VANESSA RIOS DE SOUZA

COMPOSTOS BIOATIVOS E O PROCESSAMENTO DE PEQUENAS FRUTAS VERMELHAS CULTIVADAS EM CLIMA SUBTROPICAL

LAVRAS – MG

2013

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós Graduação em Ciência dos Alimentos, para obtenção do título de Doutor.

Orientadora

Dr. Fabiana Queiroz

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Ficha Catalográfica Elaborada pela Coordenadoria de Produtos e Serviços da Biblioteca Universitária da UFLA

Souza, Vanessa Rios de.
Compostos bioativos e o processamento de pequenas frutas vermelhas cultivadas em clima subtropical / Vanessa Rios de Souza. – Lavras : UFLA, 2013.
195 p. : il.

Tese (doutorado) – Universidade Federal de Lavras, 2013.
Orientador: Fabiana Queiroz.
Bibliografia.

1. Morango. 2. Mirtilo. 3. Amora preta. 4. Framboesa. 5. Cereja 6. Pequenas frutas – Compostos bioativos. I. Universidade Federal de Lavras. II. Título.

CDD – 664.8047

VANESSA RIOS DE SOUZA

COMPOSTOS BIOATIVOS E O PROCESSAMENTO DE PEQUENAS FRUTAS VERMELHAS CULTIVADAS EM CLIMA SUBTROPICAL

(**BIOACTIVE COMPOUNDS AND THE PROCESSING OF SMALL RED FRUITS CULTURED IN SUBTROPICAL CLIMATE**)

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós Graduação em Ciência dos Alimentos, para obtenção do título de Doutor.

APROVADA em 02 de outubro de 2013.

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AGRADECIMENTOS

À Universidade Federal de Lavras e ao Departamento de Ciência dos Alimentos pela oportunidade e contribuição para a minha educação.

Ao CNPq pelo fornecimento da bolsa de estudos.

Ao Lucas, meu noivo, por todo o apoio e companheirismo, além da grande ajuda na preparação das polpas de frutas e geleias. Ao meu pai pela ajuda, amor, reconhecimento, força e incentivo para que eu siga o meu destino, minha mãe e meu irmão por toda a dedicação e carinho. Obrigada a todos pelo apoio e pela confiança depositada!

A todos os meus professores que marcaram de alguma forma a minha jornada. Em especial a minha orientadora Fabiana Queiroz, obrigada pela orientação, ensinamentos e compreensão.

À Ana Carla Marques Pinheiro, agradeço por todo apoio e auxílio incondicional em todos os momentos.

Ao Prof. Luiz Carlos de Oliveira Lima e Prof. Rafael Pio, agradeço imensamente pelo apoio no projeto e ajuda na aquisição das frutas. A ajuda de vocês foi fundamental para a realização deste trabalho.

A todos os professores mencionados e ao Pesquisador Ângelo Albérico Alvarenga pela participação da defesa e valiosos conselhos.

Aos colegas da "Planta Piloto de Processamento de Produtos de Origem Vegetal". Um agradecimento especial a Patty, Thaís, Taísa, Andressa, Jéssica, Dani e demais estagiárias pela ajuda incondicional na execução deste trabalho.

À Camila, agradeço pelos valiosos conselhos e ensinamentos. Obrigada pelo carinho.

A toda a minha família e amigos que me ajudaram de alguma forma ou torceram por mim.

Finalmente, agradeço a Deus por tudo, mas principalmente por colocar em minha vida pessoas tão especiais!

RESUMO

As pequenas frutas vermelhas, bagas ou *berries*, vêm sendo tema de diversos trabalhos devido ao alto teor de compostos fenólicos e alta capacidade antioxidante em sua composição, apresentando um importante efeito de proteção contra doenças degenerativas. O objetivo deste trabalho foi caracterizar e determinar os compostos bioativos do morango (*Fragaria* x *ananassa)*, mirtilo (*Vaccinium corymbosum*), amora preta (*Rubus* spp), framboesa (*Rubus idaeus*) e cereja (*Prunus avium* L.); desenvolver uma geleia mista de framboesa amarela, vermelha e negra; avaliar a influência da cultivar de amora preta nas características físico-qímicas, no perfil de textura e na aceitação sensorial da geleia e avaliar a degradação dos compostos bioativos no processamento de geleias elaboradas com diferentes cultivares de amora preta. A composição e teor de compostos bioativos das pequenas frutas vermelhas cultivadas em clima subtropical, no geral, foram semelhantes às frutas de climas temperados, apresentando ainda maiores teores de vitamina C. Em relação ao processamento, percebeu-se que é viável a elaboração de geleia mista de framboesa, sendo que a proporção considerada ideal é: 0-30% de framboesa amarela, 25-50% de framboesa negra e 30-75% de framboesa vermelha. Já no processamento de amora preta, conclui-se, com base na aceitação e viabilidade econômica, que as cultivares mais adequadas para a elaboração de geleia são Tupy, Comanche, Brazos, Guarani e Choctaw. Foi verificado também que há degradação dos compostos bioativos e redução da atividade antioxidante devido ao processamento das geleias de amora preta, sendo que a degradação desses compostos foi significativante diferente entre as cultivares. Destacam-se as cultivares Brazos e Caingangue como as que sofrem as menores perdas com o processamento, dando origem a geleias mais ricas em compostos bioativos e com maior capacidade antioxidante.

Palavras-chave: Morango. Mirtilo. Amora preta. Framboesa. Cereja

ABSTRACT

The small red fruits, berries or berries, have been the subject of several studies due to the high content of phenolic compounds and high antioxidant capacity in its composition, presenting a significant protective effect against degenerative diseases. The aim of this study was to characterize and determine the bioactive compounds of strawberry (*Fragaria x ananassa*), blueberry (*Vaccinium corymbosum*), blackberry (*Rubus* spp), raspberry (*Rubus idaeus*) and sweet cherry (*Prunus avium* L.), developing a mixed yellow, red and black raspberry jelly; evaluate the influence of blackberry cultivar on the physicalchemical, texture profile and sensory acceptance of jelly and evaluate the degradation of bioactive compounds in the processing of jellies prepared with different blackberry cultivars. The composition and content of bioactive compounds of small red fruit grown in subtropical climate, in general, were similar to the fruits of temperate climates, with even higher levels of vitamin C. Regarding processing, realized that it is feasible to compile mixed raspberry jam, and the ratio considered ideal is: 0-30% of yellow raspberry, 25-50% of black raspberry and 30-75% of red raspberry. In the processing of blackberry, it is concluded, based on the acceptance and economic viability, the cultivars most suitable for the preparation of jam are Tupy, Comanche, Brazos, Guarani and Choctaw. It was also verified degradation of bioactive compounds and reduction of antioxidant activity due to processing of blackberry jelly, and the degradation of these compounds was significativante different among cultivars. Noteworthy are the cultivars Brazos and Caingangue as suffering the smallest losses in processing, resulting in jams richer in bioactive compounds and higher antioxidant capacity.

Keywords: Strawberry. Blueberry. Blackberry. Raspberry. Cherry

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PRIMEIRA PARTE

1 INTRODUÇÃO

De acordo com Chang et al. (2006) e Kubota et al. (2012) pequenas frutas vermelhas, bagas ou *berries*, são frutos diminutos carnosos e suculentos, normalmente consumidos frescos ou em produtos como geleia, suco, doces, bebidas fermentadas e xarope. Entre as pequenas frutas vermelhas incluem a amora preta (*Rubus* spp.), framboesa vermelha (*Rubus idaeus*), mirtilo (*Vaccinium corymbosum*), morango (*Fragaria x ananassa*) e cereja (*Prunus avium* L.) (SEERAM, 2008). Esses frutos são oriundos, predominantemente, de regiões de clima temperado, mas com os avanços no melhoramento genético e a aprimoração do manejo cultural, algumas cultivares têm sido adaptadas a outras regiões e podem ser cultivadas em regiões com temperaturas mais elevadas no outono e inverno (PINTO et al., 2008).

As frutas, especialmente as vermelhas e azuis-escuras vem sendo alvo de vários estudos por serem excelentes fontes de compostos bioativos (CARLSEN et al., 2010; PANTELIDIS et al., 2007), apresentando benefícios adicionais à saúde (BEATTIE; CROZIER; DUTHIE, 2005; SAMEC et al., 2011; SERRA et al., 2011). A proteção contra doenças degenerativas, como câncer e doenças cardiovasculares que os vegetais desempenham nos seres humanos, está associada com uma variedade de componentes nutrientes e não nutriente, sendo muitos deles caracterizados por suas propriedades antioxidantes (ACOSTA MONTOYA et al., 2010; ALMEIDA et al., 2011; MANACH et al., 2005; PIETTA, 2000).

Os pequenos frutos vermelhos estão entre as fontes mais importantes de compostos fenólicos para as dietas humanas (HAKKINEN et al., 1999a; HAKKINEN et al., 1999b; HAKKINEN; TORRONEN, 2000). Segundo Kubota et al. (2012) as *berries* são ricas em compostos fenólicos, como os ácidos fenólicos, taninos, estilbenos, flavonoides e antocianinas, mas essas bagas, em particular, têm sido o foco de pesquisas em relação às suas propriedades ricas em antocianinas.

Embora já seja bem estabelecido que as pequenas frutas vermelhas sejam fontes de compostos bioativos, como polifenois e antocianinas, esses estudos são principalmente focados em frutos cultivados em climas temperados - Europa, Ásia e América do Norte (CHEN et al., 2013). Sabendo-se que a composição dos frutos varia com uma série de fatores – espécie, cultivar, manejo cultural, região, condições meteorológicas, maturação, tempo de colheita e as condições de armazenagem (AHERNE; O'BRIEN, 2002; HAFFNER et al., 2002; RICKMAN; BARRETT; BRUHN, 2007) - é extremamente relevante a caracterização e comparação de frutas produzidas em climas tropicais e subtropicais com frutas tradicionais de clima temperado. Maro (2011) em um estudo comparativo das propriedades químicas de framboesas produzidas em duas regiões com diferentes características climáticas encontraram diferença nos teores de sólidos solúveis e acidez da fruta. Em estudos anteriores, Acosta-Montoya et al. (2010), Mertz et al. (2007) e Szajdek e Borowska (2008) em comparação com amoras pretas de climas temperados, concluíram que as amoras pretas montanhosas tropicais apresentam propriedades nutricionais e funcionais de grande interesse para os mercados internacionais. Em outro estudo, o conteúdo de fenólicos e de antocianina da cereja foram maiores em anos de climas mais amenos do que nos anos mais frios (GONÇALVES et al., 2004).

No Brasil, a principal fruta vermelha produzida e consumida é o morango (PINELI, 2009). Porém, o cultivo de framboesas e amoras pretas, por exemplo, vem aumentando de forma constante, especialmente em áreas subtropicais dos estados de São Paulo e Minas Gerais, onde as temperaturas são maiores no outono/inverno e principalmente no verão (MARO et al., 2012). Os resultados evidenciam que as amoras pretas produzem grandes quantidades de frutos em áreas subtropicais, com algumas cultivares produzindo quantidades mais elevadas em comparação com as zonas temperadas (CAMPAGNOLO; PIO, 2012). No caso de framboesa, os resultados de desempenho produtivo de áreas subtropicais do Brasil são muito animadores, pois a produção de framboesas é constante ao longo do ano, com cultivares que produzem grandes quantidades de frutas do inverno ao outono (MOURA et al., 2012) . No Brasil, o mirtilo ainda é um fruto ainda pouco conhecido, mas com elevado valor agregado e potencial produtivo, sendo cultivado especialmente nos estados de Rio Grande do Sul, Minas Gerais e São Paulo. Assim como o mirtilo, a cereja (*Prunus avium* L.), é um fruto ainda pouco conhecido e ainda não muito cultivado no Brasil.

Devido à perecibilidade dessas frutas vermelhas (BOWER, 2007; YOUSEFI; YOUSEFI; EMAM-DJOMEH, 2013) e devido à limitada produção no Brasil (áreas subtropicais), o processamento das mesmas se torna uma importante forma de aumentar a disponibilidade e agregar ainda mais valor a esses frutos. De acordo com Byamukama et al. (2005) exceto que uma pequena quantidade que é consumida fresca ou congelada, o consumo dessas frutas se dá principalmente na forma de seus subprodutos como compotas, doces, geleias, xaropes e bebidas fermentadas. Como já é consagrada a importância nutricional dessas frutas vermelhas, em especial devido à alta capacidade antioxidante, alto teor de compostos fenólicos como as antocianinas, entre outros, a degradação dos compostos bioativos com o processamento é de fundamental importância, e vem sendo alvo de diversos estudos (GANCEL et al., 2011; MOTA, 2006a; MOTA, 2006b; PATRAS et al., 2009; WU et al., 2010).

O objetivo deste trabalho foi caracterizar e determinar os compostos bioativos do morango (*Fragaria* x *ananassa*), mirtilo (*Vaccinium corymbosum*), amora preta (*Rubus* spp), framboesa (*Rubus idaeus*) e cereja (*Prunus avium* L.) (Artigo 1); desenvolver uma geleia mista de framboesa amarela, vermelha e negra (Artigo 2); avaliar a influência da cultivar de amora preta nas características físico-qímicas, no perfil de textura e na aceitação sensorial da geleia (Artigo 3); e avaliar a degradação dos compostos bioativos no processamento de geleias elaboradas com diferentes cultivares de amora preta (Artigo 4).

2 REFENCIAL TEÓRICO

2.1 Frutas vermelhas

As pequenas frutas vermelhas são pequenos frutos carnosos e suculentos normalmente consumidos frescos ou em produtos como geleia, suco, doces, bebidas fermentadas e xarope (CHANG et al., 2006; KUBOTA et al., 2012). Entre as pequenas frutas vermelhas incluem a amora preta (*Rubus* spp.), framboesa vermelha (*Rubus idaeus*), mirtilo (*Vaccinium corymbosum*), morango (*Fragaria x ananassa*) e cereja (*Prunus avium* L.) (SEERAM, 2008).

Existem vários estudos que afirmam que a ingestão dessas frutas vermelhas tem um impacto positivo sobre a saúde humana, atuando na prevenção de diversas doenças (SEERAM, 2008). A maior parte dos benefícios para a saúde desses frutos é acreditada devido a seus compostos bioativos (BEEKWILDER; HALL; DEVOS, 2005). Essas frutas são ricas em compostos fenólicos, como os ácidos fenólicos, taninos, estilbenos, flavonoides e antocianinas, mas em particular, as bagas têm sido o foco de muitas pesquisas devido ao rico teor de antocianina (KUBOTA et al., 2012).

2.1.1 Morango

O morangueiro, (*Fragaria* spp), é uma planta da família *Rosaceae*, Ordem *Rosales*, subfamília *Rosideae*, tribo *Potentillae*, gênero *Fragaria* L. (HUTCHINSON, 1978; VIDAL; VIDAL, 2003). Há mais de 20 espécies do gênero Fragaria com o nome comum de morangueiro (DARROW, 1966) e diversas cultivares produzidas no Brasil (SANTOS, 2005). O fruto, a parte comestível do morangueiro, carnosa, suculenta, de cor rosada ou vermelha, conhecida vulgarmente como “morango”, envolve, de fato, os verdadeiros frutos, que são os pequenos prontos amarelos ou avermelhados, diminutos, superficiais (LOPES, 2005) (Figura 1a). O morango é um fruto não climatérico de consumo humano frequente (AVIGDORI-AVIDOV, 1986).

O início do cultivo do morangueiro no Brasil, segundo Camargo e Passos (1993) não é bem conhecido, entretanto, a cultura começou a se expandir desde a década de 1960 (PASSOS; TRADI, 1997). A produção de morangos no Brasil tem crescido nos últimos anos; estimando-se uma produção anual de 100 mil toneladas, com área ocupada de 3.500 ha. No Brasil, a cultura do morangueiro encontra-se difundida em regiões de clima temperado e subtropical (OLIVEIRA; NINO; SCIVITTARO, 2005), sendo sua produção concentrada nas regiões Sul e Sudeste, sendo os estados de Minas Gerais, São Paulo e Rio Grande do Sul os maiores produtores. O morangueiro apresenta grande importância econômica na comercialização de frutas tanto para seu consumo in natura como para industrialização, destacando a produção de geleias, sorvetes, balas, sucos e principalmente iogurte (SANTOS, 1993).

Ao lado da cor e sabor atrativos, o morango é também uma boa fonte de vitamina C e outros compostos antioxidantes, tais como flavonoides e outros fenólicos (ROBARDS et al., 1999). São desse modo, frutas conhecidas pelo alto teor de compostos bioativos, e de acordo com Hannum (2004), os morangos são importantes fontes de compostos fenólicos, sendo os principais, o ácido elágico e alguns flavonoides, como as antocianinas, a catequina, a quercetina e o Kaempferol. Devido essas propriedades, o consumo de morango exerce efeito antioxidante, anti-inflamatório, anticarcinogênico e antioneurodegenerativo (HANNUM, 2004).

2.1.2 Mirtilo

O mirtilo (ou "*arándano*", em espanhol, e "*blueberry*", em inglês) pertence à família das *Ericaceae*, subfamília *Vaccionoidear*, gênero *Vaccinium*, sendo nativo da América do Norte, onde é bastante cultivado e comercializado (SANTOS; RASEIRA, 2002; FACHINELLO, 2008). No Brasil, é um fruto ainda pouco conhecido, mas com elevado valor agregado e potencial produtivo. As primeiras plantas foram trazidas em 1980 pela EMBRAPA - Clima Temperado (Pelotas - RS), para avaliação de cultivares e a primeira iniciativa comercial no País começou a partir de 1990, em Vacaria – RS (HOFFMAN; ANTUNES, 2004). Assim como o estado do Rio Grande do Sul, os estados de Minas Gerais e São Paulo também possuem um potencial, porém reduzido devido ao menor acúmulo de frio hibernal, essencial para seu desenvolvimento (RASEIRA; ANTUNES, 2004).

Os frutos do mirtilo caracterizam-se por sua coloração azul-escura e formato achatado, tendo cerca de 1,0 a 2,5 cm de diâmetro e de 1,5 a 4,0 g de peso (REQUE, 2012) (Figura 1b). Possuem inúmeras pequenas sementes em seu interior e sabor doce-ácido a ácido. Podem ser consumidas tanto in natura ou após o seu processamento por congelamento, desidratação e enlatamento, quanto utilizados na fabricação de sorvetes, bolos, tortas, geleias, licores, sucos, entre outros (FACHINELLO, 2008).

De acordo com Moraes et al. (2007), do grupo das pequenas frutas que abrange, entre outras, as culturas de morango, framboesa, mirtilo e amora preta, o mirtilo é classificado como a fruta fresca mais rica em antioxidante já estudada, tendo um conteúdo elevado de polifenóis tanto na casca quanto na polpa. É uma fruta que têm chamado atenção devido à sua alta capacidade antioxidante e elevada concentração de antocianinas e outros compostos fenólicos, que rotula a fruta como um das mais desejáveis e nutritivas entre frutas e vegetais frescos (PRIOR et al., 1998). Além das antocianinas, os mirtilos também são uma boa fonte de ácido clorogênico, quercetina, kaempferol, miricetina, procianidinas, catequina, epicatequina, resveratrol e vitamina C que contribuem para a atividade antioxidante (GIOVANELLI; BURATTI, 2008).

