removed and the juice samples were diluted 1:100 with deionized water. The absorbance was read at 760 nm with a Beckman spectrometer and all analyses were carried out in duplicate.

2.3 Determination of radical scavenging activity

The DPPH (1,1-diphenyl-2-picrylhydrazil) (Sigma-Aldrich, Steinheim, BW, Germany) assay was used based on the methods of BRAND-WILLIAMS, CUVELIER & BERSET (1995), as modified by KIM et al. (2002). The spectrophotometric test is based on the reaction of a potential antioxidant with the DPPH free radical. In the radical form, $DPPH^{\bullet}$ presents a maximum absorption at 517 nm, but upon reduction by a radical scavenger, a pale-yellow non-radical form is produced. Methanolic solutions of DPPH (100 μm) were prepared daily using 80% methanol. Samples aliquots of 0.1 mL were added to 3.9 mL of fresh DPPH methanolic solution and the mixtures kept in the dark for 30 minutes at room temperature (25ºC). The absorbance was measured with a Beckman spectrometer before the addition of samples and 30 minutes after, in the presence of juice. A standard curve of the synthetic antioxidant Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) (Sigma-Aldrich, St. Louis, MO, USA) was built for concentrations from 0.08-1.28 mM/L. Analyses were carried out in duplicate and the results expressed in mM Trolox equivalent (mM TE).

2.4 Determination of total catechins [(+) catechin and (-) epicatechin]

The two monomers were analysed by HPLC with fluorescence detection. The method of Arts & Hollman (1998) for the optimised determination of catechins in fruits was used adaptated for fruit juices as described by Arts et al. (2000). Each concentrated juice sample was reconstituted to 17° Brix and if necessary, both reconstituted and commercial juices were diluted 1:2 with deionised water. Samples were filtered through a 0.45µm Millex HV (PVDF) disposable syringe filter. Standards of (+)-catechin and (-)-epicatechin were obtained from Sigma Aldrich (St Louis, MO, USA), and stock solutions containing 0.2 mg catechins/mL methanol stored below 4ºC. Standard curves were obtained using concentrations of 2, 4, 6, 8, 10 and 12 mg/L. A Perkin Elmer High Performance Liquid Chromatograph (HPLC) equipped with a binary pump and manual sampler was used. The column was a (250 mm x 4.6 mm) GL Science, Inertisil ODS – 3.5 µm with solvent flow rate of 1 mL/min. The mobile phase consisted of 5% acetonitrile (solvent A) and 25% acetonitrile (solvent B) in 0.025M phosphate buffer, pH 2.4 with the following gradient: 0-15 min, 45% B; 15-28 min, linear gradient from 45 to 70% B; 28-30 min, linear gradient from 70 to 45% B. The volume injected was 10 µl and detection was by fluorescence (280 nm excitation and 310 nm emission). The retention time of (+)-catechin varied from 11 to 15 minutes and of (-)-epicatechin from 18 to 22 minutes. Analyses were conducted in duplicate. To determine the detection limit, ten noise concentrations were taken at random and the standard deviation multiplied by three. The quantification limit was determined by injecting standard solutions and verifying the minimum concentration that provided accurate integration of the peak. In the present study, the detection limit

was 0.05 mg/L and the quantification limits were 1.00 mg/L for (+)-catechin and 2.00 mg/L for (-)-epicatechin.

2.5 Statistical analyses

The data were treated with analysis of variance and the means were compared using Tukey (LSD) test. Pearson correlation coefficients were used to investigate relationships between parameters. Data analyses were conducted using Excel 97, Microsoft Corporation, Washington, USA.

3- Results and discussion

Figures 1 to 4 show the behaviour of juice parameters analysed during 10 months.

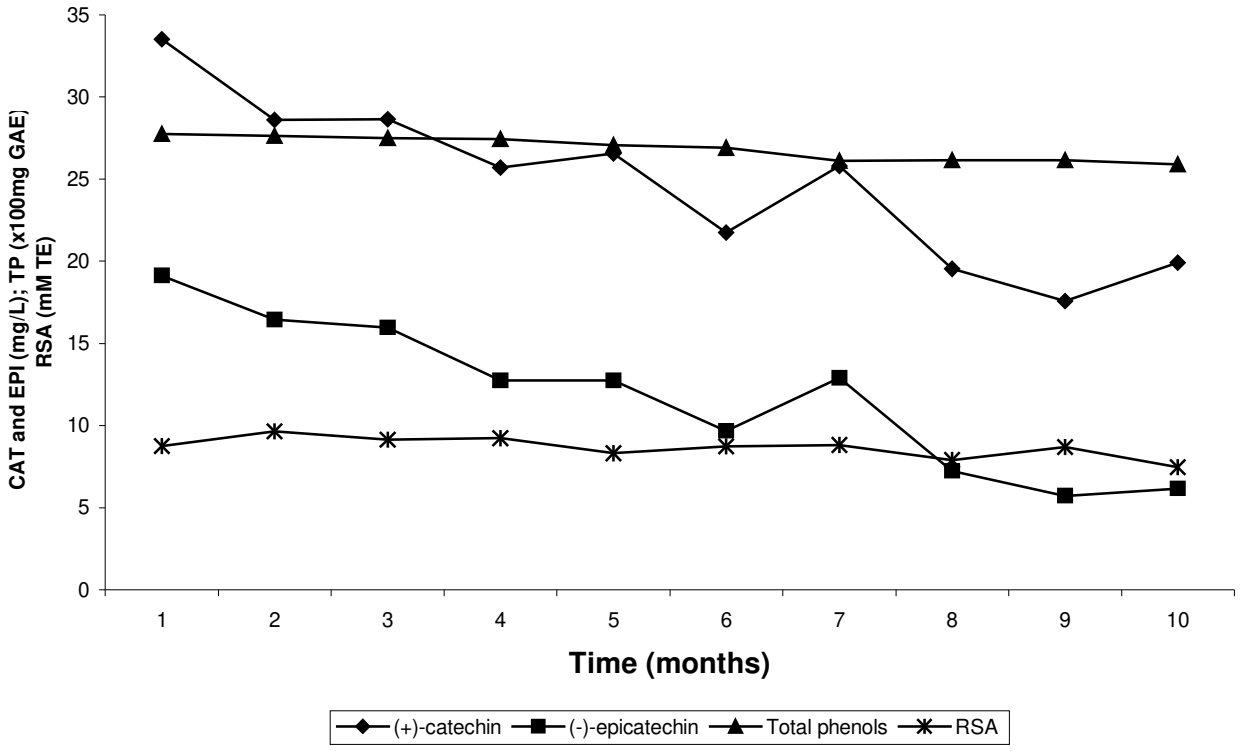

Figure 1. Concentrated Concord grape juice (CCJ): (+) catechin (CAT), (-)-epicatechin (EPI) total phenols (TP) and radical scavenging activity (RSA) behaviour during storage. Variations between duplicates were lower than 5% for TP and RSA and 10% for CAT and EPI.