2.1.3 Amora preta

A amoreira-preta pertencente à família *Rosaceae* e ao gênero *Rubus*, é uma espécie arbustiva nativa da Asia, Europa e América, bem adaptada a regiões com inverno bem definido (MOORE, 1984). O fruto verdadeiro da amoreira é denominado de minidrupa ou drupete, no qual existe uma pequena semente, sendo que a sua junção forma o que é chamado de fruto agregado (HIRSCH et al. 2012; POLING, 1996). As frutas são compostas, normalmente formadas por 75 a 85 drupetes com cerca de quatro a sete gramas (PAGOT et al., 2007). É do tipo globoso, cheio, carnoso e apresenta coloração negra quando maduro e sabor doce a doce-ácido, sendo que sua maturação ocorre entre a primavera e o verão (CURI, 2012) (Figura 1c).

No Brasil, a amora-preta foi introduzida em 1950 pela Estação Experimental de Pelotas, atual Embrapa Clima Temperado, no Rio Grande do Sul, mas somente na década de 70 houve crescendo do cultivo nos estados do Rio Grande do Sul, São Paulo e Minas Gerais, com a introdução e seleção de novas cultivares (ANTUNES, 2002). As amoras pretas estão disponíveis na forma fresca (in natura) e também congeladas e processadas termicamente na forma de geleias, sucos, polpa, entre outros produtos (ANTUNES, 2002; MOTA, 2006b).

Existem inúmeras cultivares de amoreira-preta, mas as selecionadas no Brasil são: Tupy, Guarani, Negrita, Caingangue, Brazos, Cheroke, Comanche e

Ébano (ANTUNES, 2002; CURI, 2012). As cultivares apresentam diferentes características, como diferenciada produtividade (CAMPGNOLO; PIO, 2012) e consequentemente, diferentes utilizações. As cultivares Tupy e Caigangue são recomendadas para o consumo in natura pelo fato de apresentarem baixa acidez, já a Guarani é recomendada para industrialização (SANTOS; RASEIRA, 1988).

O interesse em amoras pretas aumentou nos últimos anos, devido, em parte, sua alta concentração de compostos fenólicos, como as antocianinas e alta capacidade antioxidante, o que pode ajudar a proteger contra doenças degenerativas (BROWNMILLER; HOWARD; PRIOR, 2008; FERREIRA; ROSSO; MERCADANTE, 2010; JACQUES et al., 2010; SIRIWOHARN; WROLSTAD, 2004). Além disso, vários outros pigmentos naturais, principalmente antocianina, são usados na fabricação de produtos láteos, doces e geleias (ANTUNES, 2002).

2.1.4 Framboesa

A framboeseira, pertencente à família *Rosaceae*, gênero *Rubus*es ubgênero *Idaeobatus* é originária do norte da Ásia e Europa Oriental (ALCAYAGA, 2009). Ainda pouco cultivada no Brasil, a framboeseira (*Rubus idaeus* L.) é uma frutífera que tem despertado o interesse pelos produtores (MARO, 2011). Apesar de ter sido introduzida na década de 50 em Campos do Jordão-SP (PAGOT, 2004), as maiores áreas em cultivo encontram-se na região do município de Vacaria-RS. No entanto, em anos recentes, foram iniciados alguns plantios na Serra da Mantiqueira, tanto em São Paulo como no sul de Minas Gerais (GONÇALVES et al., 2011; MARO, 2011).

A framboeseira produz frutos agregados de tamanho diminuto, com coloração variada, e se adapta bem às regiões de inverno ameno e com temperaturas moderadas (JIN et al., 1999; MOYER et al., 2002). O fruto é constituído por muitas drupetes e uma cavidade no centro (CHANJIRAKUL et al., 2006) (Figura 1d). De acordo com as espécies e as cultivares, a coloração dos frutos varia do amarelo ao preto, incluindo os tons alaranjados, rosa, vermelho claro, intenso e púrpuro (SOUSA et al., 2007). Além das framboesas vermelhas e amarelas, pertencentes à espécie R. *idaeus*, comumente encontradas nas gôndolas de supermercados, cabe destacar a framboesa-negra (R. *niveus* Thunberg), também conhecida como *raspberry□de□mysore* e *raspberry□do□morro*.

Exceto que uma pequena quantidade de framboesas que são consumidas frescas ou congeladas, a maioria dos frutos é processada em produtos como doces, compotas, geleias, xaropes e bebidas fermentadas (BYAMUKAMA et al., 2005).

As framboesas (*Rubus idaeus* L.) são largamente consumidas, tanto frescas como processadas, não só devido às suas cores brilhantes e sabor característico, mas também devido ao seu potencial benéfico à saúde (CARVALHO; FRASER; MARTENS, 2013). As framboesas lideram o topo da lista de frutos com alto poder antioxidante, em especial devido aos altos níveis de antocianinas, flavonoides e ácidos fenólicos, sendo consideradas uma boa fonte de antioxidantes naturais, que podem fornecer proteção contra várias doenças humanas causadas por stress oxidativo (BEEKWILDER; HALL; DEVOS, 2005; KÄHKÖNEN et al., 2001; WANG; LIN, 1999). As framboesas vermelhas são conhecidas por demonstrar a capacidade antioxidante forte, principalmente como resultado de seus altos níveis de antocianinas e outros compostos fenólicos (KAFKAS et al., 2008; KAHKONEN; HOPIA; HEINONEN, 2001).

2.1.5 Cereja

A cereja, (*Prunus spp*), planta da família *Rosaceae*, ordem *Rosales*, subfamília *Rosideae* e gênero *Prunus* é um dos mais populares frutos de clima temperado, e em numerosas áreas de produção as cerejas frescas são as primeiras frutas da época, consumidas principalmente não processadas (USENIK; FABCIC; STAMPAR, 2008). Os frutos são drupas com 9-12 mm, globosos, vermelho-escuro (também amarelada, vermelho-vivo ou negra conforme as cultivares), sobor doce; endocarpo liso (Figura 1e). A distribuição da cerejeira está em quase toda a Europa, oeste da Ásia e noroeste de África (CRESPI; CASTRO; BERNARDOS, 2005a; CRESPI; CASTRO; BERNARDOS, 2005b; FRANCO, 1971).

A cereja é um fruto particularmente atrativo para o consumidor pelos seus atributos cromáticos e aromáticos, bem como pela riqueza em alguns nutrientes com um forte impacto no bem-estar humano (GONÇALVES, 2006). Além de vários componentes essenciais na dieta, tais como vitaminas, minerais, proteínas e carboidratos, as cerejas também contêm fitonutrientes que podem proporcionar benefícios, além da prevenção de deficiências nutricionais (FANIADIS; DROGOUDI; VASILAKAKIS, 2010). O consumo de cerejas doces ou amargas está relacionado com a redução do risco de câncer (KANG et al., 2003), dor de artrite e de inflamação (JACOB et al, 2003; SEERAM et al., 2002.), de sintomas de dano muscular por exercício induzido (CONNOLLY et al., 2006), o *stress* oxidativo em pessoas mais velhas (TRAUSTADÓTTIR et al., 2009), e oferecem proteção contra doenças neurodegenerativas (KIM et al., 2005). Os efeitos benéficos das cerejas podem ser atribuídos à presença de compostos fenólicos tais como antocianinas e melatonina que exercem capacidade antioxidante potente (BURKHARDT et al., 2001; SEERAM et al, 2002; VINSON et al., 2001).

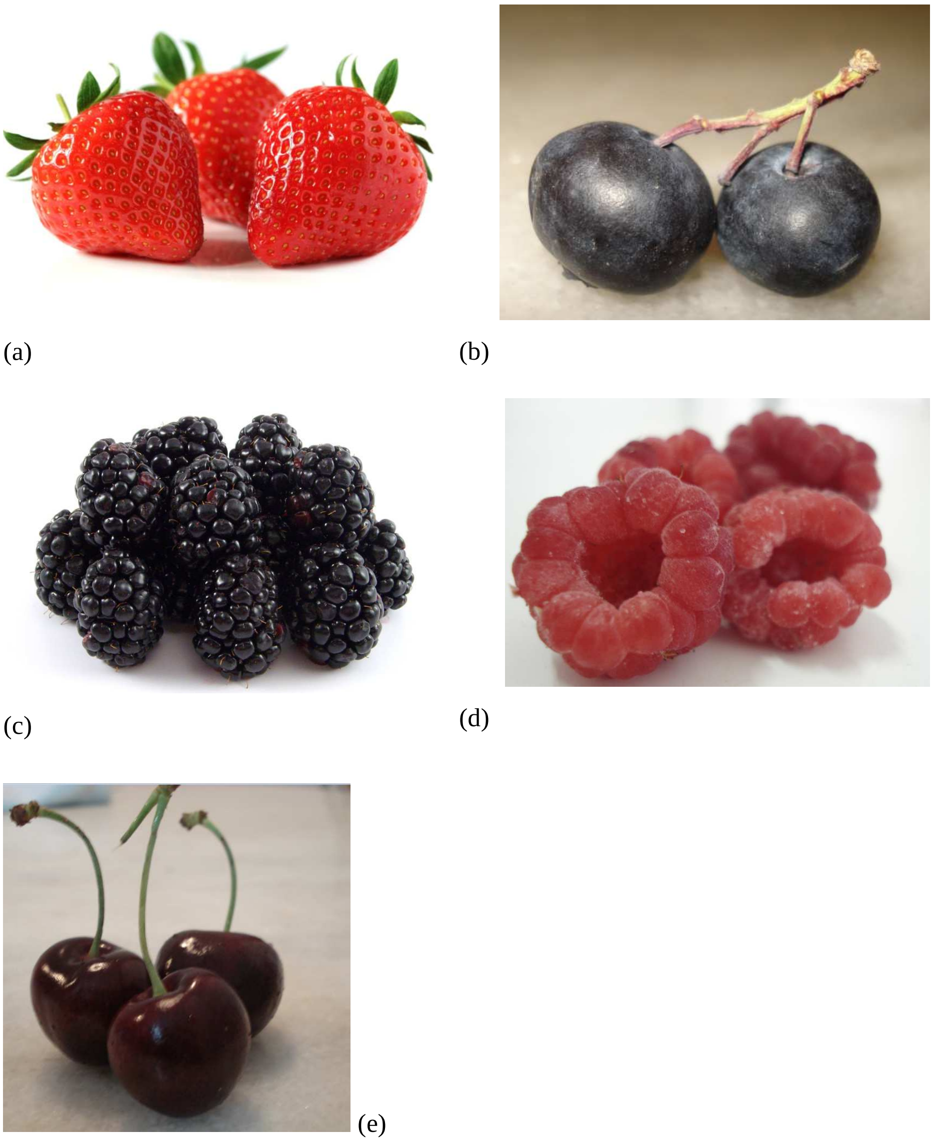

Figura 1 Frutas vermehas: morango (*Fragaria x ananassa*) (a); mirtilo (*Vaccinium corymbosum*) (b); amora preta (*Rubus* spp) (c); framboesa vermelha (*Rubus idaeus* L.) (d) e cereja (*Prunus avium* L.) (e)

Fonte: O VERDADEIRO... (2013); FREE IMAGES (2013)

2.2 Compostos Bioativos

Os alimentos funcionais se caracterizam por oferecer vários benefícios à saúde, além do valor nutritivo inerente à sua composição química, podendo desempenhar um papel potencialmente benéfico na redução do risco de doenças crônicas degenerativas (NEUMANN et al., 2002; TAIPINA; FONTS; COHEN, 2002). Segundo Moraes e Colla (2006), o papel desses alimentos em relação às doenças estará, na maioria dos casos, concentrado mais na redução dos riscos do que na prevenção.

Dentre os alimentos naturais que são considerados funcionais por suas ações benéficas no organismo humano têm-se os grãos de cereais e leguminosas, frutas e hortaliças, peixes, leite e seus derivados chás preto e verde e vinho (SCARBIERI; PACHECO, 1999). De acordo com Lajolo (2005), as substâncias ativas presentes nos alimentos, responsáveis por tais ações biológicas, são chamadas de fitoquímicos ou compostos bioativos. Exemplos desses componentes são os flavonoides (como quercetina, catequina, genisteína e antocianinas), carotenoides (licopeno, luteína, zeaxantina e β-caroteno), fotosteróis e ácidos graxos.

As "substâncias" ou "compostos bioativos" são constituintes extranutricionais, que ocorrem, tipicamente, em pequena quantidade, em alimentos de origem vegetal, com atividades biológicas ditas promotoras da saúde, tais como atividades antioxidantes, anti-inflamatória e hipocolesterolêmica (KRIS-ETHERTON et al., 2002; PINTO, 2008). A Figura 2 representa as principais classes de compostos bioativos presentes nos alimentos de origem vegetal.

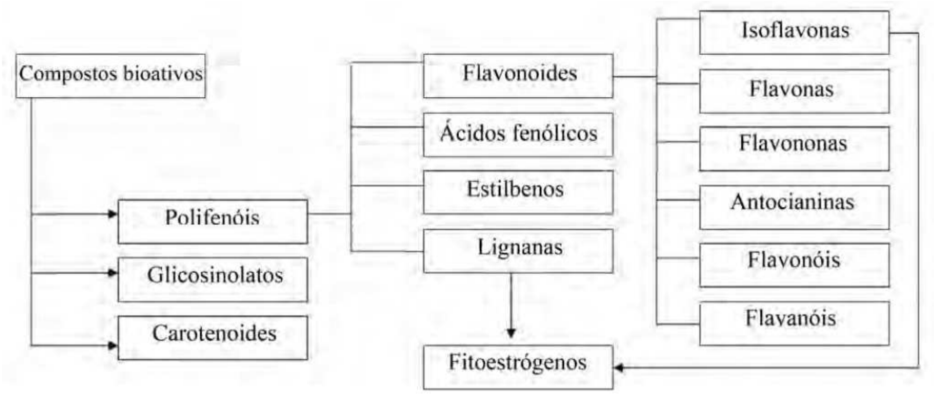

Figura 2 Subdivisão dos compostos bioativos presentes em alimentos de origem vegetal
Fonte: Horst e Lajolo (2007)

O termo “composto fenólico” abrange uma grande variedade de compostos químicos cujo aspecto estrutural comum é a presença, nas suas moléculas, de pelo menos um grupo hidroxila ligado diretamente a um anel aromático (CROTEAU; KUTCHAN; LEWIS, 2000; SHAHIDI; NACZK, 1995a; SHAHIDI; NACZK, 1995b). Esses compostos representam a maior categoria de agentes fotoquímicos e encontram-se amplamente distribuídos no reino vegetal. Eles são derivados das vias do ácido chiquímico e acetato-malonato (PINTO, 2008). Existe uma grande variabilidade na estrutura e ocorrência das substâncias de natureza fenólica, que inclui desde fenólicos muito simples, como os ácidos hidroxibenzoicos, até estruturas mais complexas, como os taninos condensados ou hidrolisáveis de elevado peso molecular (TOMÁS-BARBERÁN; ESPÍN, 2001), sendo basicamente divididos em classes; os flavonoides (que englobam antocianinas e flavonóis), os ácidos fenólicos (derivados de ácidos cinâmico e benzoico), os estilbenos (resveratrol) e uma larga variedade de polifenóis (taninos) (FRANCIS, 2000). Os compostos fenólicos naturais são metabolitos secundários e uma das principais classes de antioxidantes encontrados naturalmente em todas as plantas, e frequentemente em altas concentrações em frutas e legumes (YOU et al., 2011).

Os flavonoides constituem um grupo de pigmentos vegetais de ampla distribuição na natureza, e sua presença nos vegetais parece estar associada a funções de defesa e atração (SANTOS, 2007). Os flavonoides são compostos de baixo peso molecular, com estrutura geral C_6-C_3-C_6, caracterizados pela presença de dois anéis benzénicos ligados através de um anel pirano (IWASHINA, 2000; MARTÍNEZ-FLÓREZ et al., 2002; SHAHIDI; NACZK, 1995a; SHAHIDI; NACZK, 1995b). Esses compostos podem ser agrupados em diversas subclasses incluindo antocianinas, flavonas, flavan-3-óis, flavanonas, isoflavonas e flavonóis (KING; YOUNG, 1999)

As antocianinas, cátions de O-glicosídeos de 3,5,7,3 – tetrahidroxiflavilium, são um grupo de pigmentos naturais presentes em vegetais, sendo amplamente distribuídas na natureza. São compostos hidrossolúveis responsáveis pelas cores vermelho-alaranjado, rosa, vermelho, violeta, azul e roxo de diversas plantas (BRIDLE; TIMBERLAKE, 1997; CASTAÑEDA-OVANDO et al., 2009). Além de utilizadas como corantes naturais para alimentos, o interesse pelas antocianinas decorre de evidências relacionadas ao seu potencial benéfico à saúde em virtude de sua ação antioxidante (ESPÍN et al., 2000; WANG et al., 1997). Numerosos estudos têm mostrado os efeitos terapêuticos positivos das antocianinas, tais como antioxidante, anti-inflamatórios, protetor de DNA e protetor de doenças cardiovasculares. A cianidina (3,5,7,3,4-pentahidroxiflavilium), delfinidina (3,5,7,3,4,5-exahidroxiflavilium), malvidina (3,5,7,4-tetra-3,5-dimetoxiflavilium), pelargonidina (3,5, 7,4-tetrahidroxiflavium), peonidina peonidina (3,5,7,4-tetra- 3-metoxiflavilium), e petunidina (3,5,7,3, 4-pentahidroxi- 5 metoxiflavilium) são as seis agliconas (sem presença de açúcar ligado) mais comumente encontradas em frutas (VIZZOTO, 2012).

Muita atenção tem sido dada aos compostos bioativos, especialmente para aqueles com alto teor de antioxidantes os quais, possivelmente, são capazes de prevenir e/ou aliviar as dores de muitas doenças crônicas, como doenças cardiovasculares, câncer, doenças neurodegenerativas, inflamações, envelhecimento e problemas causados pelos radicais livres. Embora existam vários tipos de antioxidantes, os antioxidantes naturais, tais como os compostos fenólicos em frutas e vegetais têm recebido maior atenção (YOU et al., 2011).

2.2.1 Compostos com atividade antioxidante

Frutas e vegetais são uma boa fonte de recursos naturais antioxidantes, contendo vários componentes antioxidantes diferentes que fornecem proteção contra radicais livres prejudiciais, estado associado com menor incidência e mortalidade de câncer e doenças do coração, além de diversos outros benefícios à saúde (SHUI; LEONG, 2006; WANG; CAO; PRIOR, 1996).

Os antioxidantes apresentam papel importante no sistema de defesa contra espécies reativas de oxigênio que podem ser originários de compostos naturalmente presentes em alimentos, ou formados durante o processamento (GUTTERIDGE; HALLIWELL, 2000; SHAHIDI; NACZK, 1995a; SHAHIDI; NACZK, 1995b). Os antioxidantes são agentes responsáveis pela inibição e redução das lesões causadas pelos radicais livres nas células e também mecanismos de defesa contra os radicais livres que podem ser empregados nas indústrias de alimentos, cosméticos, bebidas e na medicina (DOROSHOW, 1983; WEIJL; CLETON; OSANTO, 1997).