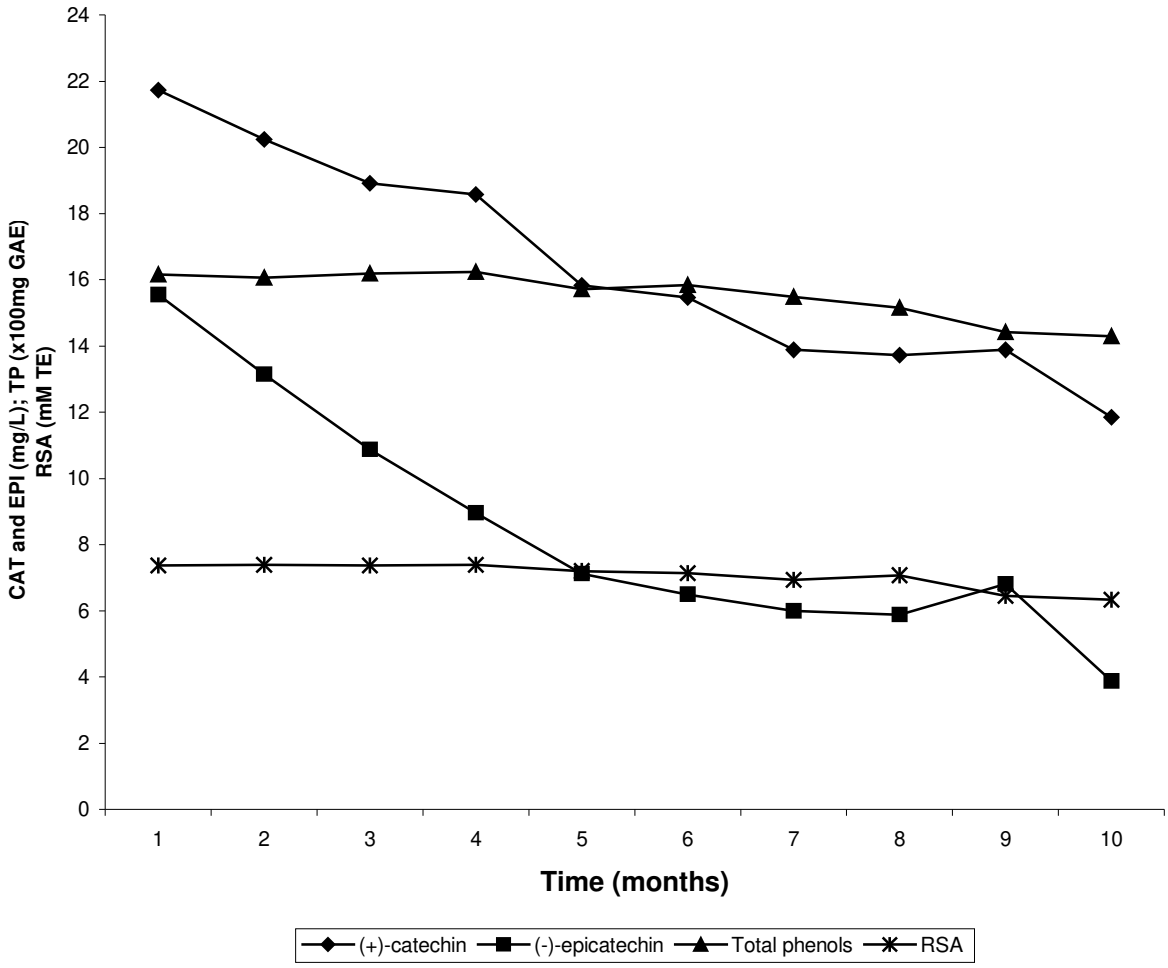

Figure 2. Concentrated Isabel grape juice (CIJ): (+) catechin (CAT), (-)-epicatechin (EPI) total phenols (TP) and radical scavenging activity (RSA) during storage. Variations between duplicates were lower than 5% for TP and RSA and 10% for CAT and EPI.

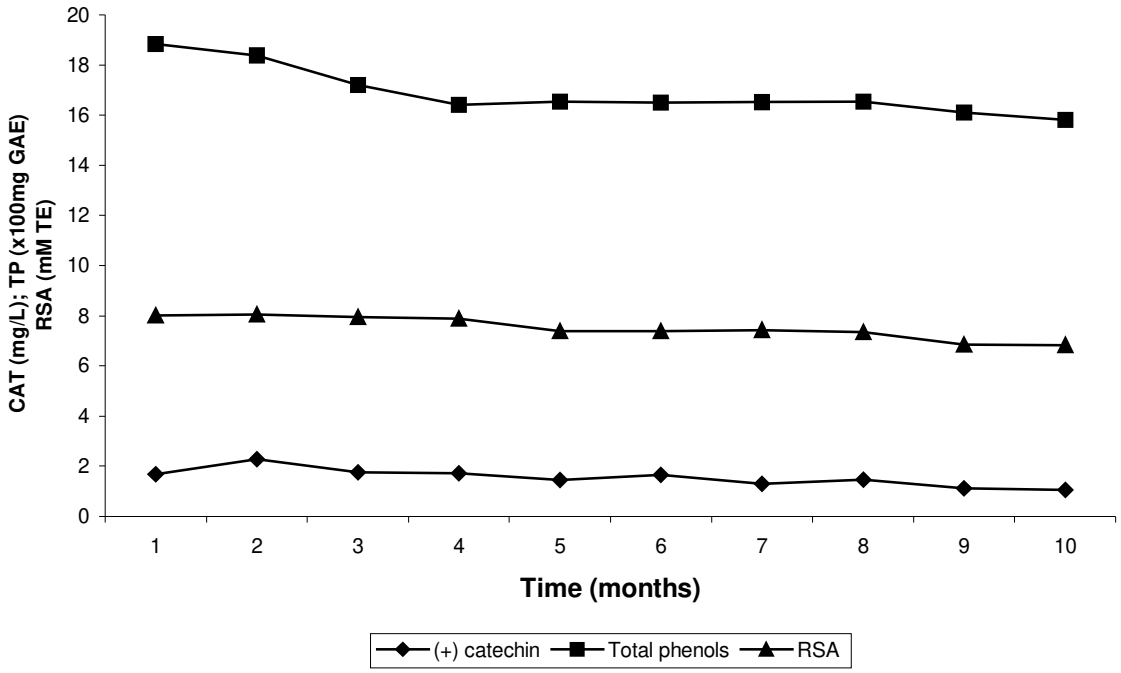

Figure 3. Pasteurized Concord grape juice (PCJ): (+) catechin (CAT), total phenols (TP) and radical scavenging activity (RSA) during storage. Note: (-) epicatechin contents were below quantification limit (2.00 mg/L). Variations between duplicates were lower than 5% for TP and RSA and 10% for CAT.