O elemento químico oxigênio pode atuar como substância tóxica originada através de processos que desencadeiam a formação de radicais livres, compostos instáveis e altamente reativos que contêm elétrons desemparelhados. Os radicais livres, altamente instáveis, reagem com moléculas estáveis, com o objetivo de capturar elétrons, transformando-as em um novo radical livre (KAUR; KAPOOR, 2001). Embora a presença desses radicais se ja crítica para a manutenção de funções fisiológicas normais (POMPELLA, 1997), a sua produção contínua durante os processos metabólicos estimula mecanismos de defesa antioxidante, para limitar os níveis intracelulares e impedir a indução de danos. O desequilíbrio entre formação e a inativação de radicais livres é denominado estresse oxidativo (SIES, 1993), que está associado ao desenvolvimento de doenças crônicas e degenerativas (AMES; SHIGENAGA; HAGEN, 1993). Sua ocorrência é acompanhada pelo aumento das defesas antioxidantes enzimáticas, mas a produção de uma elevada quantidade de radicais livres pode causar danos e morte celular (ANDERSON, 1996). Estudos clínicos e epidemiológicos têm mostrado evidências de que antioxidantes fenólicos de frutas e outros vegetais são os fatores que auxiliam na redução dessas doenças (SHAHIDI, 1996).

Desse modo, visto que os radicais livres podem causar danos oxidativos aos lipídeos, proteínas e ácidos nucleicos, podendo levar a um grande número de patologias, presume-se que a ingestão de antioxidantes capazes de neutralizar os radicais livres possa ter um papel importante na redução do risco dessas doenças (HARBORNE; WILLIAMS, 2000). Dessa forma, a identificação de fontes vegetais com alta capacidade antioxidantes é de extrema importância, sejam esses derivados de compostos bioativos como os compostos fenólicos e/ou vitaminas (PINTO, 2008).

2.3 Geleias de frutas

Embora apresentem potencial econômico, frutas finas como morango e em maior intensidade mirtilo, framboesa, cereja e amora preta, têm consumo in natura restrito devido ao alto valor agregado, em decorrência da produção limitada, difícil colheita, exigência em mão de obra, cuidados com o transporte e armazenamento e por serem altamente perecíveis. Os preços restringem seu consumo tornando-as acessíveis apenas no pico da safra, sendo que dessa maneira, uma das formas de disponibilizá-las é através do processamento das mesmas.

O processamento permite agregar valor econômico às matérias-primas, transformando produtos perecíveis em armazenáveis e comercializáveis (MONTEIRO, 2006). As frutas apresentam menor vida de prateleira e sua comercialização in natura é dificultada pelas grandes distâncias, fazendo com que as perdas pós-colheita variem de 15 a 50 % (BUENO et al., 2002).

As geleias podem ser consideradas como o segundo produto em importância comercial para a indústria de conservas de frutas brasileiras. Em outros países, principalmenteos europeus, assumem papel de destaque, tanto no consumo quanto na qualidade (SOLER et al., 1991). A transformação de frutas em produtos possibilita absorver grande parte da colheita, favorecendo o consumo de frutas durante o ano todo e a redução do desperdício de alimentos (MÉLO; LIMA; NASCIMENTO, 1999).

Segundo Resolução - CNNPA nº 12, de 1978, geleia de fruta é o produto preparado com frutas e/ou sucos ou extratos aquosos das mesmas, podendo apresentar frutas inteiras, partes e/ou pedaços sob as variadas formas, devendo tais ingredientes ser misturados com açúcares, com ou sem adição de água, pectina, ácidos e outros ingredientes permitidos por essas normas; tal mistura será convenientemente processada até uma consistência semissólida adequada e, finalmente, acondicionada de forma a assegurar sua perfeita conservação (BRASIL, 1978).

A geleia de fruta é o produto obtido pela concentração de polpa, suco ou extrato de frutas, com quantidades suficientes de açúcar, pectina e ácido, até o *brix* adequado para geleificação por ocasião do resfriamento (MÉLO; LIMA;

NASCIMENTO, 1999). Segundo Martins et al. (2007) as geleias e doces de frutas são resultantes do processamento adequado das partes comestíveis dos vegetais, adicionados de açúcares, água, pectina (0,5 a 1,5%), ajustador de pH (3 a 3,4), além de outros ingredientes e aditivos permitidos até alcançar consistência adequada (ABIA, 2001; JACKIX, 1988).

Segundo a Resolução Normativa nº15/78, as geleias podem ser elaboradas com uma ou mais espécies de fruta sendo, portanto, designadas como simples ou mistas, respectivamente (BRASIL, 1978). Esse produto pode ser do tipo comum ou extra, segundo a proporção utilizada de suco de fruta e açúcar. A primeira é elaborada com quarenta partes de suco de frutas e sessenta partes de açúcar, enquanto que na segunda essa proporção é de cinquenta partes de suco de fruta e cinquenta partes de açúcar (BRASIL, 1978). A maioria das frutas pode ser transformada em geleia, mesmo aquelas com baixo teor de pectina e ácido. Nesse caso, torna-se necessária a adição dessas substâncias, na forma de ingredientes, durante o processamento (MÉLO; LIMA; NASCIMENTO, 1999).

Os métodos de processamento térmico de geleia mais utilizados em indústrias são a cocção em vapor, em água em ebulição ou em tacho aberto. Tais processos, aplicados isoladamente ou associados a processos mecânicos e às condições de armazenamento, podem levar a alterações nas características físicas e na composição química dos alimentos, bem como em suas propriedades benéficas à saúde (PINELI, 2009).

A influência do processamento e da cultivar das bagas na degradação dos compostos bioativos e redução da capacidade antioxidante de diferentes bagas vem sendo alvo de diversos estudos. Kovačević, Levaj e Dragović-Uselac (2009), Levaj et al. (2012), Pineli (2009) e Wicklund et al. (2005) verificaram que existe diferença significativa na perda de compostos bioativos e da capacidade antioxidante entre as cultivares de morango na elaboração de doces e geleias. Wu et al. (2010) em estudos sofre os efeitos do processamento nos compostos bioativos e capacidade antioxidante de amoras *'Marion'* e *'Evergreen'* verificaram que as tecnologias de processamento mostraram efeitos sobre os compostos bioativos diferentes para as duas variedades de amora preta. Patras et al. (2009) verificaram que o tratamento térmico convencional no morango e na amora preta provoca perdas significativas de atividade antioxidante, de fenólicos totais, antocianinas e vitamina C. Kim e Padilla-Zakour (2004) verificaram que o processamento da geleia de cerejas resultou em perdas significativas de antocianinas e que não houve diferenças significativas na degradação da atividade antioxidante e compostos bioactivos. Já Šavikin et al. (2009) verificaram que o processamento em geleia de diferentes bagas causa a redução de fenólicos totais, antocianinas e também da atividade antioxidante. Arancibia-Avila et al. (2012) em estudos de diferentes durações de tempo de processamento térmico em bagas descobriram que bagas sujeitas a tratamento térmico por mais de 20 min perdem a bioatividade.

Segundo Pineli (2009), tendo em vista a importância dos métodos de conservação industrial para a redução de perdas pós-colheita de alimentos ricos com compostos fitoquímicos, é de grande relevância avaliar se as caracteristicas químicas das matérias-primas vegetais sofrem alterações após o processamento, bem como se essas alterações são significativas e desmerecem a tecnologia empregada. Além disso, é de extrema importância o estudo da viabilidade de elaboração de geleias com diferentes cultivares/espécies de bagas que atualmente são pouco utilizadas, visando seu maior aproveitamento industrial.

3 CONCLUSSÃO

A composição e teor de compostos bioativos das pequenas frutas vermelhas de clima subtropical, no geral, foram semelhantes às frutas tipicamente de climas temperados, apresentando ainda maiores teores de vitamina C. Em relação ao processamento, é viável a elaboração de geleia mista de framboesa, sendo que a proporção considerada ideal é: 0-30% de framboesa amarela, 30-50% de framboesa negra e 30-75% de framboesa vermelha. Com base na aceitação e viabilidade econômica, as cultivares mais adequadas para a elaboração de geleia são Tupy, Comanche, Brazos, Guarani e Choctaw. Foi verificado também que há degradação dos compostos bioativos e redução da atividade antioxidante devido ao processamento das geleias de amora preta, sendo que a degradação desses compostos foi significativante diferente entre as cultivares. Destacam-se as cultivares Brazos e Caingangue como as que sofrem as menores perdas com o processamento, dando origem a geleias mais ricas em compostos bioativos e com maior capacidade antioxidante.

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SEGUNDA PARTE– ARTIGOS

ARTIGO 1: DETERMINATION OF THE BIOACTIVE COMPOUNDS, ANTIOXIDANT ACTIVITY AND CHEMICAL COMPOSITION OF BRAZILIAN BLACKBERRY, RED RASPBERRY, STRAWBERRY, BLUEBERRY AND SWEET CHERRY FRUITS

Normas da Revista Científica Food Chemistry - ISSN: 0308-8146 (versão preliminar)

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ABSTRACT

This study aimed to evaluate the chemical composition, identify the bioactive compounds and measure the antioxidant activity present in blackberry, red raspberry, strawberry, sweet cherry and blueberry fruits produced in the subtropical areas of Brazil and to verify that the chemical properties of these fruit are similar when compared to the temperate production zones. Compared with berries and cherries grown in temperate climates, the greatest differences was: Brazilian fruits presented highest levels of soluble solids and total sugar; for the mineral composition, the analyzed fruits presented lower concentrations of P, K, Ca, Mg and Zn and higher levels of Fe; the phenolic content of the strawberry was higher than reported; the blackberry presented higher levels of flavonoids and raspberry was much lower than the literature; and the blackberry, strawberry and blueberry fruits showed lower anthocyanin contents than those found in the literature and in relation to ascorbic acid, as all fruits analyzed showed levels well above those found in the literature.

Keywords: bioactive compounds, antioxidant activity, berry, cherry

1. INTRODUCTION

Berry fruits, are small fleshy fruits, which are commercially cultivated and commonly consumed in fresh and processed forms, include blackberry (*Rubus* spp.), black raspberry (*Rubusoccidentalis*), red raspberry (*Rubusidaeus*), blueberry (*Vacciniumcorymbosum*) and strawberry (*Fragaria*x*ananassa*) (Seeram, 2008).

Berries are rich in phenolic compounds, such as phenolic acids, tannins, stilbenes, flavonoids and anthocyanins, but berries, in particular, have been the focus of considerable research regarding their anthocyanin-rich properties and according to Seeram (2008), there are many studies that claim that that the dietary intake of berry fruits has a positive and profound impact on human health, performance, and disease.

Although it is already well established that berries and cherries are sources of bioactive compounds such as polyphenols and anthocyanins, these studies focused mainly on berries from temperate climates, mainly in the temperate regions of Europe, Asia and North America (Chen, Xin, Zhang and Yuan, 2013). Knowing that the composition of the fruits varies with a series of factors that includes species, variety, cultivation, region, weather conditions, ripeness, time of harvest and storage conditions

(Haffner, Rosenfeld, Skrede and Wang, 2002; Faniadis, Drogoudi and Vasilakakis, 2010), is extremely relevant for the characterization and comparison of berries produced in tropical and subtropical climates with traditional berries from a temperate climate.

The raspberry and blackberry cultivation in Brazil has been increasing steadily, especially in the subtropical areas where temperatures are higher in the fall and winter and especially higher in the summer, and previous results show that blackberry plants produce large quantities of fruit in subtropical areas, with some varieties producing higher amounts compared to temperate zones (Campagnolo and Pio, 2012). For raspberries, the productive performance results of the subtropical areas in Brazil are very encouraging because the production of raspberries is constant throughout the year with certain cultivars producing large quantities of fruit in the fall and winter (Moura, Campagnolo, Pio, Curi, Assis and Silva, 2012). Thus, the determination of the nutritional composition of the berries and cherries produced in Brazilian subtropical zones is important to know the nutritional and functional properties and to verify that the chemical properties of the fruit are similar when compared to the temperate production zones.

To this end, the aims of the present study were to evaluate the chemical composition, identify the bioactive compounds and measure the antioxidant activity present in blackberry (*Rubus* spp.), red raspberry (*Rubusidaeus)*, strawberry *(Fragaria*x*ananassa)*, sweet cherry (*Prunusavium* L.) and blueberry (*Vacciniumcorymbosum*) fruits produced in the subtropical areas of the states of Minas Gerais and São Paulo, Brazil.

2. MATERIALS AND METHODS

2.1 Fruit samples

The blackberry, red raspberry and strawberry fruits were acquired from a producer in the south of Minas Gerais state, whereas the blueberry and cherry plants were acquired from a producer in São Paulo state. The fruits were harvested at their physiological maturity in the morning and transported in Styrofoam boxes to the post-harvest fruit and vegetable laboratory of the Universidade Federal de Lavras. Upon delivery, the fruits were sanitized, and all fruits were stored in a cold room at -18 °C during the analysis time.

2.2 Chemical reagents

The following chemicals were used for the experiments described later: acetone, 2,2´-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), aluminum chloride ($AlCl_3$), β-carotene, (+)-catechin, hydrochloric acid (HCl), 2,4-dinitrophenylhydrazine (2,4-DNPH), chloroform, copper sulfate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ethanol, ethyl ether, Folin–Ciocalteu reagent, gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Kjeldahl reagent, linoleic acid, methanol, nitric acid, perchloric acid, petroleum ether, phenolphthalein solution, phosphate buffer, potassium sulfate, potassium persulfate, sodium carbonate, sodium nitrate ($NaNO_3$), sodium hydroxide (NaOH), sulfuric acid and Tween 40 as well as the thermostable alpha-amylase, protease, and amyloglucosidase enzymes.

2.3 Chemical analyses

Three repetitions were performed for all chemical analyses. The values of the titratable acid, soluble solids, total sugar, pH, moisture, ash, protein, lipid, carbohydrate and total dietary fiber contents were determined (AOAC—Association of Official Analytical Chemists, 1998).

2.4 Minerals

The mineral levels were assessed in crushed and homogenized samples prepared by organic wet digestion in accordance with the methodology described by Salinas and Garcia (1985). For organic digestion, the samples were treated with a mixture of concentrated nitric and perchloric acids at a high temperature. The macro- and microelements were solubilized, subjected to different treatments and diluted for further quantitative evaluation. The quantification of elements was performed by spectrophotometry using a standard curve for each mineral. To determine the concentration of calcium, iron and manganese, we used an atomic absorption spectrophotometer and acetylene. A flame photometer was used to determine potassium (768 nm), and a visible-light spectrophotometer was used to determine phosphorus (420 nm).

2.5 Preparation of antioxidant and phenolic extracts

The extracts were obtained according to the method described by Larrauri, Ruperez and Saura-Calixto (1997). Briefly, samples were weighed (in grams) in centrifuge tubes and extracted sequentially with 40 mL of methanol/water (50:50, v/v) at room temperature for 1 hour. The tubes were centrifuged at 25,400 x *g* for 15 min, and the supernatant was recovered. Then, 40 mL of acetone/water (70:30, v/v) was added to the residue at room temperature. The samples were extracted for 60 min and centrifuged. To determine the antioxidant activity as well as total flavonoid, total monomeric anthocyanin and phenolic contents, the methanol and acetone extracts were combined and brought to a final volume of 100 mL with distilled water.

2.5.1 Antioxidant activity

The antioxidant activity was determined using the ABTS, DPPH and β-carotene methods. For the ABTS assay, the procedure followed the method of Re, Pellegrini, Proteggente, Pannala, Yang and Rice-Evans (1999) with minor modifications. The ABTS radical cation (ABTS•+) was generated by the reaction of 5 mL of aqueous ABTS solution (7 mM) with 88 μL of 140 mM (2.45 mM final concentration) potassium persulfate. The mixture was kept in the dark for 16 hours before use and then diluted with ethanol to obtain an absorbance of 0.7±0.05 units at 734 nm using a spectrophotometer. The fruit extracts (30 μL) or a reference substance (Trolox) were allowed to react with 3 mL of the resulting blue-green ABTS radical solution in the dark. The decrease of absorbance at 734 nm was measured after 6 min. Ethanolic solutions of known Trolox concentrations were used for calibration. The results are expressed as micromoles of Trolox equivalents (TEs) per gram of fresh weight (µmol of TEs/g of FW).

The DPPH free radical-scavenging capacity was estimated using the method of Brand-Williams, Cuvelier and Berset (1995). Briefly, the solution of DPPH (600 µM) was diluted with ethanol to obtain an absorbance of 0.7±0.02 units at 517 nm. The fruit extracts (0,1 ml) were allowed to react with 3,9 mL of the DPPH radical solution for 30 min in the dark, and the decrease in absorbance from the resulting solution was monitored. The absorbance of the reaction mixture was measured at 517 nm. The results were expressed as EC_{50} (g FW/g of DPPH).

The antioxidant activity was also determined by the β-carotene method, following the procedure described by Marco (1968) with minor modifications. Briefly, an aliquot (50 µl) of the β-carotene chloroform solution (20 mg/mL) was added to a flask containing 40 µl of linoleic acid, 1.0 mL of chloroform, and 530 µL of Tween 40 and then mixed. The chloroform was evaporated using an oxygenator. After the evaporation, oxygenated distilled water (approximately 100 mL) was added to obtain an absorbance of 0.65±0.5 units at 470 nm. An aliquot (0,4 mL) of Trolox solution (200 mg/L) or diluted fruit extract (200 mg/L) was added to 5 mL of the β-carotene solution and incubated in a water bath at 40 °C. The measurements were performed after 2 minutes and 120 minutes at an absorbance of 470 nm using a spectrophotometer. The antioxidant activity was calculated as the percent inhibition relative to the control.

2.5.2 Total phenolic

The total phenolic content was determined according to the adapted Folin–Ciocalteu method (Waterhouse, 2002). The extracts (0.5 mL) were mixed with 2.5 mL of Folin–Ciocalteu reagent (10%) and 2 mL of sodium carbonate solution (4%). The mixture was stirred and kept at room temperature for 2 hours in the dark. The absorbance was measured at 750 nm against a blank. Aqueous solutions of gallic acid were used for calibration. The results are expressed as g gallic acid equivalents (GAE)/100 g.

2.5.3 Total flavonoid

The total flavonoid content was measured by the aluminum chloride colorimetric assay (Zhishen, Mengcheng and Jianming, 1999). An aliquot (1 ml) of extract or catechin standard solution (5, 10, 25, 50, 100, 150 or 200 mg/l) was added to a 10 ml volumetric flask containing 4 ml of water. To the flask, 0.3 ml of 5% $NaNO_2$ and 0.3 ml of 10% $AlCl_3$ were added. After 6 minutes, 2 ml of 1 M NaOH was added and the total volume was brought to 10 ml by the addition of H_2O. The solution was mixed and the absorbance was measured against a prepared blank reagent at 510 nm. The total flavonoid contents of the fruits were expressed as mg catechin equivalents (CE)/100 g of FW. The samples were analyzed in triplicate.

2.5.4 Total monomeric anthocyanin

The total monomeric anthocyanin content (TMAC) was estimated using the pH differential method (Wrolstad, 1976). Briefly, each fruit extract was diluted with pH 1.0 and pH 4.5 buffers to attain the same dilution. The absorbance was measured at 510 nm and 700 nm in both pH

1.0 and pH 4.5 buffers. Then, the TMAC (expressed in terms of cyanidin-3-glucoside) was calculated using the following formula:

$$A = (A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5} \quad (1)$$

$$\text{TMA content} = (A \times \text{MW} \times \text{DF} \times \text{Ve} \times 1000)/(\varepsilon \times 1 \times \text{M}) \quad (2)$$

where MW is the molecular weight of cyanidin-3-glucoside (449 g mol^{-1}), DF is the dilution factor, Ve is the extract volume, ε is the molar extinction coefficient of cyanidin-3-glucoside (29,600), and M is the mass of the berries extracted.