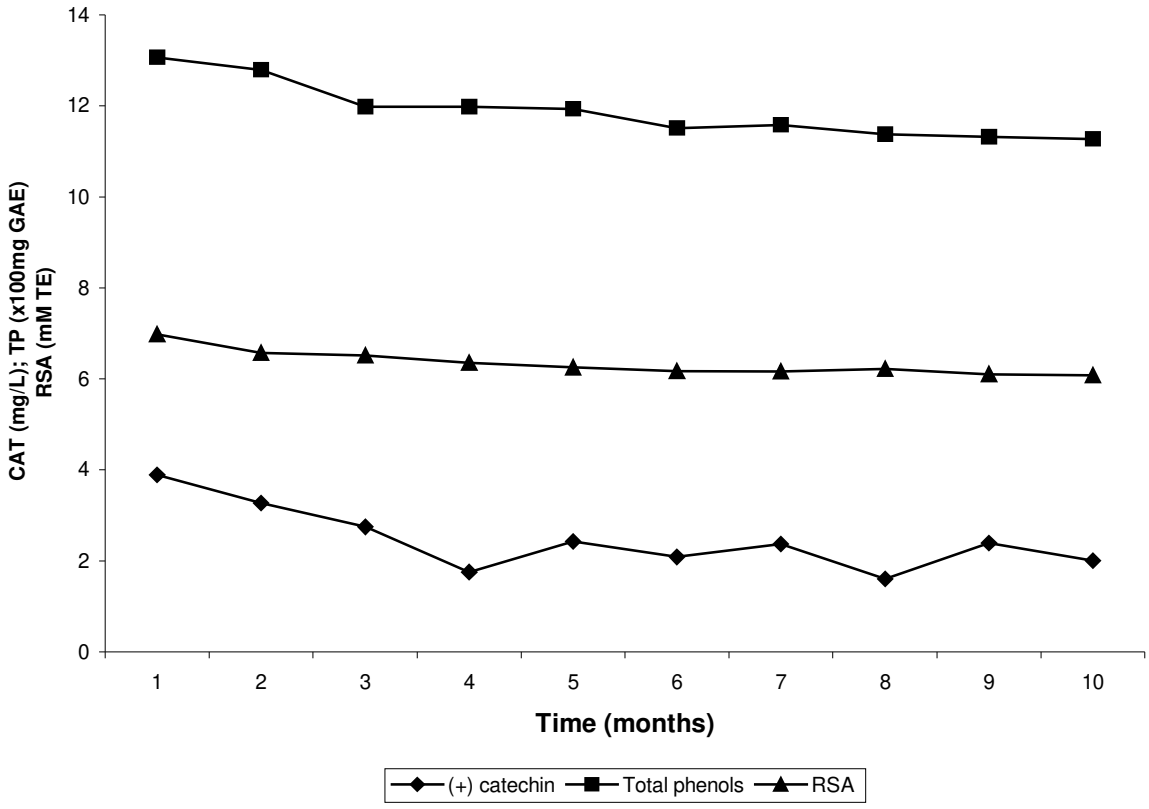

Figure 4. Pasteurized Isabel grape juice (PIJ): (+) catechin (CAT), total phenols (TP) and radical scavenging activity (RSA) during storage. Note: (-) epicatechin contents were below quantification limit (2.00 mg/L). Variations between duplicates were lower than 5% for TP and RSA and 10% for CAT.

In concentrated Concord grape juice (CCJ) CAT and EPI decreased from 33.51 to 19.93 mg/L and 19.12 to 6.15 mg/L respectively in 10 months(Figure 1). In the same period TP and RSA decreased from 2775.0 to 2587.6 mg GAE and from 8.77 to 7.45 mM TE respectively. In terms of retention during storage, CAT and EPI demonstrated preservation of 59% and 32% respectively, which was lower than preservation of TP or RSA (93% and 85%, respectively). The results for

concentrated Isabel grape juice (CIJ) under the same storage conditions showed a similar performance (Figure 2): CAT and EPI showed reductions from 21.73 to 11.85 mg/L and 25.54 to 3.88 mg/L, respectively (55% and 15% retention, respectively). TP and RSA varied from 1615.0 to 1429.0 mg GAE and from 7.38 to 6.33 mM TE, respectively (88% and 86% retention, respectively).

For pasteurised Concord juice (PCJ) (figure 3) CAT reduction was from 1.68 to 1.06 mg/L (63% retention) and EPI concentrations were below quantification limit (2.00 mg/L). TP and RSA decreased from 1884.3 to 1582.3 mg GAE and from 8.02 to 6.83 mM TE (84% and 85% retention, respectively). Pasteurised Isabel juice (PIJ), under similar storage conditions demonstrated similar modifications (Figure 4). CAT showed reduction from 3.89 to 2.01 mg/L (52% retention) and EPI concentrations were below quantification limit. TP and RSA decreased from 1306.9 to 1126.9 mg GAE and from 6.98 to 6.08 mM TE (86% and 87% retention, respectively).

Considering the four juices investigated, CAT and EPI (detected only in concentrated juices), demonstrated the lowest preservation (maximum 63% and 32%, respectively), regardless of cultivar and storage differences. EPI showed less preservation than its isomer CAT, probably due to higher reactivity, which agrees with the results of Freitas, Glories & Laguerre (1998) showing (-)-epicatechin as being more oxidizable than (+)-catechin in a model experiment.

TP and RSA showed greater preservation: TP retention ranged from 93% to 84% and RSA from 87% to 85%, demonstrating that other phenolic compounds, rather than monomeric catechins contributed to the radical scavenging capacity. It was previously verified that teaflavins, originating from oxidized and dimerized

catechins in teas possessed the same radical scavenging activity as the initial monomers (Leung et al., 2001). As for the decrease in phenolic content, this could be attributed to enzymatic and non-enzymatic reactions (ES-SAFI et al., 2003). Talcott & Lee (2002) found that processing methods rather than storage conditions were important for retention of radical scavenging properties in Muscadine grape juices. This partially agrees with the present investigations, in which neither storage nor process impacted RSA, which was more related to cultivar, with Concord juices showing the highest activity.

Statistical differences ($p < 0.05$) amongst juices were found: for (+) catechins CCJ>CIJ>PCJ=PIJ; for (-)-epicatechins CCJ=CIJ; for total phenols CCJ>PCJ>CIJ>PIJ and for radical scavenging activity CCJ>CIJ=PCJ>PIJ. Concentrated juices showed notably higher catechin contents, possibly due to processing (hot pressing). In contrast, pasteurised juices (cold pressing) showed only about 10% of the amount. The findings agree with Fuleki & Ricardo-da-Silva (2003) who demonstrated that hot pressing enhanced extraction of flavan-3-ols. Regarding TP contents, grape cultivar rather than process was the relevant factor in the present study, with Concord grapes showing greater contents than Isabel grapes.