The results were expressed as mg cyanidin-3-glucoside equivalents/100 g of FW.

2.6 Ascorbic acid

The vitamin C content of each fruit pulp was determined by a colorimetric method with 2,4-dinitrophenylhydrazine (2,4-DNPH) according to Strohecker and Henning (1967). The samples were analyzed in a spectrophotometer at an absorbance of 520 nm. The results are expressed as mg ascorbic acid/100 g of fresh weight.

2.7 Statistical analysis

The data were reported as the means ± the standard deviation (SD) experiments run in triplicate and were analyzed using SPSS 17.0. A Pearson correlation test was conducted to determine the correlation between variables. Significance levels were defined $p < 0.05$.

3. RESULTS AND DISCUSSION

Table 1 presents the centesimal composition of blackberry, red raspberry, strawberry, blueberry and sweet cherry fruits and compares the composition listed in the National Nutrient Database for Standard Reference (USDA – United States Department of Agriculture, 2013) to these fruits.

TABLE 1 - The composition (g/100g fresh weight) of blackberry, red raspberry, strawberry, blueberry and cherry and the USDA database.

	FRUITS				
	Blackberry	**Redraspberry**	**Strawberry**	**Blueberry**	**Cherry**
MOISTURE	**87.92±0.59**	**88.60±0.19**	**92.68±0.17**	**87.70±0.14**	**86.43±0.31**
USDA database	88.15	85.75	90.95	84.21	82.25
PROTEIN	**1.27±0.06**	**1.00±0.08**	**0.50±0.02**	**0.48±0.01**	**1.00±0.05**
USDA database	1.39	1.20	0.67	0.74	1.06
LIPIDS	**0.42±0.05**	**0.28±0.02**	**0.25±0.02**	**0.19±0.01**	**0.20±0.01**
USDA database	0.49	0.65	0.30	0.33	0.20
CARBOHYDRATES	**10.18±0.61**	**9.88±0.11**	**6.30±0.13**	**11.54±0.13**	**11.94±0.28**
USDA database	9.61	11.94	7.68	14.49	16.01
DIETARY FIBER	**4.47±0.67**	**5.77±0.57**	**1.31±0.18**	**1.90±0.46**	**2.07±0.22**
USDA database	5.30	6.50	2.0	2.40	2.10
ASH	**0.21±0.02**	**0.25±0.00**	**0.27±0.01**	**0.08±0.00**	**0.42±0.01**
USDA database	-	-	-	-	-
ENERGY VALUE (kcal)	**49.57±2.18**	**46.00±0.85**	**29.4±0.75**	**49.86±0.59**	**53.59±1.22**
USDA database	43	52	32	57	63

Mean value±standard deviation of fruit weight; n=3.

All fruits had high moisture content, ranging from 86.43% (cherry) to 92.68% (strawberry). The protein content ranged from 0.48% (blueberry) to 1.27% (blackberry). All fruits were low in fat content; the blueberry had the lowest fat content (0.19%) and the blackberry had the highest (0.42%). The carbohydrate content ranged from 6.30% (strawberry) to 11.94% (cherry). Regarding dietary fiber, the levels were between 1.31% (strawberry) and 5.77% (red raspberry). The ash ranged from 0.08% (blueberry) to 0.42% (cherry). Based on these results, the energy value was found to range from 29.4 kcal (strawberry) to 49.57 kcal (blackberry).

In general, the Brazilian berries and cherry showed similar centesimal composition to the database values provided by the USDA with slightly higher moisture content and slightly lower energy, protein, lipid, carbohydrate and dietary fiber values. Thus, although the climate, soil, management, insolation and others conditions were different, these differences seems to have not affect affect the composition of these fruits.

Table 2 presents the physical-chemical characteristics of blackberry, red raspberry, strawberry, blueberry and sweet cherry fruits. The range of values found in the literature is also presented.

TABLE 2 - The pH, titratable acidity (TA), soluble solids (SS), total sugar (TS)and the ratio of total sugar/titratable acidity (TS/TA) of blackberry, red raspberry, strawberry, blueberry and cherry.

	FRUITS				
	Blackberry[1]	**Red raspberry[2]**	**Strawberry[3]**	**Blueberry[4]**	**Cherry[5]**
pH	**2.99±0.04**	**2.86±0.04**	**3.73±0.01**	**3.64±0.05**	**4.08±0.01**
Literature	2.51-4.12	3.11-3.65	3.27-3.43	2.56-3.15	3.11-4.81
TA/ (g citric acid/100g)	**1.51±0.04**	**1.88±0.09**	**0.86±0.10**	**0.58±0.07**	**0.55±0.07**
Literature	1.26-1.54	0.62-3.59	0.60-1.31	0.68-0.84	0.57-2.53
SS (°Brix)	**10.17±0.29**	**10.33±0.58**	**10.50±0.50**	**14.67±0.58**	**18.67±0.58**
Literature	6.19-11.11	8.4-14.7	6.33-10.86	10.67-13.2	12.5-22.73
TS (%)	**4.47±1.35**	**6.38±1.71**	**5.08±0.39**	**12.74±1.06**	**13.73±1.01**
Literature	2.75-22.1	2.62-9.24	4.50-6.52	9.96	7.68-14.40
SS/TA	**6.71±0.18**	**5.50±0.55**	**12.27±1.39**	**28.61±6.27**	**34.07±3.85**

Mean value±standard deviation of pulp weight; n=3.

[1] Literature data for blackberry: Hassimotto et al. (2008), Tosun, Ustun and Tekguler(2008); Acosta-Montoya et. al (2010), Wu et al. (2010)

[2] Literature data for red raspberry: Haffner et al. (2002); Çekiç and Ozgen (2010), Moura et al. (2012)

[3] Literature data for strawberry: Kafkas, Kosar, Paydas, Kafkas and Baser (2007)

[4] Literature data for blueberry: Almenar, Samsudin, Auras, Harte and Rubino (2008)

[5] Literature data for cherry: Ballistreri, Continella, Gentile, Amenta, Fabroni and Rapisarda(2012); Benalti, Sabio, Hernández and Gervasini(2003); Serradilla et al. (2012); Faniadis et al. (2010); Serradilla, Lozano, Bernalte, Ayuso, López-Corralesand González-Cómez, (2011)

USDA (2013)

The pH values ranged from 2.86 to 4.08 (red raspberry and cherry, respectively), and the levels of acidity ranged from 0.55 g of citric acid/100 g in the cherry to 1.88 g of citric acid/100 g in the red raspberry. With respect to soluble solids and total sugars, the blackberry had the lowest levels (10.17 °Brix and 4.47%, respectively) and cherry had the highest levels (18.67 °Brix and 13.73%, respectively). Among the fruits analyzed, the blueberry and the cherry stand out for presenting the highest levels of soluble solids and sugars and the lowest levels of acidity; consequently, they have the highest ratios of soluble solids/acid (28.61 to blueberry and 34.07 to cherry).

In relation to physical-chemical characteristics, the main difference observed, compared with the literature, is that the fruits evaluated showed higher content of soluble solids and total sugars, which can mean a tastier fruit and a fruit with greater potential for sensory acceptance. This characteristic can be explained by the climatic conditions to which this fruits have been cultivated - fruit grown in subtropical climate are subjected to higher intensity light and temperatures than those cultivated in temperate climates.

The mineral compositions, including P, K, Ca, Mg, Zn and Fe, of blackberry, red raspberry, strawberry, blueberry and sweet cherry fruits are shown in Table 3. The mineral contribution of each fruit to the Dietary Reference Intake (DRI) for a healthy adult male in % per 100 g of pulp (Institute of Medicine, 1999-2011) and the literature range of these values are also presented in Table 3.

TABLE 3 - The minerals contents and the %DRI contribution per 100 g of pulp of blackberry, red raspberry, strawberry, blueberry and cherry.

	Blackberry	Red raspberry	Strawberry	Blueberry	Cherry
P (mg/100g f.w)	**7.25±0.35**	**5.70±0.10**	**6.59±0.16**	**8.61±0.10**	**12.21±0.28**
DRI*	1.25	0.98	1.14	1.48	2.10
Literature**			12.00-29.00		
K (mg/100g f.w.)	**79.73±3.87**	**71.84±1.22**	**51.24±1.21**	**70.13±0.81**	**90.92±2.06**
DRI*	1.70	1.53	1.09	1.49	1.93
Literature**			77.00-349.79		
Ca(mg/100g f.w.)	**7.25±0.35**	**1.14±0.02**	**2.20±0.05**	**0.00±0.00**	**0.00±0.00**
DRI*	0.91	0.14	0.27	0.00	0.00
Literature**			6.00-29.00		
Mg(mg/100g f.w.)	**15.70±0.76**	**15.96±0.27**	**8.78±0.21**	**4.92±0.06**	**12.21±0.28**
DRI*	4.49	4.56	2.51	1.41	3.49
Literature**			6.00-44.80		
Zn(mg/100g f.w.)	**0.20±0.01**	**0.37±0.01**	**0.13±0.00**	**0.13±0.00**	**0.69±0.02**
DRI*	2.13	3.94	1.38	1.38	7.34
Literature**	0.07-0.44				
Fe (mg/100g f.w.)	**1.28±0.066**	**1.06±0.02**	**1.00±0.02**	**1.24±0.01**	**1.16±0.03**
DRI*	21.33	17.67	16.67	20.67	19.33
Literature**	0.28-1.08				

* Institute of Medicine, 1999-2011
**Literature: USDA (2013), Hakala et al. (2003),Tosun et al. (2008)

Among the fruits analyzed, the concentration of minerals (measured in mg/100 g FW) was found to range between 5.70 (red raspberry) and 12.21 (cherry) for P; 51.24 (strawberry) and 90.92 (cherry) for K; 0.00 (blueberry and cherry) and 7.25 (blackberry) for Ca; 4.92 (blueberry) and 15.96 (red raspberry) for Mg; 0.13 (strawberry and blueberry) and 0.69 (cherry) for Zn; and 1.00 (strawberry) and 1.28 (blackberry) for Fe.

In comparison to the USDA database and data from the literature, the analyzed fruits presented lower concentrations of the minerals P, K, Ca, Mg and Zn and higher levels of Fe. This difference is justified because, as already mentioned, we compared the composition of fruits of different cultivars subjected to different climatic conditions and post-harvest handling techniques. In studies of the effects of cultivars and cultivation conditions on the composition of strawberries, Hakala, Lapvetelainen, Houpalahti, Kallio and Tahvonen (2003) analyzed several cultivars of strawberries for two consecutive years and concluded that the cultivar and, to a lesser extent, the climatic conditions and soil influenced the mineral composition of the strawberries. The soils of temperate regions such as Europe, are typically basic, since the soils of Brazil are typically acidic, with low levels of P, Ca, K and Mg and high contents of Al, Mn and Fe (Santos et al. 2006); fact that can justify the results.

In general, the fruits analyzed do not significantly contribute to the DRI of Ca, P or K, but have a significant contribution to the DRI of Fe and an intermediate contribution to the DRI for Zn and Mg.

The results for the total phenolic, total flavonoid, total monomeric anthocyanin and ascorbic acid contents as well as the antioxidant capacity of blackberry, red raspberry, strawberry, blueberry and cherry fruits are shown in Table 4. The range found for the total phenolic compounds, anthocyanin and ascorbic acid contents, which are the bioactive compounds most often found in the literature, are also expressed in Table 4. We chose not to include the literature values for antioxidant activity due to the differences between the methods and the presentation of the results, which did not allow for a proper comparison. Additionally, because data found for the flavonoids were not abundant in the literature, these parameters was also not expressed.

TABLE 4 - The antioxidant capacity (ABTS, DPPH and β-carotene method), total phenolic, total flavonoid, total monomeric anthocyanin, ascorbic acid and carotenoid content of blackberry, red raspberry, strawberry, blueberry and cherry.

	FRUITS				
	Blackberry[1]	Red raspberry[2]	Strawberry[3]	Blueberry[4]	Cherry[5]
Antioxidant Capacity – ABTS (μmol/g f.w.)	**13.23 ±1.37**	**6.27 ±0.02**	**7.87 ±0.87**	**5.88 ±1.17**	**8.83 ±1.32**
Antioxidant Capacity – DPPH (EC_{50} – g f.w/g DPPH)	**2142.42 ±125.64**	**4960.58 ±157.33**	**3778.94 ±333.88**	**7775.45 ±1009.60**	**6065.68 ±563.46**
Antioxidant Capacity – β-carotene (% protection)	**87.46 ±3.09**	**75.19 ±3.92**	**67.13 ±0.42**	**59.88 ±1.06**	**61.93 ±0.83**
Total Phenolics (mg GAEs/100g f.w.)	**850.52 ±4.77**	**357.83 ±7.06**	**621.92 ±15.51**	**305.38 ±5.09**	**314.45 ±5.95**
Literature	176-1020	148-714	200-300	44.4-394	74-501.58
Total Flavonoid (mg CE/100g f.w.)	**87.03 ±4.85**	**9.61 ±2.15**	**38.17 ±2.76**	**47.53 ±2.40**	**59.92 ±3.76**
Total anthocyanin (mg of cyanidin 3-glucoside equivalent/ 100 g of f.w.)	**58.61 ±2.19**	**14.69 ±2.03**	**16.03 ±0.50**	**29.72 ±4.20**	**26.72 ±3.22**
Literature	77-188	1.3-437	20-32	140-224	6-85
AscorbicAcid (mg/100g f.w)	**52.41 ±11.31**	**92.17 ±10.11**	**90.13 ±2.24**	**73.21 ±0.35**	**62.42 ±7.69**
Literature	10-17	15-38	32-85	10	7-103

Mean value±standard deviation of fruit weight; n=3.

*Abreviations:*TEAC: Trolox equivalent antioxidant capacity (µM Trolox equiv./g fw); DPPH: 2-diphenyl-1-picryhydrazyl radical scavenging activity; GAE: Gallic acid equivalent; CE: catechin equivalent.

[1] Literature data for blackberry: Wang and Lin (2000); Pantelidis et al. (2007); Hassimoto et al. (2008); Koca and Karadeniz, (2009); Wu et al. (2010); Acosta-Montoya et al. (2010); Samecand Zegarac(2011)

[2] Literature data for red raspberry:Haffner et al. (2002); Pantelidis et al. (2007);Jin et al. (2012); Bobimaitè, Viskelis and Venskutonis(2012); Çekiç and Ozgenet al. (2010); Chen et al. (2013)

[3] Literature data for strawberry: Wang and Lin (2000); Robert and Gordon (2003); Hakala et al. (2003); Pantelidis et al. (2007)

[4] Literature data for blueberry: Koca and Karadeniz (2009); You, Wang, Chen, Huang, Wang and Lin (2000)

[5] Literature data for cherry: Benalti et al. (2003); Pantelidis et al. (2007); Faniadis et al. (2010); Samecand Zegarac(2011); Serradilla et al. (2011); Ballistreri et al. (2012); Serradilla et al. (2012) USDA (2013)

In general, the antioxidant methods utilized are in agreement; the blackberry has the highest antioxidant activity, the strawberry the intermediate and the blueberry the lowest. The descending order of antioxidant capacity of the fruits for each antioxidant method used is:

ABTS method: Blackberry > Cherry > Strawberry > Red Raspberry > Blueberry

DPPH method: Blackberry > Strawberry > Red Raspberry > Cherry > Blueberry

β-carotene method: Blackberry > Red Raspberry > Strawberry > Cherry > Blueberry

According to Hassimoto, Genovese and Lajolo (2005), one of the major problems with the antioxidant activity of biological materials is the choice of the method of analysis because typically the analysis is specific for only one property. The three methods used presented coherent results for the fruits evaluated, which was mainly seen with the DPPH and β-carotene methods. The ABTS, DPPH, β-carotene methods all show that the blackberry is the richest source of antioxidants and the blueberry is the poorest.

According to Hassimoto et al. (2005), the values of antioxidant activity are classified as high (>70% inhibition), intermediate (40-70% inhibition), and low (<40% inhibition). According to this classification, blackberries and red raspberries are good sources of antioxidants and the other fruits (strawberry, cherry and blueberry) have intermediate antioxidant activity. The red raspberry and the cherry antioxidant activity (TEAC and DPPH) is in agreement with the range found in the literature (Çekiç and Ozgenet, 2010; Jin, Wang, Gao, Chen, Zhang and Wang 2012).

The total phenolic content ranged from 305.38 (blueberry) to 850.52 mg GAE/100 g (blackberry). Following the polyphenol classification proposed by Vasco, Ruales and Kamal-Eldin(2008) using low (<100 mg GAE/100 g), medium (100–500 mg GAE/100 g) and high (>500 mg GAE/100 g) denominations, the blackberry (850.52 mg GAE/100 g) and the strawberry (621.92 mg GAE/100 g) can be categorized as having a high concentration of phenols. This classification indicates that this fruit is an excellent source of phenols. Red raspberries (357.82 mg GAE/100 g), blueberries (305.38 mg GAE/100 g) and cherries (314.45 mg GAE/100 g) can be categorized as having an average phenol content, and they may also be considered a good source of phenols. According to the data from the literature for berries and cherries, the blackberry, raspberry, blueberry and cherry all showed phenolic contents consistent with ranges previously reported. Additionally, the phenolic content of the strawberry was higher than that reported in the literature (Table 4).

The total flavonoids ranged from 9.61 (red raspberry) to 87.03 mg CE/100 g (blackberry), with the cherry (59.92 mg CE/100 g), blueberry (47.53 mg CE/100 g) and strawberry (38.17 mg CE/100 g) presenting the intermediate values. Compared to literature data, the blackberry (87.03 mg CE/100 g) had higher levels than those found by Samec and Zegarac (2011) (66.13 mg CE/100 g).

For the total monomeric anthocyanin contents, the blackberry presented the highest value (58.61 mg of cyanidin 3-glucoside equivalent/g), the blueberry and cherry presented the intermediate values (29.72 and 26.72 mg of cyanidin 3-glucoside equivalent/g, respectively) and the red raspberry showed the lowest value (14.69 mg of cyanidin 3-glucoside equivalent/g). The blackberry, strawberry and blueberry anthocyanin contents were lower than those found in the literature, and the raspberry and cherry were within the range previously found (Table 4). Compared with blackberries grown in temperate climates, the tropical blackberry presents a lower anthocyanin content (Acosta-Montoya et al., 2010).

The ascorbic acid levels ranged from 52.41 (blackberry) to 92.17 mg/100 g f.w. (red raspberry). Ramful, Tarnus, Aruoma, Bourdan and Bahorun, (2011) classified fruits into three categories according to the ascorbic acid content: low (<30 mg/100 g), medium (30 - 50 mg/100 g) and high (> 50 mg/100 g). According to this classification, all fruits analyzed qualify as fruits with high ascorbic acid content because all berries fruits analyzed exhibited ascorbic acid contents well above the ranges found in the literature (Table 4).

The Pearson's correlation coefficients between antioxidant activity, total phenolic contents and ascorbic acid levels are presented in Table 5.

TABLE 5 Pearson's correlation coefficients ($p<0.05$) between antioxidant capacity parameters, total phenolic contents, total flavonoid, total monomeric anthocyanin and ascorbic acid.

	Antioxidant						
	TEAC	DPPH	β-carot.	TPC	TFC	TMA	AA
TEAC	-	-	-	0.83	0.84	0.85	-
DPPH	-	-	-.086	0.91	-	-	-
β-carot.	-	-0.86	-	-	-	-	-
TPC	0.83	0.91	-	-	-	-	-
TFC	0.84	-	-	-	-	0.89	-0.93
TMA	0.85	-	-	-	0.89	-	-0.88
AA	-	-	-	-	-0.93	-0.88	-

Abbreviations: TEAC: Trolox equivalent antioxidant capacity; DPPH: 2-diphenyl-1-picryhydrazyl radical scavenging activity; β-carot: β-carotene method; TPC: total phenolic contents; TF: total flavonoid contents; TMA: total monomeric anthocyanin; AA: ascorbic acid

The total antioxidant capacity from the TEAC and DPPH tests was highly and positively correlated to total phenolic contents. The total antioxidant capacity (TEAC) was also highly and positively correlated total monomeric anthocyanin contents and DPPH to the total flavonoid contents. Several studies with berries and cherries have reported relationships between the antioxidant activity and the phenolic compounds and anthocyanin contents (Pantelidis et al., 2007; Hassimoto, Mota, Cordenunsi and Lajolo, 2008; Koca and Karadeniz, 2009; Wu, Frei, Kennedy and Zhao, 2010).

The total flavonoid content was highly and positively correlated to the total monomeric anthocyanin content, and both of these parameters were highly and negatively correlated to the ascorbic acid content. Anthocyanins belong to a class of flavonoids that are the water-soluble pigments responsible for the orange, red and blue colors of many fruits (Hassimoto et al. 2008); therefore, one would expect this strong correlation between the flavonoids and anthocyanins.

4. CONCLUSION

The blackberry stands out among the fruits evaluated by exhibiting the highest antioxidant activity and the highest levels of phenols, flavonoids, anthocyanins and carotenoids. Compared with berries and cherries grown in temperate climates, the greatest differences was:

Brazilian fruits presented highest levels of soluble solids and total sugar; for the mineral composition, the analyzed fruits presented lower concentrations of P, K, Ca, Mg and Zn and higher levels of Fe; the phenolic content of the strawberry was higher than reported; the blackberry presented higher levels of flavonoids and raspberry was much lower than the literature; and the blackberry, strawberry and blueberry fruits showed lower anthocyanin contents than those found in the literature and in relation to ascorbic acid, as all fruits analyzed showed levels well above those found in the literature.

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ARTIGO 2: EVALUATION OF THE JELLY PROCESSING POTENTIAL OF RASPBERRIES ADAPTED IN BRAZIL

Normas da Revista Científica LWT- Food Science and Technology - ISSN: 0023-6438 (versão preliminar)

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ABSTRACT

Generally raspberry products as jams, jellies and preserves are made with red raspberry, however, yellow raspberry and especially black raspberry are also fruits adapted in Brazil, presenting even better productivity and quality. Thus, the aim of this study was to evaluate the processing potential of other varieties of raspberry, but the red, in the preparation of jellies through mixture design and response surface methodology (RSM). These techniques were used to optimize the following three variables: yellow (Golden Bliss cultivar, *Rubus idaeus*), black (*Rubus niveus*) and red raspberries (Batum cultivar, *Rubus idaeus*) to formulate a mixed raspberry fruit jelly through sensory evaluations. It was found that jelly formulated with a mix of colored raspberries grown in subtropical regions is a viable and alternative way to use yellow and black raspberries. The mixed raspberry jelly must have 0-30% yellow raspberries, 25-50% black raspberries and 30-75% red raspberries. Within this region, the optimum formulation has ideal characteristics that are often not observed in formulations with 100% black or yellow raspberries.

Keywords: optimization, response surface methodology, mixture design, PARAFAC, red raspberry

INTRODUCTION

In recent decades, the cultivation of temperate fruit species has significantly increased in specific regions. Cultivation is no longer practiced only in the coldest areas but has moved into non-traditional growing regions that experience mild winters and high-temperature summers. A recent example in Brazil is the cultivation of raspberries in subtropical regions (Maro, Pio, Silva and Patto, 2012; Moura, Campagnolo, Pio, Curi, Assis and Silva, 2012).

Raspberries (*Rubus idaeus* L.) are among the most popular berries in the world, and they are consumed fresh or frozen mainly in processed forms (juices, jams, jellies, syrups and wines) not only because of their bright color and characteristic flavor but increasingly because of their beneficial health properties (Byamukama, Kiremire, Andersen and Steigen, 2005; Bobimaitè, Viskelis and Venskutonis, 2012; Lim, Jeong and Shin, 2013; Carvalho, Fraser and Martens, 2013). The fruits of the raspberry plant have a high free radical-scavenging capacity, and they contain high levels of anthocyanins, flavonoids, and phenolic acids that offer significant health benefits to consumers (Ochoa, Kesselera, Vulliouda and Lozano, 1999; Wang and Lin, 2000; Kahkonen, Hopia and Heinonen, 2001; Jakobek, Seruga, Seruga, Novak and Medvidovic-Kosanovic, 2009; Khanizadeh et al. 2009). In addition, these compounds stand out for their natural pigments, mainly anthocyanin, which confers an attractive coloration when fruits are processed into dairy products, jams, jellies and fruit preserves (Nour, Trandafir and Ionica, 2011).

Raspberries have a short market life because of their high perishability and high contents of water make them susceptible for microbial or enzymatic degradation (Yousefi, Yousefi and Emam-Djomeh, 2013). Because of their perishability and the limited berry production in Brazil (only in subtropical areas), one way to increase raspberry availability and add even more value is to create new products such as jellies and jams (Souza et al., 2012). According to Zotarelli, Zanatta and Clemente (2008), mixed fruit jams, jellies and preserves are an interesting way of processing fruits because the nutritional characteristics of two or more fruits are combined and they provide pleasant sensory characteristics, which help to gradually gain prime space in the consumer market.

The cultivation of berries, such as raspberries, has increased steadily in Brazil, especially in subtropical areas, where temperatures are higher in autumn-winter and especially high during the summer (Maro et al., 2012; Campagnolo and Pio, 2012). In the case of raspberries, the productive performance results from subtropical Brazil are very encouraging because the production of raspberries is constant throughout the year, a variety of plants producing large quantities of fruit during fall and winter (Moura et al., 2012).

In addition to the red and yellow raspberries of *R. idaeus*, which are commonly found on supermarket shelves, we highlight the black raspberry (*R. niveus* Thunberg), which produces clusters of small fruit with a dark purple color and is well suited to regions with mild winters and moderate temperatures (Jin, Yin□Chun, Gui□Qin and Wen□Dun, 1999; Moyer, Hummer, Wrolstad and Finn 2002). Regarding raspberry productivity in subtropical Brazil, Moura et al. (2012) found that the black raspberry stands out among the yellow and red berries as an excellent choice for cultivation, with excellent adaptability and high productivity and fruit quality.

Thus it becomes extremely important studies of the processing of red, yellow and black raspberries, when isolated and in combination to assess the potential processing of these fruits. The production of mixed jams prepared with red, yellow and black raspberries may add value to the final product or enable the production of a superior quality jelly (Silva et al. 2012).

In this context, this work aimed to evaluate the processing potential of yellow (Golden Bliss cultivar, *Rubus idaeus*), black (*Rubus niveus*) and red raspberries (Batum cultivar, *Rubus idaeus*) in the preparation of jellies through mixture design and response surface methodology (RSM). This study also aimed to determine the physicochemical parameters for the optimized formulation from the resulting models.

MATERIALS AND METHODS

Ingredients

The pulp of yellow (Golden Bliss cultivar, *Rubus idaeus*), black (*Rubus niveus*) and red raspberries (Batum cultivar, *Rubus idaeus*) was used to make jelly. Fruits for jelly preparation were acquired from the orchard of the Federal University of Lavras in Minas Gerais, Brazil. These fruits were harvested in the morning at their physiological maturity on the basis of their color and size and were refrigerated in a cold room at 18°C until processing. In addition to the fruits, sucrose and high-methoxyl pectin were also added (Danisco, SP, Brazil). Citric acid was not added to the jellies because the pH of the fruits (2.98-3.52) was not suitable for its use.

Experimental Design

In the present study, a centroid mixture design (Cornell, 1983) was used to evaluate the effects of and to optimize the proportions of the yellow (X_1), black (X_2) and red raspberries (X_3) in the jellies based on their physicochemical and sensory characteristics. The design and experimental levels for the three factors are presented in Table 1.

Table 1 Level and composition of fruits in formulation of jelly

Formula	Level (%)			Yellow raspberry*	Black raspberry*	Red raspberry*
	X_1	X_2	X_3			
1	100	0	0	60	0	0
2	0	100	0	0	60	0
3	0	0	100	0	0	60
4	50	50	0	30	30	0
5	0	50	50	0	30	30
6	50	0	50	30	0	30
7	33	33	33	20	20	20

*Percentage of fruit in the jelly, considering that the pulp represents 60% of the formulation

Jelly Preparation

The preparation of mixed raspberry jellies was carried out in the Laboratory of Processing Plant Products at the Federal University of Lavras. The percentages of ingredients used to make the jellies formulations, as expressed in relation to the total weight (sugar and pulp), were 60% fruit pulp, 40% sugar and 1.5% pectin.

To process the jellies, a blend of fruit pulps was prepared (according to Table 1) and added to sucrose. The jellies were processed in an open pan heated by GLP (Macanuda, SC, Brazil). High-methoxyl pectin was added after the mixture reached a boil. At the end of the process, after the soluble solids reached 65° Brix, the cooking was stopped. The total soluble solids were determined by using a portable refractometer model RT-82 and the °Brix were measured at ± 25°C. The hot jellies were then poured into 250 mL sterile bottles, cooled in a container of water and ice and stored in a refrigerator at ± 7°C. Figure 1 describes the jelly processing.

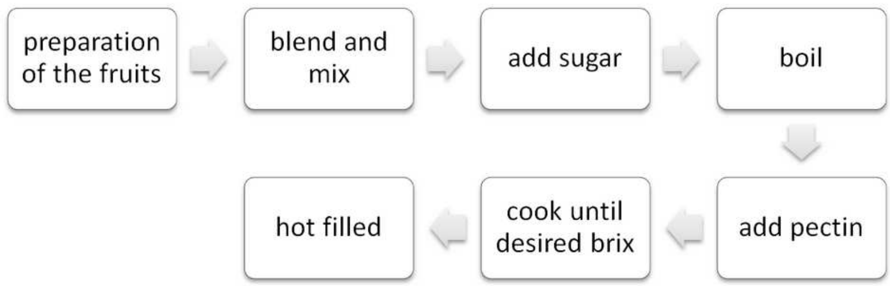

FIG. 1. Steps used in the preparation of jellies.

Sensory Analysis

Sensory analysis was performed in the laboratory of Sensory Analysis in the Food Science Department at the Federal University of Lavras. An acceptance test was conducted for attributes of color, taste, consistency and overall desirability using a hedonic 9-point scale (1 = dislike extremely 9 = like extremely) (Stone and Sidel, 1993).

The test was conducted with 90 participants (48 women and 42 men) who were students and office staff 18 and 40 years of age. Panelists were selected based on their regular consumption of fruit jams, jellies and preserves. In the sensory evaluation, each panelist evaluated 7 formulations in two sessions spread over two consecutive days. Four formulations were evaluated during the first session, and three formulations were evaluated during the final session.

Samples of approximately 5 g of jellies (Acosta, Víquez and Cubero 2008) were served in 50 mL cups at refrigerator temperature (7°C) in a balanced manner (Wakeling and MacFie, 1995). These samples were coded with three-digit numbers drawn from a table of random numbers. The test was conducted in individual booths under white light with adequate ventilation. Tasters were offered sufficient water for the analysis. The laboratory temperature was set at 23°C. The panelists were instructed to taste and evaluate each set of samples from left to right and to rinse their mouths with water between samples. In addition, the testers were instructed on the use of the hedonic scale.

Physicochemical analysis

Physicochemical analyses of the color (L*, a* and b*), pH, total acidity, soluble solids and soluble pectin were performed on the fruit blends (Table 1), and the same analyses, except for the soluble solids, total sugar and soluble pectin tests, were also conducted on the jelly formulations prepared with the fruit-pulp blends.

Three repetitions were performed for each physicochemical analysis. The titratable acids, soluble solids and pH values were determined according to the IAL, or Instituto Adolfo Lutz (2005). The method proposed by Dische (1962) was used for the determination of total sugar. The colors of the fruit pulps and jelies were determined according to the method described by Gennadios, Weller, Hanna and Froning (1996). The values of L*, a* and b* were determined using a Minolta CR 400 colorimeter with D65 (daylight) and CIELab patterns, where L* ranges from 0 (black) to 100 (white), a* varies from green (-) to red (+) and b* ranges from blue (-) to yellow (+). The pectin extraction was performed as indicated by McCready and McComb (1952) and was quantified as a percentage of galacturonic acid according to the colorimetric method described by Kintner and Van Buren (1982).

Statistical Analysis

Initially, the proportions of yellow (X_1), black (X_2) and red raspberry (X_3) in the mixed fruit jelly were compared to consumer acceptance and physicochemical parameters by a three-way external preference map obtained by PARAFAC (Nunes, Pinheiro and Bastos 2011; Nunes, Bastos, Pinheiro, Pimenta and Pimenta, 2012) using the SensoMaker software, version 1.6 (Pinheiro, Nunes and Vietoris, 2013).

A three-way array was arranged from matrices of i rows (i samples) and j + m columns (j consumers + m physicochemical measurements). These matrices were staked according to K consumer attributes (color, taste, consistency and overall liking), resulting in the three way array with i, j + m and k. The individual i x j + m matrices of the consumer acceptance attributes were previously standardized (correlation matrix). The i x m portion was the same for each i x j portion of the individual matrices (Nunes, et al. 2012). The PARAFAC model was optimized using the value of Core Consistency Diagnostics (CORCONDIA) to choose the number of factors (Bro, 1997; Nunes et al., 2011). PARAFAC procedures and the construction of a three-way preference map and three-way external preference map were previously reported in detail (Nunes et al. 2011; Nunes et al. 2012).

The other statistical analysis was based on the predicted model equation. A contour plot of the sensory attributes was generated and then superimposed to obtain the optimum region for better sensory acceptance. From the predicted optimum region, the optimum predicted point was selected, and the physicochemical parameters were estimated. Both the analyses of variance used to examine the significance of the data fit to the model and the triangular contour plots generated from the polynomial equations for each response were created using Statistica 6.0 for Windows (StatSoft Inc., USA, 2001).

RESULTS AND DISCUSSION

Physicochemical properties of the mixed raspberry pulp samples

Mean scores for the physicochemical properties of the mixed pulp samples from the raspberry jelly formulations are shown in Table 2. There was a significant difference ($p \leq 0.05$) in all of the studied physicochemical properties.

Table 2 Physicochemical properties of the pulp samples

	Physicochemical analysis							
Pulp samples	L^*	a^*	b^*	pH	Total acidity	Soluble solid	Total sugar	Pectin
P1	57.91^{a}	0.40^{f}	30.01^{c}	3.04^{bc}	1.87^{a}	10.00^{a}	6.52^{a}	0.56^{b}
P2	15.54^{f}	19.02^{e}	7.17^{f}	3.52^{a}	0.79^{c}	8.23^{b}	4.74^{b}	0.82^{a}
P3	32.45^{c}	37.09^{d}	20.77^{d}	2.98^{cd}	1.82^{a}	10.10^{a}	6.04^{a}	0.59^{b}
P4	22.42^{d}	34.62^{d}	13.00^{e}	3.07^{b}	1.28^{b}	8.67^{b}	4.69^{b}	0.54^{b}
P5	19.25^{e}	33.16^{de}	11.93^{e}	3.12^{b}	1.25^{b}	7.00^{c}	3.23^{c}	0.35^{c}
P6	37.35^{b}	33.14^{de}	21.28^{d}	2.93^{d}	1.65^{a}	8.67^{b}	4.58^{b}	0.39^{c}
P7	23.46^{d}	35.20^{d}	14.07^{e}	3.04^{bc}	1.54^{ab}	7.80^{bc}	3.00^{c}	0.54^{b}

In a column, means with no common superscripts are significantly different ($p < 0.05$).

Total acidity- g citric acidy/100g fw, soluble solid - °Brix; total sugar and pectin – g/100g fw.

P1- yellow raspberry; P2 – black raspberry; P3- red raspberry; P4 – 50% yellow and 50% black raspberry; P5- 50% black and 50% red raspberry; P6 – 50% yellow and 50% red raspberry; P7 – 33% yellow, 33% black and 33% red raspberry.

With regards to color, the yellow raspberry (P1) has larger parameter values for L* and lower values for b* and a*; these values refer to its main characteristic, a clear yellow fruit coloration. The black raspberry (P2) presented lower values for color parameters L* and b*, which refer to its intense black color. In turn, the red raspberry (P3) presented L* and b* color parameters situated between yellow and black raspberries and a higher a* color parameter. With regards to the pulp mixture, the combination of characteristics for each fruit alone creates new colorations; for example, when yellow raspberry (P1) is mixed with black or red (P4, P6 and P7) there is a general decrease in the L* and b* parameters and a significant increase in the a* parameter.

In relation to the pH and titratable acidity, the black raspberry (P2) has the highest pH and the lowest acidity, and the pulps from yellow (P1), red (P3) and combinations of the three raspberries generally showed similar pH and acidity levels. The pH of the fruit alone or in combination ranged from 2.98 to 3.52; this is the ideal range for the formulation of jams and jellies because, according to Jackix (1988), the optimum pH for jam and jelly gelation is between 3.0 and 3.4.

The values for soluble solids and sugar content varied from 7.00 to 10.10 (°Brix) and from 3.00 to 6.52%, respectively. Generally, the black raspberry (P2) had the lowest soluble solids and total sugars, and the yellow (P1) and red (P3) raspberries had similar values and the highest total solids and sugars. In addition to contributing to the formation of a gel, sugar acts as a preservative to inhibit the growth of microorganisms because it increases the osmotic pressure with a consequent reduction in the water activity (Ferreira et al. 2004).

The black raspberry (P2) had the highest levels of soluble pectin, and yellow (P1) and red raspberries (P3) did not differ between themselves, and they had the lowest levels. Pectin plays an essential role in the formulation of jams and jellies, contributing to the formation of gel and consequently influencing the texture and consistency of the final product. The pectin content needed for preparing jams and jellies varies from 0.5% to 1.5% for pulp mass + sugar (Jackix, 1988).

Sensory and physicochemical analysis of raspberry jelly formulations

Figure 2 shows the three-way external map that represents the distribution of consumers, samples, consumers' sensory attributes related to acceptance and physicochemical properties. The PARAFAC was fixed with two factors, which led to a concordia value of 92.06% and a variance value of 42.32%.