Aging was associated with decreases in CAT, EPI, TP and RSA in all four juices ($p<0.05$). Despite the high reduction suffered by catechins, retention of TP and RSA was above 84% regardless of process and cultivar. Zafrilla et al. (2003) observed similar changes in wines: antioxidant activity was maintained after seven months storage, although a decrease in the concentration of some phenols was observed. In CCJ, CIJ and PIJ, radical scavenging activities were better correlated

with TP than with CAT or EPI ($p<0.05$). Catechin monomers (CAT and EPI) were strongly correlated during aging ($r>0.96$, $p<0.01$).

The differences in the TP contents of the grape juices found no correspondence in RSA. Hence, in order to verify radical scavenging potential of each product, the mean ratio TP:RSA was calculated. It decreased in the following order: PIJ>CIJ>PCJ>CCJ. Dávalos, Bartolomé & Gómez-Cordovés (2005) called this assessment "antioxidant activity provided by a unit of polyphenol" and in our study it indicated that Isabel juices contained or retained specific phenolic compounds with higher antioxidant activity "per phenolic unit".

4- Conclusions

Storage impacted catechin contents in grape juices but not total phenolics and radical scavenging activity. Both storage conditions revealed similar retention of total phenolics and radical scavenging activity *in vitro* after 10-months. This study demonstrated that the antioxidant power per phenolic unit must be considered when evaluating antioxidant activity of phenolic-rich foodstuffs.

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Capítulo 5: **ARTIGO DE PESQUISA: "Sensory stability of concentrated Concord and Isabel grape juices during storage"**

Submetido ao *Journal of Sensory Studies*

Sensory stability of concentrated Concord and Isabel grape juices during storage

Abstract

The aim of the present study was to evaluate sensory characteristics and phenolic contents of concentrated red grape juice from two cultivars (Concord and Isabel), over an 8-month storage period. The quantitative descriptive analysis method (QDA) was used to evaluate the sensory aspects. Total phenols were quantified by the Folin-Ciocalteu assay and catechins [(+)-catechin and (-)-epicatechin] by HPLC with fluorescence detection. The results showed a fairly stable sensory behaviour for Concord and Isabel grape juices. Total phenols of Concord and Isabel juices showed 93% and 88% retentions, respectively and total catechin retentions of 60% and 85% respectively. Concord juice was more stable to storage showing sensory characteristics of astringency, bitterness and a higher colour intensity, while Isabel juice was characterized by sweetness and juice flavour. Under the storage conditions investigated, concentrated juices were capable of maintaining their sensory standards.

1- Introduction

Colour, bitterness and astringency are sensory perceptions related to phenolic compounds in grapes and wines. Bitterness is considered to be a taste mediated by receptors while astringency is more a sensation that leads to a mouth-feel perception (Roussef, 1990). Vidal et. al.(2004) investigated the bitter and astringent properties of tannin-like polyphenols in wines using descriptive analysis.

The authors found that monomeric flavanols contributed to the astringency and bitterness when in adequate concentrations and that polymeric substances formed during wine aging could lead to a decrease in both sensory properties. More recently, Preys et. al. (2006) characterized and analyzed 30 French and 29 German commercial red wines for phenolic compounds and sensory properties finding a strong relationship between phenolics and astringency.

Phenolic compounds are highly reactive and undergo several changes during processing and storage of food products. The newly-formed products show specific sensory properties that are usually different from the original substances. Es-Safi; Cheynier & Moutounet (2003) demonstrated that (+) catechin incubated with glyoxylic acid (an oxidation product of tartaric acid present in grapes) formed brown products during aging. Enzymatic browning is also responsible for changes in colour and the enzymatic inactivation of grape-derived products has already been studied (Es-Safi et al., 2000b). Changes in the sensory parameters compromise product quality in the long run and the study of such modifications may provide the industry with the information required to take the necessary measures to improve quality.

In spite of the extensive studies on wines, not much has been published on the sensory changes in red grape juice during storage with respect to the phenolic composition. Due to recent discoveries about the health benefits related to its consumption, grape juice is a beverage showing a rising trend. The antioxidant properties and other physiological effects of grape juices have recently been studied by several authors (Osman et al., 1998; Stein et al., 1999; Keevil et al., 2000; O´Byrne et al., 2002; Devara; Grundy & Jialal, 2002). Brazil is a grape juice

producer (87 million litres in 2006) with an expanding consumer and export market. Sensory quality assurance during storage is a concern for grape juice producers due to the reactive character of this phenolic-rich beverage. Nevertheless, sensory modifications of concentrated grape juices during aging have not yet been reported.

Many interactions among phenolic compounds with an impact on sensory properties have been evaluated by model assays or by accelerated aging of food matrices. Recently, the perceptions most extensive studied have been colour (Es-Safi, Cheynier & Moutounet, 2000a; Es-Safi et al., 2000b) and astringency (Fukui et al., 2002; Monteleone et al., 2004; Gambuti et al., 2006, François et. al., 2006). However, fruit juices are essentially complex mixtures and experiments with food matrices (particularly commercial food products) and real storage time seem to be necessary in order to measure aging/maturation processes. Considering the lack of investigations on the sensory aspects of concentrated grape juice during storage, the main objective of the present study was to evaluate the sensory stability of concentrated red grape juice with respect to its phenolic compounds during aging.

2. Material and methods

2.1 Samples

Concentrated and commercial grape juices of the Concord and Isabel cultivars (*Vitis labrusca* species) were received in February and March of 2006. Concentrated juices were supplied by a manufacturer after a process which consisted of pressing with simultaneous pasteurization followed by concentration to 68°Brix. Concentrated juices were stored at 5°C in the dark. Commercial

pasteurized grape juices (soluble solids contents ranging from 14 to 19° Brix) were obtained from a different manufacturer after grape pressing, pasteurization and bottling. Juices were stored in their own transparent green glass bottles under indirect lighting at room temperature (20-25℃). Both storage situations simulated the typical conditions found in the industry/warehouse. Every 60 days two samples of concentrated juice from each cultivar were taken from storage and placed at -18℃ in the dark until analysed. Concentrated juices were evaluated after 0, 2, 4, 6 and 8 months of storage, whereas ready-to-drink grape juices were analyzed just once after eight months storage as a comparable standard. Prior to analyses, concentrated juices were reconstituted to 17° Brix by mixing 1mL juice with 3.85 mL of deionized water.

2.2. Sensory Evaluation

University students from the Nutrition Sciences Department were invited to form a group of panelists to discriminate and quantify certain attributes of grape juice using the quantitative descriptive analysis (QDA). A pre-determined list of attributes was established taking into account the perceptions related to phenolic compounds in beverages according to several authors and most recently by Preys et al. (2006) and Gambuti et al. (2006). The list of attributes included the following descriptors: colour, astringency, bitterness, sweetness and characteristic grape juice flavour. The last two, although not related to phenols were included in order to minimize the interactive effect, which may interfere in the perception of other attributes when panelists are not free to express a sensation, according to Lawless & Heymann (1999) and Stone & Sidel (2004). For measurement, a non-structured

9 cm rating scale was used with anchors at each end, representing the minimum and maximum intensities. The judges were asked to draw a vertical line through the scale at the point where it best represented the intensity of each attribute and product. Evaluation by the panelists and sensory tests took place in the individual testing booths at the University under white lighting.

2.2.1 Panel selection and training

The purposes of the investigation were described to 60 volunteers and a questionnaire based on Meilgaard, Civille & Carr, (1999) was applied to verify their sensory relation to grape juice and to detect any impediments in participating. After signing the consent form approved by the local Ethics Commission on Human Research (Process n°. 4096.7.2006), 30 volunteers were evaluated on the basis of their ability to differentiate basic tastes (salty, sour, sweet, acid) and two distinct solutions of monosodium glutamate and water. Sixteen volunteers were then chosen amongst those able to differentiate all six solutions or with up to two errors not related to the tastes under investigation. As a result, 16 volunteers formed the sensory panel, attending a four-day training of 60 minutes per day, followed by discrimination tests after each session.

Since the attributes and descriptors were pre-determined, the panel leader was asked to verify with the panelists the adequacies of the language as well as of the reference solutions and their concentrations. References were based on Behrens & da Silva (2000) for the evaluation of white wines. On the fourth day, the five attributes were presented and all references were available for consultation by the panelists. At the end of the training period, panelists were asked to evaluate

three samples of commercial grape juice with three repetitions in sensory cabins. The analysis of variance (ANOVA) was used to investigate the discriminative ability and reproducibility of each judge for every attribute. The participants who showed discriminative ability ($p < 0.50$) and reproducibility ($p > 0.05$) immediately made up the final panelist group. The test group was thus composed of 12 judges.

2.2.2 Tests

Samples were presented at room temperature, in plastic cups coded with three digits containing 20 mL of juices and balanced for position effects. The panel evaluated 12 samples in triplicates in three sessions of three samples per day interrupted by a 10-minute break during four consecutive days. Concentrated juices were reconstituted to 17°Brix by mixing 1mL juice to 3.85 mL of deionised water prior to analysis while commercial juices were served at the natural juice concentration. The judges were asked to clean their mouths with a piece of cracker and mineral water.

2.3 Determination of total phenols

Total phenols were measured by the Folin-Ciocalteu assay (SINGLETON & ROSSI, 1965) using gallic acid (Sigma-Aldrich, St Louis, MO, USA) for the standard curve and the results were expressed in mg gallic acid equivalents/L (GAE). Floating particles were removed by centrifugation and juice samples diluted 1:100 with deionized water, followed by reading of absorbance (in duplicate) at 760 nm in a Beckman spectrometer.

2.4 Determination of total catechins [(+) catechin and (-) epicatechin]

The two monomers were analysed by HPLC with fluorescence detection according to ARTS & HOLLMAN (1998) and ARTS et al., (2000). The standards of (+)-catechin and (-)-epicatechin were obtained from Sigma-Aldrich (St Louis, MO, USA). A Perkin Elmer High Performance Liquid Chromatograph (HPLC) equipped with a 250 mm x 4.6 mm GL Science, Inertisil ODS – 3.5 μm column was used. Analyses were carried out in duplicate. Detection limit was 0.05 mg/L and quantification limits were 1.00 mg/L for (+)-catechin and 2.00 mg/L for (-)-epicatechin.

2.5 Statistical analysis

For the sensory data, the responses on the rating scale were converted to numerical numbers from 0 to 9. Data from the training evaluation was analyzed using a two-way analysis of variance (ANOVA) for samples and judges. As for the sensory tests, the mean attribute ratings were calculated and treated with ANOVA to determine possible statistical differences at a 5% significance level. Tukey's test was chosen to identify statistical separation amongst means since it minimizes Type I errors and the same statistical significance level was applied. Principal component analysis (PCA) was carried out to provide a visual interpretation of the results. To determine possible relationships among the sensory, chemical and instrumental data, Pearson correlation coefficients were applied. Statistical analyses were performed with SAS (Statistical Analysis System, 6.0 - Cary, NC USA).

3. Results and Discussion

3.1 Sensory evaluation

After trials and discussions about the references and descriptors of attributes, panelists agreed on the set of substances and concentrations seen in Table 1. Table 2 shows the intensity scores for the concentrated Concord (CCJ) and Isabel (CIJ) juice.

Table 1. Reference solutions for given attributes used in training sessions.

Attribute	Reference substances	Concentrations	
		Minimum: "none"	Maximum: "very much"
Astringency	Tannic acid	0	3 g/L
Colour	Grape juice	water	Concentrated juice
Bitterness	Caffeine	0	1g/L
Sweetness	Sucrose	0	60 g/L
Characteristic grape juice flavour	Commercial grape juice	diluted 1:4	Sugar added commercial juice

Table 2. Means for the attribute obtained for concentrated Concord (CCJ) and Isabel (CIJ) juices at five aging timepoints.

	CCJ					CIJ				
Months	0	2	4	6	8	0	2	4	6	8
Colour	8.4^{abcd}[a]	8.5^{ab}	8.6^{ab}	8.3^{bcd}	8.7^{a}	8.6^{ab}	8.5^{ab}	8.2^{d}	8.4^{abc}	8.2^{cd}
Sweetness	5.3^{ef}	5.4^{de}	5.8^{cde}	5.1^{e}	6.2^{cd}	7.2^{ab}	7.5^{a}	6.5^{bc}	7.3^{ab}	6.1^{cd}
Adstring.	6.3^{a}	5.7^{ab}	6.0^{ab}	6.2^{a}	5.7^{ab}	4.8^{bc}	3.2^{de}	3.3^{de}	4.2^{cd}	2.8^{e}
Bitterness	4.2^{abc}	4.5^{a}	4.3^{ab}	4.1^{abc}	3.5^{bcd}	3.3^{cde}	2.2^{f}	2.5^{ef}	2.4^{ef}	2.7^{def}
Flavour	5.7^{bc}	5.7^{c}	5.8^{bc}	5.6^{cd}	5.7^{bc}	6.5^{ab}	6.3^{abc}	6.7^{a}	6.6^{a}	6.1^{abc}

[a] Means within the same line followed by the same letter (s) are not significantly different at $p< 0.05$.

The colour of CCJ was not affected by storage time and some alterations were observed for CIJ. Sweetness was stable during aging with differences between cultivars: CIJ was sweeter than CCJ for the first six months, but not at the eighth-month storage point. Astringency and bitterness were the attributes with the

lowest overall scores, with CCJ being more astringent and bitter than CIJ. For the characteristic grape juice flavour no differences were observed during storage, with higher intensities for CIJ with respect to this attribute.

Figure 1 shows a comparison between pasteurized Concord (PCJ) and Isabel (PIJ) juices after eight months of storage under different storage conditions.