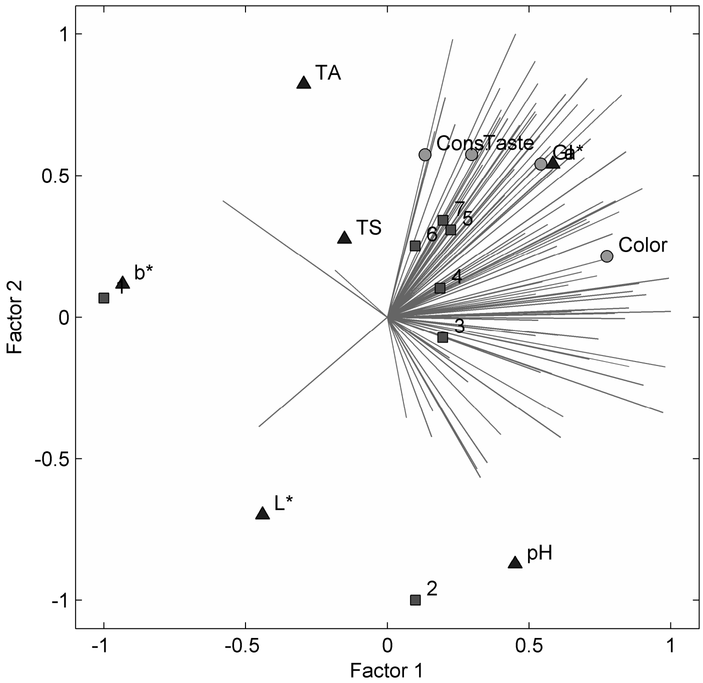

FIG. 2. Three-way external preference map for sensory attributes (color, taste, consistency and overall liking (OL)) and physicochemical properties (color L*, a* and b*, pH, total acidity (TA) and total sugar (TS)) for the mixed raspberry jelly formulations.

F1- yellow raspberry; F2 – black raspberry; F3- red raspberry; F4 – 50% yellow and 50% black raspberry; F5- 50% black and 50% red raspberry; F6 – 50% yellow and 50% red raspberry; F7 – 33% yellow, 33% black and 33% red raspberry

Mean scores for the sensory characteristics and the physicochemical properties of the mixed raspberry jelly formulations are shown in Table 3.

Table 3 Sensory characteristics and physicochemical properties of the mixed raspberry jelly

	Sensory attributes				Physicochemical parameters					
F	Color	Taste	Consistency	OL	L^*	a^*	b^*	pH	TA	TS
1	3.05	6.15	6.69	5.37	24.45	1.97	0.83	3.17	1.32	52.88
2	6.99	6.10	5.85	6.22	13.50	3.22	-0.59	3.59	0.68	43.14
3	7.70	6.69	6.74	6.91	20.70	3.82	-0.25	3.24	1.32	52.75
4	7.52	6.95	6.85	7.08	21.72	2.34	-0.88	3.37	0.94	37.56
5	7.77	7.31	7.00	7.32	16.31	6.40	-0,70	3.27	1.37	60.17
6	7.24	6.84	6.80	6.94	21.59	5.89	-0.02	3.23	1.06	66.15
7	7.70	7.03	6.94	7.22	16.53	6.22	-0.58	3.36	1.15	35.15

Overall liking (OL); Total acidity (TA) - g citric acidy/100g fw, total sugar (TS) - g/100g fw.

F1- yellow raspberry; F2 – black raspberry; F3- red raspberry; F4 – 50% yellow and 50% black raspberry; F5- 50% black and 50% red raspberry; F6 – 50% yellow and 50% red raspberry; F7 – 33% yellow, 33% black and 33% red raspberry.

As shown by the three-way external preference map-TWEPM (Figure 2), samples F1 and F2, which correspond to the jellies with 100% yellow raspberry and 100% black raspberry, respectively, were less preferred.

Analyzing the TWEPM and the numerical values shown in Table 3, it is observed that F1 is mainly characterized by higher b* values (0.83), which is explained by the specific color of the yellow raspberry. F2 stood out in relation to the lower color parameter L* (13.50) and higher pH (3.59), which is characteristic of black raspberry pulp (Table 2). The remaining formulations (F3, F4, F5, F6 and F7), which correspond to 100% red raspberry and combinations of three raspberry types, were preferred to the color, taste, consistency and overall liking attributes (Figure 2). These formulations stood out in relation to the higher a* values (between 2:34 and 6:40), which are associated with intense red coloration (Figure 2 and Table 3).

Table 3 shows that the yellow raspberry jelly (F1) generally resulted in higher acceptances for taste and consistency and was similar to other formulations, with an average hedonic term of "like slightly" and "like moderately." However, the acceptance was well below average for the color attribute, which was situated between the hedonic terms "dislike moderately" and "slightly disliked". This low acceptance for the overall impression - which was also lower than that of other formulations, with an average score between "neither liked/disliked”and "like slightly” - was most likely influenced by thecolor parameter. Thus, it is clear that F1 had a lower acceptance than the others because of its yellow color, which negatively influenced the acceptance of the color attribute and consequently influenced the overall impression. The yellow color is most likely not associated with the typical red raspberry color to which consumers are accustomed.

From Figure 2 and Table 3, it is clear that the formulation with 100% black raspberries (F2) had lower average acceptance for all of the analyzed sensory parameters and presented average scores for color, flavor and overall impression ranging between the hedonic terms "like slightly" and "like moderately," and F2 had a medium consistency score ranging between "not liked/disliked" and "like slightly." Through TWEPM (Figure 2), it is clear that this formulation had markedly lower values for the color parameter L* and higher pH values. The very dark coloring and high pH (less acidity) in the jelly are characteristic of black raspberries (Table 2) and led to a lower acceptance score than the other formulations. The consumers appear to prefer a raspberry jelly with a certain acidity, such as that obtained with the yellow raspberry, whereas the black color of raspberry jam seems to be desirable although it does not remind the consumer of the red raspberry to which they are accustomed.

Although a texture analysis was not performed, 100% black raspberry jelly possibly presents a superior consistency to the others, due to the higher pectin content in the black raspberry compared to other raspberries and their mixtures (Table 2). This may explain the lower than average grade for the sensory attribute consistency for this formulation over the others.

The other formulations (F3, F4, F5, F6 and F7) were the most widely accepted for all of the sensory attributes (Figure 2), with average scores located between the hedonic terms "like slightly" and "like very much" (Table 3). Figure 2 illustrates that the physicochemical attribute that correlates with these formulations was the color parameter a*. Table 2 shows that these formulations generally had higher values for this parameter. This finding reinforces the idea that the consumer has a preference for raspberry jellies with a reddish color.

From Table 3 and Figure 2, it is observed that when raspberry jellies are prepared only with yellow or black raspberries, they do not result in good consumer acceptance, but when you combine two or three raspberries (including red raspberries), acceptance is noticeably greater.

Sensory optimization of raspberry jelly

The sensory and physicochemical data were subjected to response surface methodology analysis using response surface regression (RSREG), and a predicted equation was developed for each attribute (Table 4). A complete quadratic model was fit to the dependent variables, except for color L^* and pH, which were fit to linear models. All models presented R^2 values greater than 0.8 and significant ($p \leq 0.05$) regressions, indicating that they were suitable for predictions (Henika, 1982).

A contour curve was plotted (Figure 3) using the equation for predicted overall liking (Table 4).

Table 4 Predicted model for sensory and physicochemical data to the raspberry reserve formulations

Attribute	Predicted model	R^2 value
Color	$Y=3.08X_1+7.01X_2+7.73X_3+9.52X_1X_2+6.99X_1X_3+1.25X_2X_3$	0.99
Taste	$Y=6.17X_1+6.12X_2+6.70X_3+2.98X_1X_2+1.35X_1X_3+3.33X_2X_3$	0.97
Consistency	$Y=6.69X_1+5.86X_2+6.75X_3+2.21X_1X_2+0.21X_1X_3+2.67X_2X_3$	0.99
Overall Liking	$Y=5.38X_1+6.24X_2+6.92X_3+4.85X_1X_2+2.94X_1X_3+2.74X_2X_3$	0.99
L^*	$Y=24.88X_1+13.61X_2+19.71X_3$	0.89
a^*	$Y=1.92X_1+3.17X_2+3.77X_3-0.04X_1X_2+12.96X_1X_3+12.50X_2X_3$	0.98
b^*	$Y=0.82X_1-0.60X_2-0.26X_3-3.85X_1X_2-0.93X_1X_3-0.97X_2X_3$	0.99
pH	$Y=3.19X_1+3.55X_2+2.21X_3$	0.84
Total acidity	$Y=1.31X_1+0.68X_2+1.32X_3-0.21X_1X_2-0.97X_1X_3+1.47X_2X_3$	0.99

X_1 – yellow raspberry; X_2 – black raspberry; X_3 – red raspberry

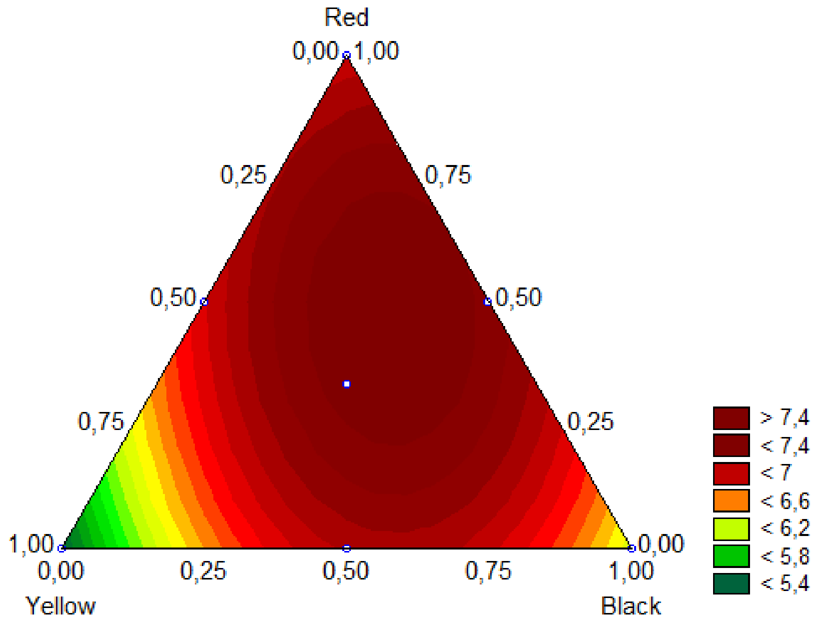

FIG. 3. Contour plot for overall liking to the raspberry jelly

The contour curve shows the optimal region where obtainable middle notes of sensory acceptance were at 7.4, containing 0-40% yellow raspberries, 20-50% black raspberries and 25-75% red raspberries. The response surface confirms the hypothesis proposed earlier, in which the yellow and black raspberries contribute less to the sensory acceptance of the jellies, and the larger their proportions in a given formulation, the lower its acceptance.

During studies on the phenology and yield of raspberry cultivars in subtropical Brazil, Moura et al (2012) concluded that the black raspberry stands out, is an excellent option for cultivation, has excellent adaptability, is high yielding (18.2 to 25 Mg ha^{-1}) and has good fruit quality when compared with the yellow (5 Mg ha^{-1}) and red raspberries 'Batum' (4.4 Mg ha^{-1}).

Based on the common optimal region of acceptance and economic feasibility (as related to the adaptation, productivity and quality of raspberries), the optimal jelly formulation had the following proportions: 50% of black raspberries, 20% of yellow raspberries and 30% of red raspberries. These levels were put into the predicted models to determine the average acceptance score and the value of the physical chemical characteristics for the mixed raspberry jelly within the optimum region of acceptance. Thus, for a formulation containing 50% black, 20% yellow and 30% red raspberries, the average sensory attribute scores are as follows: 8.18 for color, 7.18 for flavor, 6.92 for consistency and 7.34 for overall liking. For the physical and chemical attributes, the jelly presents color parameters L* of 17,69, a* of 5.76, b* of -0.80. The pH is 3.08, and the acidity is 1.14 g/100 g of citric acid.

From these results, producing a mixed raspberry jelly seems feasible and is an interesting alternative to use the yellow and black raspberries. When combined, raspberries make a jelly with better acceptability than when processed alone, the justification for the lower acceptance of raspberry jelly prepared from just black or yellow fruits is a consequence of their coloring.

CONCLUSION

A jelly formulated with a mix of colored raspberries grown in subtropical regions is a viable and alternative way to use yellow and black raspberries. The mixed raspberry jelly must have 0-30% yellow raspberries, 25-50% black raspberries and 30-75% red raspberries. Within this region, the optimum formulation has ideal characteristics that are often not observed in formulations with 100% black or yellow raspberries, thus, it is clear that it is not feasible the jelly processing with black and yellow raspberry only, however when in combination with red raspberry the jelly presented high sensory acceptability.

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ARTIGO 3: ANALYSIS OF THE SUBTROPICAL BLACKBERRY CULTIVAR POTENTIAL IN JELLY PROCESSING

Normas da Revista Científica Food Research International -
ISSN: 0963-9969 (versão preliminar)

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ABSTRACT

In Brazil, there are numerous blackberry cultivars under cultivation, however, only a few cultivars, such as the Guarani, are displayed/used for processing. In this context the aim of this work was to study the effects of different Brazilian blackberry cultivars (Guarani, Brazos, Comanche, Tupy, Cherokee, Caingangue and Choctaw) on the physicochemical characteristics, texture profiles and the consumer acceptance of the resulting jelly to identify the potential use of these cultivars in the jelly industry. It is feasible to produce blackberry jellies with the Tupy, Comanche, Brazos, Guarani and Choctaw cultivars because these jellies demonstrated good acceptability combined with good productivity. Consumers were shown to have a preference for a softer blackberry and less consistent jelly.

Keywords: blackberry, Brazilian cultivars, jelly

1. INTRODUCTION

Berries are small fleshy fruits that are typically eaten fresh or in products such as juice, jam, jelly, wine and syrup (Kubota, Ishikawa, Sugyyama, Fukumoto & Miyagi, 2012). Berry fruits are widely consumed in our diet and have attracted much attention due to their potential human health benefits, been and excellent source of bioactive compounds (Seeram, 2006 and Seeram, 2008).

Blackberry (*Rubus* sp.) fruit contains high levels of anthocyanins and other phenolic compounds, mainly flavonols and ellagitannins, which contribute to its high antioxidant capacity and other biological activities (Ali, Svensson, Alsanius & Olsson, 2011 and Kaume, Howard & Latha, 2012). The blackberries also have natural pigments, especially anthocyanin, which imparts an attractive color during fruit processing (Acosta-Montoya, Vaillant, Cozzano, Mertz, Pérez & Castro 2010).

The fragility and high post-harvest respiration rate of blackberries contributes significantly to their nutritional and microbiological deterioration, resulting in limited shelf-life and diminished quality and health benefits (Bower, 2007). Although blackberries are available in a fresh form (*in natura*), they are mainly consumed as frozen or thermally processed pulp in juices, jellies, jams and other products (Antunes, 2002 and Mota, 2006b). Berry jams, jellies and preserves are an important dietary form of berry fruits (Figuerola, 2007). Previous studies have indicated that a small portion of flavonols were lost during strawberry jam production (Häkkinen, Kärenlampi, Mykkänen & Torronen, 2000), and total phenolics were preserved during blackberry jam processing (Amakura, Umino, Tsuji & Tonogai 2000).

The cultivation of blackberries in Brazil began with the launch of the first Brazilian cultivars (Tupy, Guarani and Caingangue) by the Embrapa Temperate Climate breeding program (Fachinello, Pasa, Schmtiz & Betemps, 2011). An estimated 400 blackberry cultivars were produced by this breeding program (Clark & Finn, 2011), but the most commonly cultivated types in Brazil are: Tupy, Guarani, Negrita, Caingangue, Brazos, Cherokee, Comanche and Ébano (Antunes, 2002). The cultivars have different characteristics in terms of productivity (Campagnolo and Pio, 2012b) and use. The cultivars Tupy and Guarani, for example, are recommended for fresh consumption because of their low acidity, and the Guarani is recommended for industrial production (Santos & Raseira, 1988). The blackberry has adapted well to the subtropical climate of Brazil, and has good productivity. Campagnolo and Pio (2012a) recorded 6430 kg/ha^{-1} with the Tupy cultivar in Helena, PR. In the municipality of Marechal Candido Rondon, PR, which has similar climatic conditions, the grain yields for Brazos was 18602.5 kg ha^{-1}, 15129.8 kg ha^{-1} for Guarani, 11395.9 kg ha^{-1} for Choctaw and 9412.5 kg ha^{-1} for Comanche (Campagnolo & Pio, 2012b).

According to Mota (2006b), blackberry cultivation has been encouraged because of its potential for commercialization and industrialization. In Brazil, there are numerous blackberry cultivars under cultivation (Antunes, 2002); however, only a few cultivars, such as the Guarani, are displayed/used for processing (Santos and Raseira, 1988).

In this context, the aim of this work was to study the effects of different blackberry cultivars (Guarani, Brazos, Comanche, Tupy, Cherokee, Caingangue and Choctaw) on the physicochemical and texture profiles and the consumer acceptance of the resulting jelly to identify the potential use of these cultivars in the jelly industry.

2. MATERIALS AND METHODS

2.1 Ingredients

Jellies were made from the pulp of seven blackberry cultivars grown in the subtropical climate of Minas Gerais state in Brazil, namely, Guarani, Brazos, Comanche, Tupy, Cherokee, Caingangue and Choctaw. The fruits used for jelly preparation were acquired from an orchard at the Federal University of Lavras, Minas Gerais, Brazil. The fruits were harvested at physiological maturity, which was determined by their color and size in the morning, and kept refrigerated in a cold room at 18°C until processing. In addition to the fruits, sucrose and high-methoxyl pectin were also used in the jelly (Danisco®, SP, Brazil). Citric acid was not added during preserve preparation because the pH of the fruits (3.32-3.41) was not suitable.

2.2 Jelly formulation

Seven blackberry jellies were prepared and the only variation between the formulations was the blackberry cultivar. The prepared jellies were then subjected to physicochemical, texture and sensory analyses.

Following Acosta, Víquez, Cubrero & Morales (2006), the blackberries were thawed by immersion in potable water at 25°C for 30 min. The fruit was drained in a plastic colander to eliminate excess water and manually sorted for apparent physical and microbiological damage. The fruits were then beaten in a blender and were sieved following homogenization to obtain clarified juice, and finally, the clarified juice was packaged immediately in high-density polyethylene bags (250 g per bag) and stored at -20°C for 2 days (the time necessary to process all formulations).

The blackberry jelly preparation was conducted in the Laboratory of Processing Plant Products at the Federal University of Lavras. The percentages of ingredients used to formulate jellies, as expressed in relation to their total weights (sugar and pulp), were 60% fruit pulp, 40% sugar and 1.5% pectin.

To process the jellies, fruit pulps were added to sucrose and were then processed in an open pan heated by a gas flame (Macanuda, SC, Brazil). After reaching a boil, high-methoxyl pectin was added. At the end of the process, after the soluble solids reached 65° Brix, the cooking was halted. The total soluble solids were determined by using a portable refractometer model RT-82 and the °Brix was measured at ± 25°C. The hot jellies were then poured into 250 mL sterile bottles, cooled in a container of water and ice and stored in a refrigerator at ± 7°C. Figure 1 describes the jelly processing.