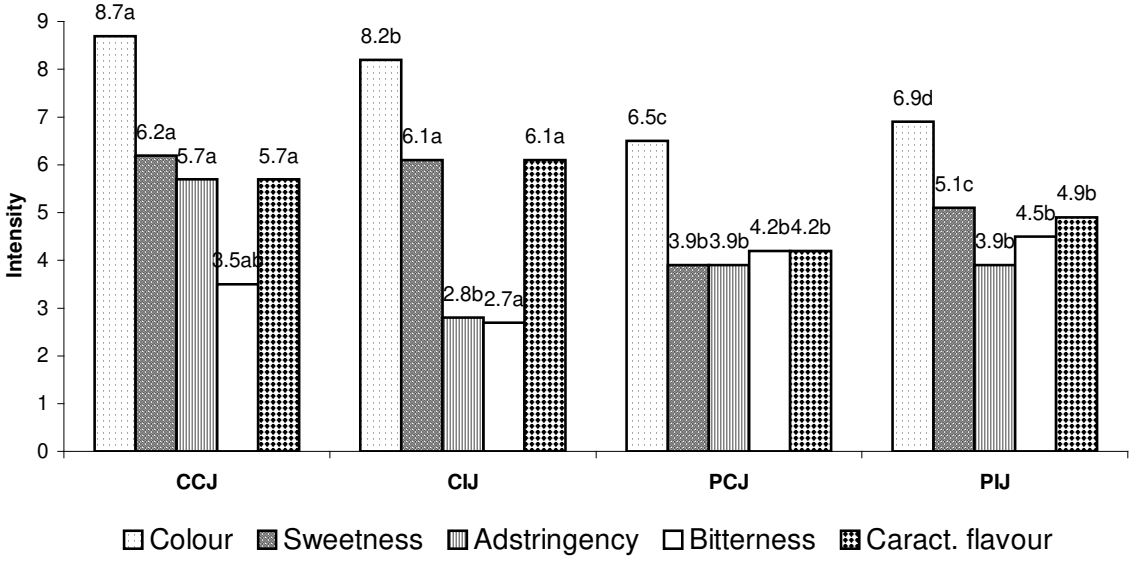

Figure 1. Means for the attributes for concentrated Concord (CCJ) and Isabel (CIJ) juices and pasteurized Concord (PCJ) and Isabel (PIJ) juies after 8 months of storage under different conditions. Different letters under the same legends represent significant differences ($p<0.05$).

Colour was the most intense attribute for all the products, followed by sweetness. The intensities of these two attributes were significantly higher in concentrated juices of both cultivars. CCJ showed the highest intensity of astringency and CIJ the lowest for this attribute and also for bitterness. The sensory panel found concentrated juices significantly more intense in the

characteristic grape juice flavour than pasteurized ones. Concentrated juices stored under refrigeration received higher ratings for desirable attributes such as colour, sweetness and flavour when compared to juices kept in bottles at room temperature.

Sensory ratings were plotted on the principal component analysis (PCA) graph for better visualization of sample distributions (Figure 2).

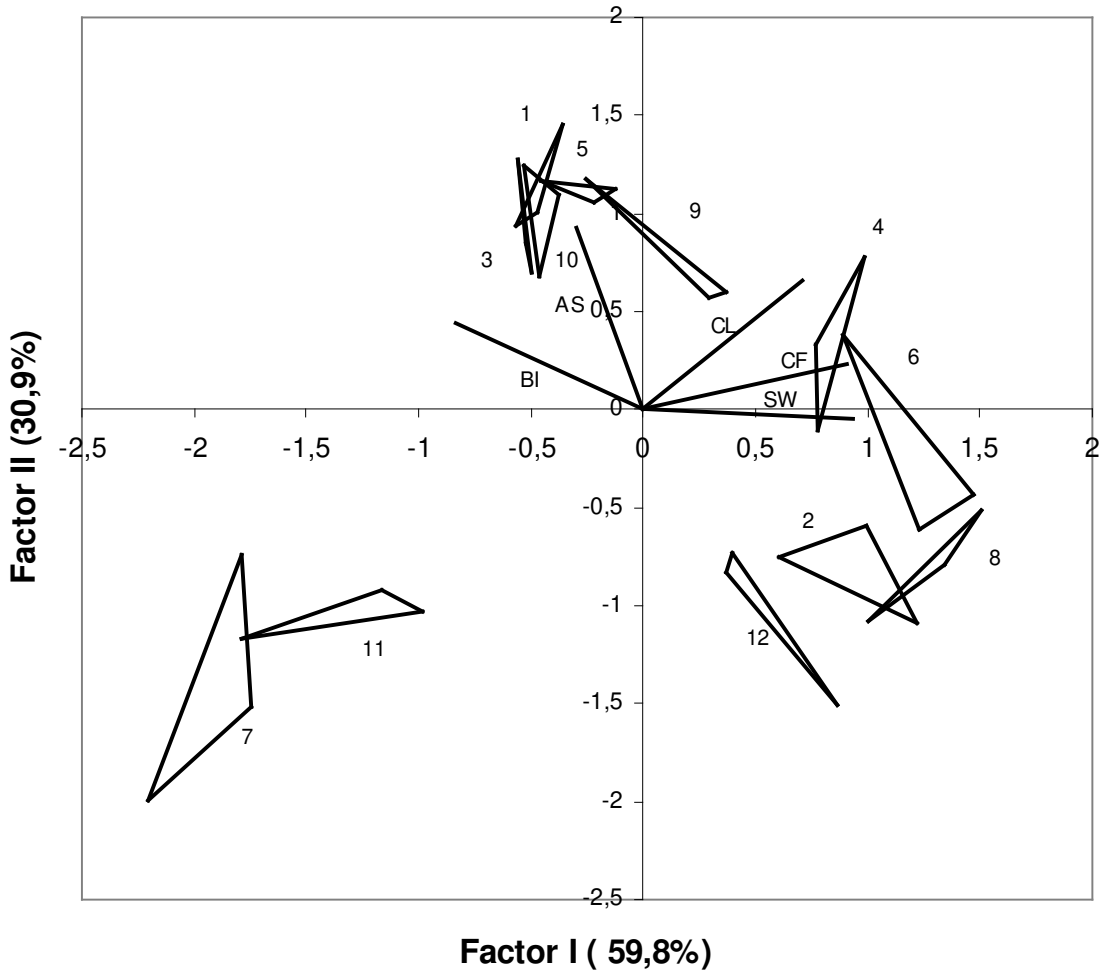

Figure 2. Principal component analysis of the sensory attributes of 12 grape juice samples in relation to sweetness (SW), characteristic grape juice flavour (CF), bitterness (BI), colour (CL) and astringency (AS).

Each juice is represented by a triangle and their vertexes are the means of each repetition. The greater variation amongst samples (axis I) was in relation to sweetness (SW), characteristic grape juice flavour (CF) and bitterness (BI) and to a

lesser extent for colour (CL) and astringency (AS) as represented by axis II. Samples 1,3,5,9 and 10 represent five storage moments of CCJ. Their positions on the PCA indicate uniform sensory aspects, which were mainly characterized by bitterness and astringency. CIJs represented by samples 2,4,6,8 and 12 were less homogeneous and better described by their sweetness and characteristic grape juice flavour. At the bottom-left of the graph samples 7 and 11 of PCJ and PIJ respectively at the 8th-month storage are represented, showing less intensity for the attributes. Figure 2 describes the sensory profiles during storage as follows: i) CCJ characterized by astringency and bitterness stability, and higher colour intensity; ii) CIJ distinguished by sweetness and juice flavour; iii) PCJ and PIJ differentiated by low intensities for the desirable attributes.

3.2 Concentrations of total phenols and catechins during aging

In 8 months, total phenol contents of CCJ showed a slight decline from 2775.0 to 2587.6 mg GAE (93% retention) and of CIJ from 1615.0 to 1428.9 mg GAE (88% retention). Total catechins (sum of isomers) decreased from 44.64 to 26.79 mg/L in CCJ (60% retention) and from 23.14 to 19.62 mg/L in CIJ (85% retention). Variation coefficients between duplicates were lower than 10% in catechins and 5% in total phenol determinations.

Musingo et al. (2001) showed a reduction of catechins during storage of grape juices. Es-Safi; Cheynier & Moutounet (2003) observed a decrease in (+) catechins and the formation of unknown brown products after incubation with glyoxylic acid under accelerated aging. The relationships between chemical and sensory parameters are shown in Table 3.

Table 3. Correlations between sensory and chemical results for CCJ: storage time (ST), (+) catechin (CA), (-) epicatechin (EP); total phenols (TP), colour (CL), sweetness (SW), astringency (AS), bitterness (BI), characteristic flavour (CF).

	ST	CA	EP	TP	CL	SW	AS	BI	CF
ST	1.00								
CA	-0.99[a]	1.00							
EP	-0.91	0.95	1.00						
TP	-0.98	0.94	0.82	1.00					
CL	0.40	-0.31	-0.28	-0.47	1.00				
SW	0.54	-0.47	-0.49	-0.57	0.97	1.00			
AS	-0.40	0.29	0.10	0.57	-0.74	-0.64	1.00		
BI	-0.76	0.80	0.90	0.68	-0.46	-0.64	0.22	1.00	
CF	-0.22	0.31	0.29	0.19	0.67	0.56	-0.25	0.19	1.00

[a] Coefficients are significant at 5% for r≥0.88.

(+)-Catechin was positively related with (-)-epicatechin and total phenols. A positive relation was observed between colour and sweetness, while bitterness correlated positively with (-) epicatechin. Kielhorn & Thorngate (1999) and Thorngate & Noble (1995) demonstrated that oral sensations produced by monomeric flavan-3-ols are preferentially bitter rather than astringent, especially (-)-epicatechin. Total phenols were not significantly related to colour during aging. Colour depicted a slight increase in intensity at the 8th month, whereas phenols depicted a 7% decrease during storage. Enzimatic browning probably caused a decrease in phenolic contents with no effect on the perception of colour intensity due to formation of brown pigments. Siler and Morris (1993) previously demonstrated some degree of browning in Concord grape juice after accelerated aging. Correlations regarding CIJ are shown in Table 4.

Table 4. Correlations between sensory and chemical results for CIJ: storage time (ST), (+) catechin (CA), (-) epicatechin (EP); total phenols (TP), colour (CL), sweetness (SW), astringency (AS), bitterness (BI), characteristic flavour (CF).

	ST	*CA*	*EP*	*TP*	*CL*	*SW*	*AS*	*BI*	*CF*
ST	1.00								
CA	-0.27[a]	1.00							
EP	-0.75	0.76	1.00						
TP	-0.91	-0.08	0.40	1.00					
CL	-0.80	-0.02	0.53	0.73	1.00				
SW	-0.64	0.35	0.60	0.49	0.85	1.00			
AS	-0.58	-0.39	-0.02	0.80	0.73	0.57	1.00		
BI	-0.38	-0.74	-0.25	0.66	0.34	-0.15	0.60	1.00	
CF	-0.33	0.22	0.03	0.48	0.08	0.29	0.53	0.01	1.00

[a] Coefficients are significant at 5% for r≥0.88.

CIJ depicted the differences with respect to (+) catechin: the monomer was not correlated with time or with phenols, due to a peak in substance content after two months storage. Colour intensity in CIJ was also closely related to sweetness. Phenolic compounds showed a weaker relation with bitterness and a stronger one with astringency, though not significant. An explanation for this is the difference in absolute quantities of phenolic compounds in each cultivar: CCJ had almost two times more total phenols than CIJ. The findings agree with Vidal at. al. (2004) demonstrating that polyphenols needed to be present in sufficient amounts or proportions to provoke bitterness or astringency. Colour showed some relation with phenols and stronger negative correlation with storage time, implying in a decline in colour during aging. This observation agrees with the findings of Es-Safi et. al. (2003): using a model assay, the authors demonstrated that high concentrations of phenolics, especially catechins produced more stable coloured compounds due to interactions among them and other substances present in the grapes. Therefore

the reduced phenolic content in CIJ possibly limited the production of brown pigments during aging and affected colour stability.

4. Conclusion

Phenolic-rich beverages are complex matrices and this study revealed important differences in both sensory and chemical aspects of the two grape cultivars. Concentration and refrigeration demonstrated effectiveness as storage conditions for grape juice: desirable attributes such as colour, sweetness and characteristic flavour received higher ratings when compared to pasteurized juices under different aging conditions. Concentrated Concord juice was more stable than Isabel during the storage period. Both cultivars showed significant differences in their sensory attributes as well as in the contents of total catechins and phenols. The knowledge of such characteristics may be useful to juice producers when elaborating blends.

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Capítulo 6: CONCLUSÃO GERAL

Conclusões e Considerações Finais

O suco concentrado de uva é um produto com alto teor de fenólicos e potencial de produção, consumo e exportação pelo Brasil. É uma fonte de antioxidantes para dieta e uso farmacológico / cosmético. Efeitos fisiológicos benéficos em humanos já foram demonstrados, embora os mecanismos e os compostos relacionados à ação ainda não estejam esclarecidos. Alimentos ricos em polifenóis sofrem alterações químicas e sensoriais no processamento e armazenamento.

Os resultados desta tese demonstraram que os compostos fenólicos mais abundantes nos sucos de uva concentrados foram malvidina, piceatanol-O-glicosídeo e flavilium dimetoxilado. Antes do tratamento térmico, o perfil de massa revelou substâncias de baixo peso molecular não identificadas, possivelmente ácidos fenólicos ou produtos de degradação. A peonidina foi o fenólico mais abundante nesta fase, substituída por malvidina e flavilium dimetoxilado nas etapas seguintes, indicando alteração molecular em direção à estabilidade. Ao contrário do encontrado na literatura, o aumento de polifenóis metoxilados não diminuiu a capacidade de seqüestrar radicais livres do suco, que mostrou pouca variação.