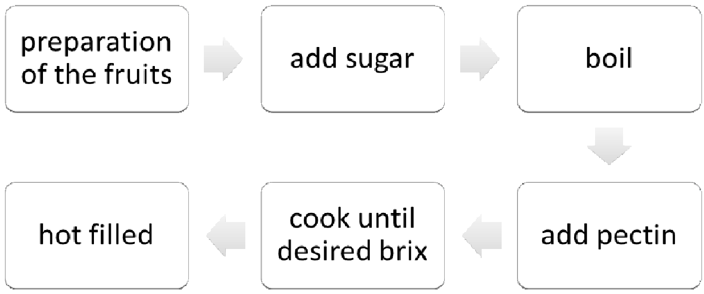

Figure 1 Steps used in the preparation of blackberry jellies

2.3 Sensory Analysis

Sensory analysis was performed in the laboratory of Sensory Analysis at the Food Science Department of the Federal University of Lavras. An acceptance test was conducted on the attributes of color, taste, consistency and overall desirability by using a hedonic scale of 9 points (1 = dislike extremely 9 = like extremely) (Stone & Sidel, 1993).

The test was conducted with 90 participants (55 women and 35 men) who were students and office staff aged between 18 and 45 years. Panelists were selected based on their regular consumption of fruit jams, jellies and preserves. During the sensory evaluation, each panelist evaluated seven formulations over two sessions spread over two consecutive days. Four formulations were evaluated during the first session, and three formulations were evaluated for the final session.

Samples of approximately 5 g of jelly (Acosta, Víquez & Cubero, 2008) were served in 50 mL cups at refrigerator temperature (7°C) in a balanced manner (Wakeling & MacFie, 1995). These samples were coded with three-digit numbers drawn from a table of random numbers. The test was conducted in individual booths under white light with adequate ventilation. Tasters were offered sufficient water for the analysis. The laboratory temperature was set at 23°C. The panelists were instructed to taste and evaluate each set of samples from left to right and rinse their mouths with water between samples. In addition, testers were instructed on the use of the hedonic scale.

2.3 Physicochemical analysis

Physicochemical analyses of color (L^*, a^* and b^*), pH, total acidity, soluble solids, total sugar and soluble pectin were performed for the blackberry cultivars, as well as physicochemical analyses of color (L^*, a^* and b^*), pH and total acidity were performed in the jellies.

Three repetitions were performed for each physicochemical analysis. The titratable acidity, soluble solids and pH values were determined according to the IAL- Instituto Adolfo Lutz (2005). The method proposed by Dische (1962) was used for the determination of total sugar. The colors of the fruit pulps and jellies were determined according to the method described by Gennadios, Weller, Hanna & Froning (1996). The values of L*, a* and b* were determined by using a Minolta CR 400 colorimeter with D65 (daylight) and CIELab patterns, where L * ranges from 0 (black) to 100 (white), a * varies from green (-) to red (+) and b* ranges from blue (-) to yellow (+). The pectin extraction was performed as indicated by McCready & McComb (1952) and quantified as a percentage of galacturonic acid according to the colorimetric method of Kintner & Van Buren (1982).

2.4 Texture profile analysis

Texture profile analysis (TPA) is a method for evaluating sensory properties. The test consists of compressing the food (study sample) twice in a reciprocating motion to mimic the action of the mandible; a first compression and relaxation followed by a second compression are performed during testing. This test yields a graph of force versus time from which the texture parameters are calculated (Bourne, 2002; Herrero, Ordónez, Avila, Herranz, Hoz & Cambero, 2007; Honikel, 1998 and Lau et al., 2000).

The texture profile analyses (TPA) were performed in penetration mode under the following conditions (Pereira et al. 2013): a pre-test speed of 1.0 mm/s, a test speed of 1.0 mm/s, a post-test speed of 1.0 mm/s, a time interval between penetration cycles of 5.0 s, a distance of 20.0 mm and a compression with a 6.0 mm diameter cylindrical aluminum probe using the Stable Micro Systems Model TA-XT2i texturometer (Goldaming, England). The jelly samples were compressed by approximately 30%. The parameters analyzed were hardness, adhesiveness, springiness, cohesiveness, gumminess and chewiness. The test was performed in triplicate. The analyses were conducted in the packaging containing the blackberry jellies (height: 50.50 mm, diameter: 100.70 mm).

2.5 Statistical analysis

To correlate physicochemical characteristics with the blackberry fruit pulps and the physicochemical and texture characteristics with jelly formulations, the physicochemical and texture data were analyzed by a principal component analysis (PCA). The data set was arranged in a matrix of 7 lines (samples) and 8 (pulp physicochemical analysis) or 11 columns (jelly physicochemical and texture parameters). The data were standardized (correlation matrix) and the PCA was applied. The PCA score plot and loading was built from the first two principal components.

The acceptance data for the different formulations were analyzed by using an internal preference map. Matrices to each attribute with 7 lines (samples) and 90 columns (consumers) were staked according to acceptance attributes, resulting in a 7 x 90 array. The individual matrices of the acceptance attributes were previously standardized (correlation matrix) and then plotted.

Data analysis was performed with SensoMaker software version 1.6 (Pinheiro, Nunes & Vietoris, 2013).

3. RESULTS AND DISCUSSION

3.1 Physicochemical analysis of blackberry cultivars

A principal component analysis (PCA) was generated to correlate the physicochemical analyses with the different blackberry cultivars (Figure 2).

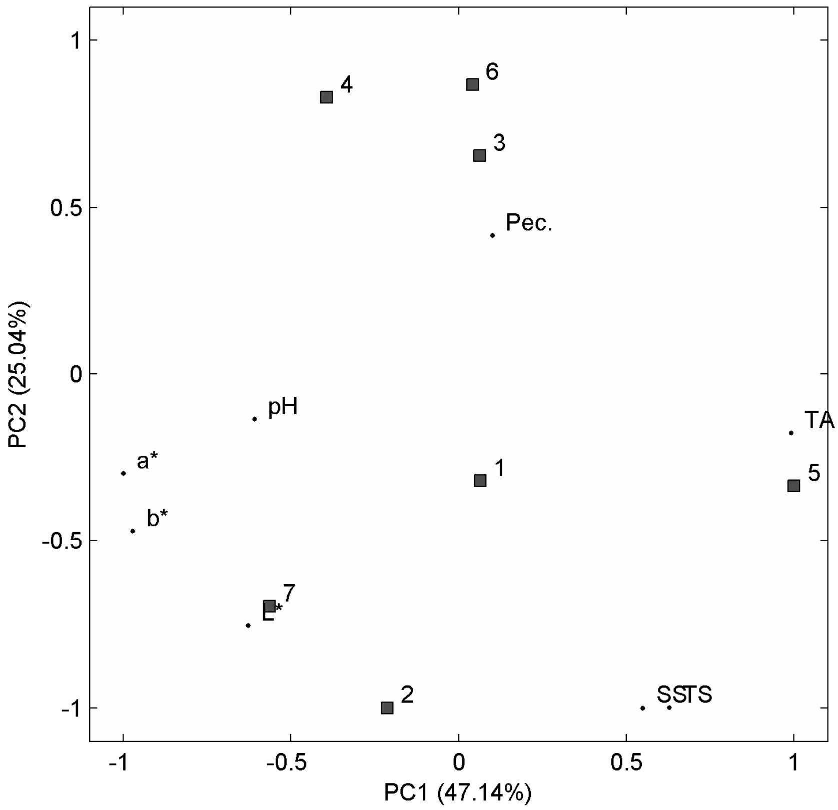

Figure 2 Principal Component Analysis (PCA) for the physical and chemical attributes of different blackberry samples

Physical and physical chemical parameters: TA - total acidity; SS – soluble solids; TS – total sugar.
Sampleformulations: 1- Guarani cultivar; 2 –Brazos cultivar; 3- Comanche cultivar; 4 – Tupy cultivar; 5- Cherokee cultivar; 6 –Caingangue cultivar; 7 –Choctaw cultivar.

The mean scores for the different blackberry cultivar physicochemical properties used in the formulations of blackberry jelly are shown in Table 1.

Table 1 Physicochemical properties of the different blackberry cultivars

Blackberry Cultivar	Physicochemical analysis							
	L^*	**a^***	**b^***	**pH**	**Total acidity**	**Soluble solid**	**Total sugar**	**Pectin**
C1	16.58	22.67	4.01	3.32	1.28	8.0	4.68	0.36
C2	17.30	25.89	6.22	3.32	1.15	8.7	5.35	0.35
C3	14.71	20.56	2.91	3.41	1.15	7.3	4.11	0.56
C4	15.72	25.73	5.40	3.34	1.19	4.9	3.21	0.48
C5	15.41	16.34	1.47	3.27	1.73	9.7	5.86	0.52
C6	16.32	20.38	3.34	3.25	1.17	5.0	3.75	0.45
C7	20.12	25.62	6.51	3.41	1.09	7.7	4.57	0.55

Total acidity- g citric acidy/100 g fw; soluble solid - °Brix; total sugar and pectin – g/100 g fw.
C1- Guarani cultivar; C2 –Brazos cultivar; C3- Comanche cultivar; C4 –Tupy cultivar; C5- Cherokee cultivar; C6 –Caingangue cultivar; C7 –Choctaw cultivar.

Through the PCA (Figure 2), it is possible to see that cultivars C7 (Choctaw) and the lower intensity C2 (Brazos) were characterized by higher Hunter L* values (20.12 and 17.30, respectively), a* values (25.62 and 25.89, respectively) and b* values (6.51 and 6.22, respectively) (Table 1). These samples have a less intense black and a more intense red coloration than the other cultivars, which is typical of blackberries at the less mature stages.

The cultivars analyzed in this study showed a higher intensity of black (L*) and Hunter a* and b* values, which were relatively similar to those found in studies by Hirsch, Facco, Rodrigues, Vizzotto & Emanuelli (2012) for different blackberry cultivars developed in Brazil. The difference between the color parameters of different cultivars can be explained by differences inherent to the fruit or between the stages of maturation, although care has been taken to collect all cultivars at similar maturation levels. According to Tosun, Ustun & Tekguler (2008), the Hunter L* value of blackberries tends to decrease with fruit ripening as the color becomes deep/dark. According to Tosun, Ustun & Tekguler (2008), because it is an index of redness and greenness, the Hunter a^* value increases during the early fruit ripening stages, but the Hunter a value decreases during the ripe mature stage because of its violet color. The Hunter b^* value, which expresses yellowness and blueness, decreased with maturity.

Cultivar C5 (Cherokee) was run through the PCA (Figure 2), and it was characterized by a higher acidity (1.73g citric acidy/100 g fw).Cultivars C2 (Brazos) and C5 (Cherokee) had higher soluble solids (8.7 and 9.7, respectively) and sugars (5.35 and 5.86, respectively) (Table 1).

The different blackberry cultivars had pH values ranging 3.25 to 3.41 and acidity ranging from 1.09 to 1.73 g citric acid/100 g (Table 1). Mota (2006b); Hassimoto, Mota, Cordenunsi & Lajolo (2008) and Hirsch, Facco, Rodrigues, Vizzotto & Emanuelli (2012) found that the pH of the fruits ranged from 2.78 to 3.83 and the titratable acidity ranged 1:24 to 1:58, values similar to those found in the studies of the physicochemical characteristics from similar blackberry cultivars. The pH of the fruit is considered ideal for formulating jams and jellies because, according

Jackix (1988), the optimum pH for jam and jelly gelation should be between 3.0 and 3.4.

The soluble solids and total sugars in the blackberry cultivars ranged from 5.0 to 9.7 °Brix and 3.21 to 5.86%, respectively (Table 1). The soluble solid and total sugar levels are consistent with the range found in the literature for studies on different Brazilian cultivars and different blackberry cultivars from Turkey at the optimum stage of maturity (Mota, 2006b; Hassimoto, Mota, Cordenunsi & Lajolo, 2008; Tosun, Ustun & Tekguler 2,008; Hirsch, Facco, Rodrigues, Vizzotto & Emanuelli, 2012).

Through the PCA (Figure 2), blackberry cultivars C3 (Comanche), C4 (Tupy) and C6 (Caingangue) were characterized by a higher pectin content (0.45 to 0.56) (Table 1). In the jam and jelly formulation, pectin plays an essential role in the formation of the gel and consequently influences the texture and consistency of the final product, and the ideal pectin content for preparing jams and jellies varies from 0.5% and 1.5% (Jackix, 1988).

3.2 Physicochemical and textural analyses of blackberry jelly formulations

The principal component analysis (PCA) was generated to correlate the physicochemical and texture analyses with the blackberry jellies (Figure 3).

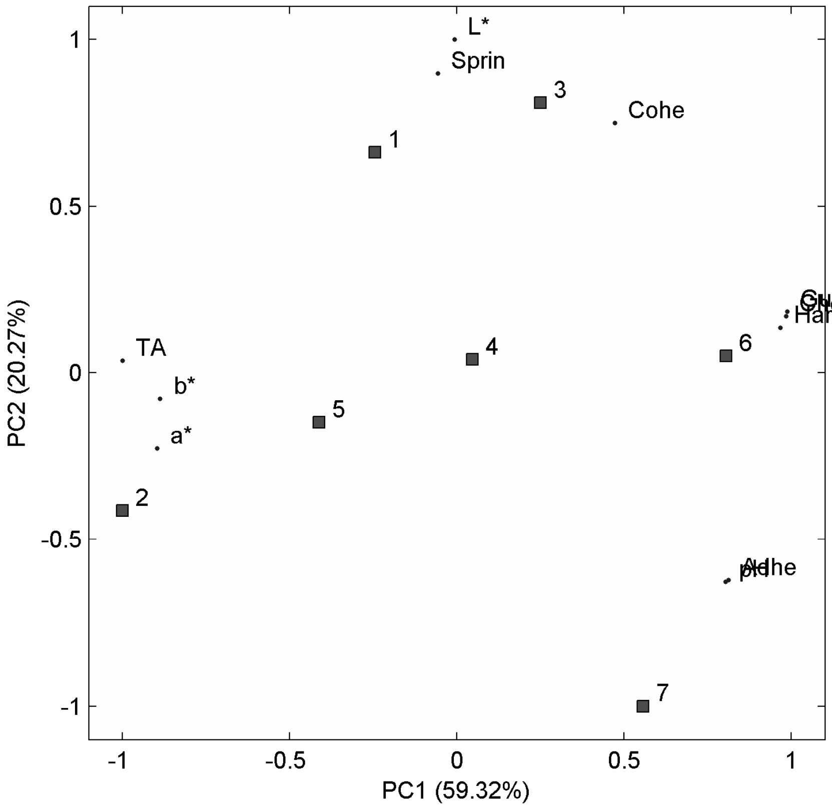

Figure 3 Principal Component Analysis (PCA) for the different samples of blackberry jelly and physical and chemical attributes of texture.

Physicochemical and texture parameters: Gumm - gumminess; Chew- chewiness; Hard - hardness; Sprin - springiness; Cohe - cohesiveness; Adhe - adhesiveness; TA - total acidity

Samples: 1- Guarani cultivar; 2 –Brazos cultivar; 3- Comanche cultivar; 4 –Tupy cultivar; 5- Cherokee cultivar; 6 –Caingangue cultivar; 7 –Choctaw cultivar.

The mean scores for physicochemical properties and the texture analysis of the blackberry jelly formulations are shown in Table 2.

Table 2 Physicochemical properties and texture parameters of the blackberry jelly formulations

	Physicochemical properties					Texture Parameters					
F	**L***	**a***	**b***	**pH**	**TA**	**Hard.**	**Adhe.**	**Spr**	**Coh**	**Gum**	**Chew**
1	23.98	4.95	-0.25	3.29	0.81	15.67	-17.45	0.95	0.34	54.15	47.92
2	21.59	8.34	0.76	3.33	0.90	11.57	-12.75	0.95	0.30	29.50	28.45
3	23.95	3.64	-0.54	3.33	0.77	28.76	-15.33	0.98	0.32	91.23	85.04
4	23.50	4.72	-0.17	3.36	0.81	24.25	-29.56	0.95	0.31	74.11	71.63
5	23.39	4.21	-0.34	3.29	0.81	13.26	-17.03	0.93	0.30	38.20	37.93
6	23.44	3.28	-0.53	3.48	0.72	32.80	-32.41	0.94	0.33	113.96	104.85
7	20.38	3.69	-0.79	3.49	0.72	26.14	-37.50	0.93	0.31	83.74	77.15

Total acidity (TA) - g citric acidy/100 g fw; total sugar (TS) - g/100 g fw.
Hard. – Hardness (N); Adhe – Adhesiveness (N/s); Spr – Springiness; Coh – Cohesiveness; Gum – Gumminess (N); Chew – Chewiness.
Samples: 1- Guarani cultivar; 2 –Brazos cultivar; 3- Comanche cultivar; 4 –Tupy cultivar; 5- Cherokee cultivar; 6 –Caingangue cultivar; 7 –Choctaw cultivar.

With regards to color, the jelly formulations had Hunter L* values (Table 2) ranging from 20.38 to 23.98, a* values ranging from 3.28 to 8.34 and b* values ranging from -0.25 to 0.76 (Table 2). According to the PCA (Figure 3) and Table 2, formulation F1 (Guaraní) and F3 (Comanche) stood out as having the highest Hunter L* value (23.98 and 23.95), so this jelly formulations has the lowest intensity of black coloration. In Table 1, it is apparent that this cultivars were not among those with a lower intensity black color; however, factors such as the reduced time required to acquire the final jam Brix could lead to a clearer appearance because of the lower intensity of the reactions, such as the Maillard. In relation to the Hunter a* and b* values, formulation F2

(Brazos) stood out for its higher rates of these parameters (8.34 and 0.76, respectively) (Figure 3 and Table 2). According to Table 1, it was this cultivar that had the highest values for these color parameters.

When compared with the samples without processing, the Hunter L* value for jellies increased and the Hunter a* and b* values decreased, indicating that the jelly had a lower intensity of black and red colors compared to the fresh fruit. Patras, Brunton, Pieve & Butler, (2009) observed that the thermal treatment caused the decrease of Hunter a^* value (redness) in blackberry purées compared to unprocessed samples, and according to these authors, the change in the instrumental parameters can be explained by the degradation of anthocyanin with processing because this pigment is mainly responsible for the red color of these fruits. Mota (2006a); Mota (2006b); Wu, Frei, Kennedy & Zhao (2010) and Gancel, Feneuil, Acosta, Pérez & Vaillant (2011) found that the cooling and heating of blackberry pulp causes considerable anthocyanin losses, and this degradation may vary from 8.8% to 52%. Among other factors, the loss of color at higher temperatures has also been attributed to increased rates of enzyme-mediated losses via enzymes, such as peroxidase, polyphenol oxidase and glucosidase (Cano, Hernandez & De Ancos, 1997).

With regards to acidity, the pH of the jellies ranged from 3.29 to 3.49 and acidity ranged from 0.72 to 0.90 g citric acid/100 g (Table 2). The PCA (Figure 3) and Table 2 show that formulation F7 (Choctaw) stood out for having the highest pH (3.49) and formulation F2 (cultivar Brazos) had the highest acidity (0.90) From Table 1, the Choctaw cultivar actually presented the lowest acidity (higher pH and lower titratable acidity) and the Brazos cultivar was not among those with higher acidity but might have acquired this trait with processing.