Em geral, estudos que mostram decréscimo de antocianinas monoméricas durante o armazenamento o atribuem à condensação e polimerização. Contudo, a caracterização dos compostos dos sucos de uva nesta investigação demonstrou ocorrência de deglicolisação e metoxilação. Poucos estudos revelam a presença

de antocianidinas em produtos de uva, talvez pelo uso de padrões glicosilados e ou pela instabilidade das moléculas.

No espectro de massa, o íon de m/z 293 de identidade não confirmada no capítulo 3, é presumivelmente catequina/epicatequina, que possuem massa molecular idêntica e demonstraram comportamento compatível com o apresentado nos resultados cromatográficos do capítulo 4. Não foram encontradas referências sobre a presença de piceatanol glicosídeo em vinhos tintos ou sucos de uva. A substância foi caraterística do suco de uva Concord após a concentração. Ao relacionar os achados de espectrometria de massa à caracterização sensorial (capítulo 5), vê-se que a variedade de uva Concord apresentou proporção maior de malvidina e menor de hexose, compatível com sua cor mais intensa e menor dulçor.

Neste trabalho, a espectrometria de massa com infusão direta mostrou-se uma técnica eficaz para caracterização do perfil fenólico de sucos de uva. Sua aplicação, embora não quantitativa, dispensa substâncias-padrão como referência, que ocorre em outros métodos. Para certos compostos fenólicos como procianidinas e outros oligômeros a inexistência de referências requer a extração de padrões da própria amostra. Por se tratarem de substâncias altamente reativas, a caracterização de grupos fenólicos deve utilizar uma técnica rápida e que evite extrações com solventes.

Este estudo mostrou que a retenção de fenólicos totais e atividade antioxidante ultrapassou 84% durante o armazenamento dos sucos e que a preservação de catequinas foi mais modesta, independentemente de cultivar e forma de armazenagem. Sucos concentrados apresentaram teores de catequinas

notavelmente superiores, possivelmente devido à extração a quente. Epicatequinas confirmaram possuir reatividade superior a seu isômero e a ausência da substância em sucos pasteurizados se deve possivelmente a sua baixa extração a frio.

Os sucos concentrados demonstraram ser ricos em compostos fenólicos com teores comparáveis a vinhos tintos e chá verde e capacidade similar de seqüestrar radicais livres. O suco pasteurizado da cultivar Isabel com metade do teor de fenólicos mostrou atividade antioxidante apenas 20% menor, sendo o suco com maior taxa de antioxidação por unidade de fenólico. A propósito, este dado colabora na interpretação da atividade antioxidante de alimentos. Sugiro que seja abordado em estudos futuros, pois quantifica a contribuição específica dos fenólicos presentes em cada alimento.

Não foram encontrados outros estudos sobre a estabilidade sensorial de sucos de uva em relação à alteração fenólica. Grande parte das investigações sobre percepções sensoriais associadas a fenólicos é realizada com ensaios modelo. No entanto, alimentos ricos em fenólicos reúnem substâncias complexas e altamente reativas, implicando em interações muitas vezes imprevisíveis e desconhecidas. Assim, a observação dos fenômenos em produtos de consumo é indispensável.

O teste sensorial apontou que não houve relação entre concentração de fenólicos e cor do suco, neste estudo. A redução no teor de fenóis confirma achados anteriores onde o escurecimento enzimático sofrido por estas substâncias contribui para a estabilidade da cor. As relações entre atributos sensoriais e compostos fenólicos foram distintas nas cultivares Concord e Isabel,

demonstrando os efeitos específicos de cada perfil. O alto teor de catequinas na variedade Concord demonstrou maior associação com gosto amargo do que com adstringência, ao contrário da uva Isabel.

Sucos concentrados demonstraram maior intensidade em atributos desejáveis como cor, gosto doce e sabor característico, com especificidades para cada cultivar. A variedade Concord apresentou maior estabilidade ao armazenamento com atributos característicos de adstringência, gosto amargo e intensidade de cor. A uva Isabel foi caracterizada por gosto doce e sabor característico. Portanto, a preparação de "blends" entre estas cultivares é a oportunidade para a indústria unir estabilidade e qualidade sensorial em um produto. O tempo de estocagem não esteve associado à alteração das percepções sensoriais investigadas.

Concluiu-se que os sucos de uva são alimentos com altos teores de fenólicos totais e capacidade antioxidante. O processamento e armazenagem promoveram pouca alteração quantitativa em fenóis e no estado oxidativo dos sucos concentrados de uva Concord e Isabel. Os perfis fenólicos sofreram alterações em ambas situações e diferem entre as cultivares. O armazenamento refrigerado no período de entressafra preservou a qualidade sensorial e a capacidade antioxidante dos sucos.

Anexos: ANEXO I**: Parecer Consubstanciado do Comitê de Ética em Pesquisa com Seres Humanos**

UNIVERSIDADE CATÓLICA DE SANTOS
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24/07/06

UNIVERSIDADE CATÓLICA DE SANTOS
COMITÊ DE ÉTICA EM PESQUISA – COMET

PARECER CONSUBSTANCIADO

Protocolo Geral nº **4096.7.2006**

Interessada: Andréa Pitelli Boiago Golücke

Título do Projeto: *"Estudo sobre o efeito do processamento nos teores de catequinas e atributos sensoriais de sucos de uva".*

Sobre o projeto:

Justificativa: Ressalta-se a importância das catequinas, presente no suco de uva, para a saúde dos seres humanos.
Objetivos: Quantificar catequinas [(+)-catequina, (-)-epicatequina] em suco de uva produzido em larga escala (concentrado) durante o período de estocagem.
Avaliar determinados atributos sensoriais do suco de uva concentrado.
Desenho e Metodologia: A partir de amostras, realizar-se-ão análises quantitativas, a partir do método de Arts e Hollman (1998) e adaptado por Arts (2000). As análises sensoriais serão efetuadas pelo método de Análise Descritiva Quantitativa (ADQ), de Stone e Sidel (2004), por uma equipe treinada
Avaliação dos riscos e benefícios da pesquisa: O projeto apresenta cuidados com a saúde e o bem estar dos consumidores do suco de uva, tanto na população como com os voluntários da pesquisa. Apresenta o Termo de Consentimento Livre e Esclarecido (TCLE), com os requisitos estabelecidos na Resolução 196/96, com a referência a inexistência de riscos e a presença de desconforto mínimo (persistência do gosto do suco de uva, após o teste). Foram anexados os documentos exigidos pelo COMET.
O Parecer:

Baseado nas normas da Resolução 196/96 (Ministério da Saúde) e com todos os documentos exigidos anexados, o colegiado do COMET considera o presente protocolo de pesquisa APROVADO.

Santos, 15 de julho de 2006

Prof. Dr.Catulo César Pestana de Barros Magalhães
Presidente do COMET - UNISANTOS