Mota (2006a) characterized different Brazilian blackberry jellies and found that the pH ranged from 3.36 to 3.47, and the acidity ranged from 1.22 to 1.79. Compared with fresh fruits, the jelly pH was slightly higher and the acidity was considerably lower. The acidity was inversely correlated to the pH, and the increase in pH and consequent decrease in total acidity may be explained by the changes that occurred during processing because the organic acids can be converted to sugars (Tosun, Ustun & Tekguler, 2008).

In relation to the texture profile of jellies in Figure 2, it is clear that formulation F6 (Caingangue) is highly correlated with the textural characteristics of hardness, gumminess and chewiness, and in Table 2, it is clear that this sample had the highest values for these parameters (32.80, 113.96 and 104.85, respectively). Hardness measures the force required to achieve a given deformation (Friedman, Whitney & Szczesniak, 1963, Bourne, 1968; Van Vliet, 1991), gumminess determines the force required to chew a semi-solid food (Oliveira et al., 2009) and chewiness is the quantity of energy required to simulate the mastication of a semi-solid sample to a steady state of swallowing (Huang, Kennedy, Li, Xu & Xie 2007). Thus, the Caingangue cultivar was used to produce a more rigid and firm jelly.

Jellies F1 (cultivar Guarani) and F3 (Comanche cultivar) are correlated with the texture parameters of elasticity (0.95 and 0.98, respectively) and cohesiveness (0:34 and 0:32, respectively). The cohesiveness of the rheological parameters is correlated with the properties of the food as it is swallowed, especially if it is in a solid state (Ishihara, Nakauma, Funami, Odake & Nishinari, 2011 and Lucas, Prinz, Agrawal & Bruce 2002). The cohesiveness parameters are then correlated with the food disintegration, for example, the lower the cohesiveness, the greater the disintegration during the first compression cycle (Extralab, 2010). Elasticity measures the speed with which the deformed material returns to its original condition after the deforming force is removed (Friedman, Whitney & Szczesniak, 1963, Bourne 1968; Van Vliet, 1991).

The jelly F7 (Choctaw) is correlated with the adhesiveness (Figure 3), presenting highest module value of this parameter (Table 2). The adhesion measures the amount of force to simulate the work needed to overcome the forces of attraction between the surface and the surface of food in contact with it (Bourne 1968; Van Vliet, 1991), thus the Choctaw cultivars produce a more adhesive jelly.

Several factors may explain the variation in texture between the jellies prepared with different blackberry cultivars; among them are the amount of sugar naturally present in each cultivar, pH and acidity, soluble pectin content, factors that can influence gelation and, consequently, the texture of the final product. In addition, other factors such as the moisture content and chemical composition of the fruit can influence the texture profile by influencing the cooking time, yield, and, hence, the moisture content of the final product (Oakenfull, 1987; Jackix, 1988; Gava, 1998 and Löfgren & Hermansson, 2007).

3.3 Sensory analysis of the blackberry jelly formulations

Figure 4 shows the internal preference map to the sensory attributes, which is a representation of the distribution of consumers and samples evaluated for consumer acceptance.

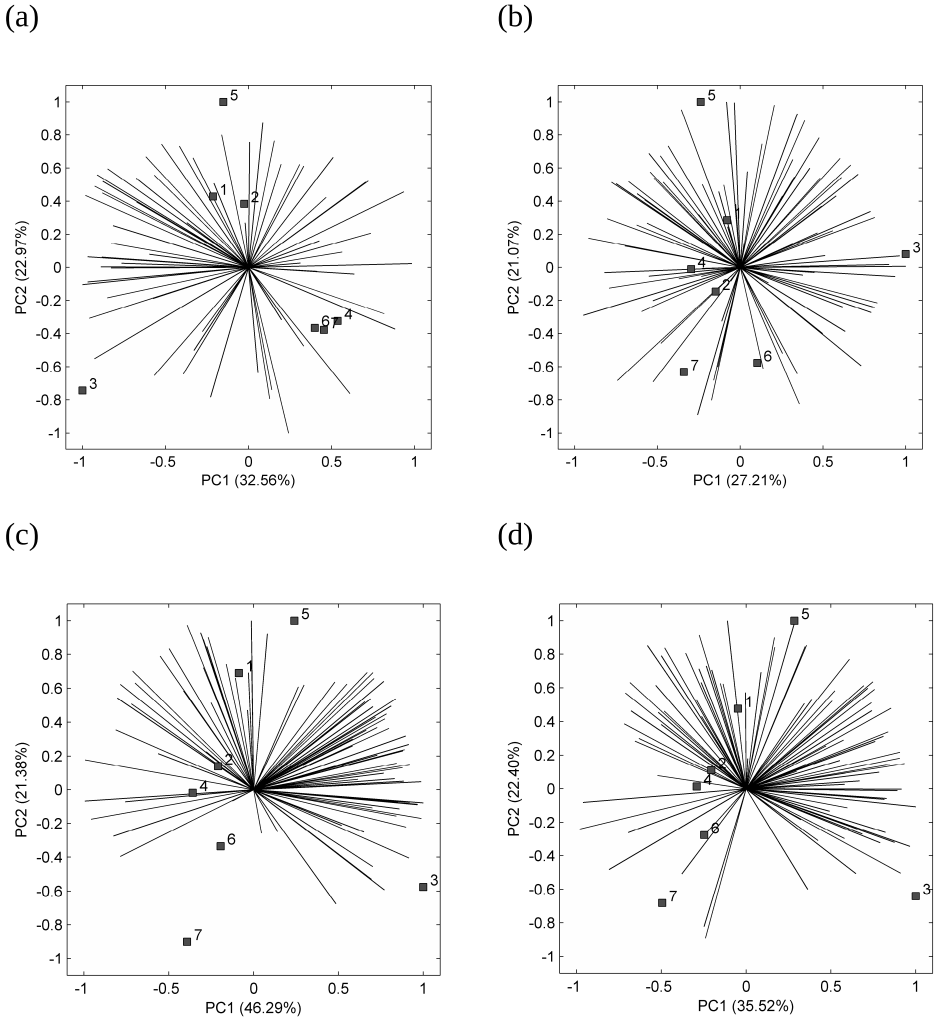

Figure 4 Internal preference map for the color (a), taste (b), consistency (c) and overall desirability (d) of the blackberry jelly formulations.

Samples: 1- Guarani cultivar; 2 –Brazos cultivar; 3- Comanche cultivar; 4 –Tupy cultivar; 5- Cherokee cultivar; 6 –Caingangue cultivar; 7 –Choctaw cultivar.

The mean scores for the sensory characteristics of the blackberry jelly formulations are shown in Table 3.

Table 3 Sensory characteristics of the blackberry jelly formulations

	Sensory Parameters			
Samples	**Color**	**Taste**	**Consistency**	**Overall Liking**
1	7.55	6.81	6.58	6.74
2	7.39	6.78	6.09	6.58
3	7.55	6.69	6.73	6.85
4	7.27	6.55	5.75	6.32
5	7.52	6.92	6.78	7.05
6	7.15	6.79	5.92	6.47
7	7.07	6.20	5.09	5.85

Samples: 1- Guarani cultivar; 2 –Brazos cultivar; 3- Comanche cultivar; 4 –Tupy cultivar; 5- Cherokee cultivar; 6 –Caingangue cultivar; 7 –Choctaw cultivar.

Through internal maps (Figure 4), it is clear that there was no preference for any of the formulations for the color (Figure 4a) or taste (Figure 4b). For consistency attributes and overall desirability, Figure 4c and Figure 4d indicate that consumers have well distributed around the samples, but formulations F6 (Caingangue) and F7 (Choctaw) were the least accepted. From Table 4, it is clear that, in general, all formulations had good acceptance grades for all sensory attributes evaluated, and the average scores for color range between the hedonic terms "liked moderately" and "liked very much",the scores for taste range between "liked slightly" and "liked moderately", and the scores for consistency and overall desirability range from "not liked/not disliked" and "liked very much".

Formulation F6 (Caingangue), which was among the least preferred for consistency and overall liking attributes (Figure 4 c and 4d), is characterized by a firmer and more rigid jelly because of its hardness, gumminess and chewiness (Figure 2 and Table 2); thus, consumers seem to have a preference for a jelly that is softer and less consistent. Negative results for the acceptance of jelly derived from Caingangue, coupled with a low production (Campagnolo & Pio, 2012B), indicate that this cultivar is not suitable for processing.

Formulation F7 (Choctaw), which together with F6 had the lowest acceptance, is characterized by the highest adhesion (Figure 2 and Table 2). Thus, consumers in addition to not want rigid and firm jelly, do not want a very adhesive jelly. This conclusion drawn earlier, that consumer prefers a softer and less consistent jelly.

Formulation F6 (Caingangue) and F7 (Choctaw) were also characterized by its pH (Figure 2) presenting the lowest acidity (Table 2). This fact may had contributed to the negative texture parameters to the consumers and probably an adjustment in the amount of added acid can improve their texture characteristics

Regarding the most preferred samples (F1, F2, F3, F4 and F5) (Figure 4), it is clear from the PCA, as shown in Figure 2, that formulation F1 (cultivar Guarani) is characterized by the color parameter L* (Figure 2), having presented the highest values for this parameter (Table 2) and F2 formulation (Brazos) was characterized by having the highest Hunter a* and b* color and higher acidity. However, because the sensory acceptance for this color and taste was similar for all samples, we cannot correlate consumer preference for these parameters. The other physicochemical attributes for the jelly formulations have not been further characterized (Figure 2).

In general, all blackberry cultivars showed good acceptability. Although acceptance for texture and overall liking for jellies elaborated with the Caingangue and Choctaw cultivars was below the other formulations, this fact can probably be reversed by adjusting the ingredients and process. Regarding the economic side, the cultivar Caingangue and Cherokee had low productivity, 3.44 kg/ha^{-1} and 3.01 kg/ha^{-1}, respectively (Campagnolo & Pio, 2012b), being that way, unviable for processing. Thus, based on their high acceptability and good productivity, Tupy, Comanche, Brazos, Guarani and Choctaw cultivars have the potential to be processed into jelly.

4. CONCLUSION

Although the different blackberry cultivars studied had different physicochemical characteristics, which were reflected in jellies with different physicochemical characteristics and texture profiles, the sensory acceptance was high and quite similar between samples. In conclusion, it is feasible to produce blackberry jellies with the Tupy, Comanche, Brazos, Guarani and Choctaw cultivars because these jellies demonstrated good acceptability combined with good productivity. Consumers were shown to have a preference for a softer blackberry and less consistent jelly.

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ARTIGO 4: JELLY PROCESSING EFFECT ON THE ANTIOXIDANT CAPACITY AND BIOACTIVE COMPOUNDS IN DIFFERENT BLACKBERRY CULTIVARS

Normas da Revista Científica Food Research International - ISSN: 0963-9969 (versão preliminar)

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ABSTRACT

The berries are very recognized by high antioxidant capacity and high content of bioactive compounds such as phenols, anthocyanins, flavonoids and vitamin C, which bring many health benefits. The effect of thermal processing and cultivar berries has been the subject of several studies, so that it can serve as a basis in order to lose as little as possible of bioactive compounds and antioxidant capacity of these fruits. In Brazil, there are numerous blackberry cultivars under cultivation, however, only a few cultivars, such as the Guarani, are displayed/used for processing. In this context, the aim of this work was to study how the bioactive compounds contents and antioxidant capacity of blackberry jellies processing were influenced by cultivars (Guarani, Brazos, Comanche, Tupy, Cherokee, Caingangue and Choctaw). It is concluded that occurs degradation of the bioactive compounds and reduction of antioxidant activity due to the jam processing and the degradation of these compounds was significantly different between the different blackberry cultivars. The Brazos and Caingangue cultivars stand out as suffering the smallest losses in processing, resulting in richer jellies in bioactive compounds and higher antioxidant capacity.

KEYWORDS: blackberry, jelly, antioxidant activity, bioactive compounds

1. INTRODUCTION

Berries are small fleshy fruits characterized by a high content and wide diversity of bioactive compounds (Szajdek & Borowska, 2008). Berry fruits are widely consumed in our diet and have attracted much attention due to their potential human health benefits (Seeram, Adams, Zhang, Lee, Sand, Scheuller & Heber, 2006; Seeram, 2008). Most of the health benefits of these fruits are believed to be a result of their antioxidant properties (Beekwilder, Hall & de Vos, 2005). Berries are rich in phenolic compounds, such as phenolic acids, tannins, stilbenes, flavonoids and anthocyanins, but berries, in particular, have been the focus of considerable research regarding their anthocyanin-rich properties (Kubota, Ishikawa, Sugyyama, Fukumoto & Miyagi, 2012).

Blackberry (*Rubus* sp.) fruit contains high levels of anthocyanins and other phenolic compounds, mainly flavonoids and ellagitannins, which contribute to its high antioxidant capacity and other biological activities (Ali, Svensson, Alsanius & Olsson, 2011; Kaume, Howard & Latha, 2012). However, the fragility and high postharvest respiration rate of blackberries contributes significantly to their nutritional and microbiological deterioration, resulting in limited shelf-life and diminished quality and health benefits (Bower, 2007). Due to the fact that these fruit are highly perishable most fresh blackberries are converted into frozen, dried, and canned products, or processed into jams, jellies, and juices for longer storage to satisfy various markets and consumer demands (Rickman, Barrett & Bruhn, 2007).

Berry jams, jellies and preserves are an important dietary form of berry fruit, been an excellent sources of nutritional substances with antioxidant potential. (Kim & Padilla-Zakour, 2004; Figuerola, 2007; Šavikin, Zdunić, Janković, Tasić, Menković, Stević & Đorđević, 2009; Levaj, Kovačević, Bituh & Dragovic-Uzelać, 2012). The extent to which bioactive compounds are preserved in blackberry products depends heavily on the specific processing technology, blackberry variety, production location, maturity, time of harvest and storage conditions (Rickman et al., 2007). The effect of thermal processing and cultivar berries has been the subject of several studies, so that it can serve as a basis in order to lose as little as possible of bioactive compounds and antioxidant capacity of these fruits. (Kim & Padilla-Zakour, 2004; Wicklund, Rosenfeld, Martinsen, Sundfor, Lea, Bruun, Blomhoff & Haffner, 2005; Kovačević, Bituh & Dragovic-Uzelać, 2009; Šavikin, Zdunić, Janković, Tasić, Menković, Stević & Đorđević, 2009; Patras, Brunton, Pieve & Butler 2009; Wu, Frei, Kennedy & Zhao 2010; Gancel, Feneuil, Acosta, Pérez & Vaillant, 2011; Levaj, Kovačević, Bituh & Dragovic-Uzelać, 2012; Arancibia-Avila, Namiesnik, Toledo, Werner, Martinez-Ayala, Rocha-Guzmán, Gallegos-Infante & Gorinstein, 2012).

According to Mota (2006b) blackberry cultivation has been encouraged because of its potential for commercialization and industrialization. The cultivation of blackberries in Brazil began with the launch of the first Brazilian cultivars (Tupy, Guarani and Caingangue) by the Embrapa Temperate Climate breeding program (Fachinello, Pasa, Schmtiz & Betemps, 2011). An estimated 400 blackberry cultivars were produced by this breeding program (Clark & Finn, 2011), but the most commonly cultivated types in Brazil are: Tupy, Guarani, Negrita, Caingangue, Brazos, Cherokee, Comanche and Ebony (Antunes, 2002). In Brazil, only a few cultivars, such as the Guarani, are displayed/used for processing (Santos & Raseira, 1988). Thus, it is extremely important to evaluate the feasibility of processing other cultivars that are not used for this purpose.

In this context, the aim of this work was to study how the bioactive compounds contents (phenolic, anthocyanin and ascorbic acid) and antioxidant capacity of blackberry jellies processing were influenced by cultivars (Guarani, Brazos, Comanche, Tupy, Cherokeee, Caingangue and Choctaw).

2. MATERIALS AND METHODS

2.1 Ingredients

To elaborate the jellies were used the pulps of 7 blackberry cultivar grown in subtropical climate of Minas Gerais state - Brazil: Guarani, Brazos, Comanche, Tupy, Cherokee, Caingangue and Choctaw. The fruits used in the preparation of jellies were acquired from the orchard of the Federal University of Lavras– Minas Gerais –Brazil. This fruits were harvested at their physiological maturity, based on color and size, in the morning and kept refrigerated in a cold room at 18°C until the time of processing. In addition, to process the jelly were used also sucrose and high-methoxyl pectin (Danisco, SP, Brazil). Citric acid was not added in the preparation due to the suitable pH of the fruits (3.32-3.41) for the processing this product.

2.2 Formulation of the jellies

Were prepared seven blackberry jellies and the only variation between the formulations was in relation to the blackberry cultivate. Fowling Acosta et al. (2006) blackberries were thawed by immersion in potable water at 25°C for 30 min. Fruit was drained in a plastic colander to eliminate excess water and manually sorted for physical damage. The fruits were then beaten in a blender, and after homogenization were sieved in order to obtain clarified juice.

The percentages of ingredients used to elaborate the jellies formulations, expressed relative to total weight (sugar and pulp) were: 60% of fruit pulp, 40% sugar and 1.5% pectin. Figure 1 describes the processing of the jellies. Initially fruit pulps were added to sucrose, and then were processed in open pan heated by gas flame (Macanuda, SC, Brazil). After a boil was reached, high-methoxyl pectin was added. At the end of the process, after the soluble solids reached 65° Brix, the cooking was stopped. The total soluble solids were determined using a portable refractometer model RT-82 and °Brix was measured at ± 25°C. The jellies were then poured hot into 250 mL sterile bottles, cooled in a container with water and ice and stored in a refrigerator at ± 7°C.

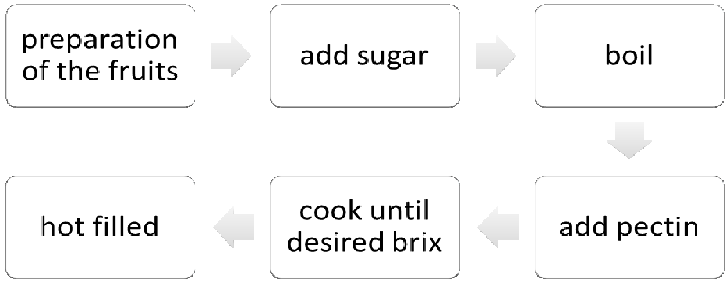

Figure 1 Steps used in the preparation of blackberry jellies

The parameter to finish the jellies cooking was the soluble solids of the product, however, all cultivars reached 65 °Brix with the same processing time – between 28-29 minutes. Thus, the time of thermal processing cannot be considered as a relevant study variable.

2.3 Analysis

Analysis was made of 7 cultivars of fresh blackberry and 7 blackberry jellies formulations at time 0 (24 h after processing) in triplicate. Was performed the analysis of antioxidant activity, total phenolics, total anthocyanins and ascorbic acid.

2.3.1 Preparation of antioxidant and phenolic extracts

The extracts were obtained according to the method described by Larrauri, Ruperez & Saura-Calixto (1997). Samples were weighed (g) in centrifuge tubes and extracted sequentially with 40 mL of methanol/water (50:50, v/v) at room temperature for 1 hour. The tubes were centrifuged at

25,400 g for 15 min, and the supernatant was recovered. Then, 40 mL of acetone/water (70:30, v/v) was added to the residue at room temperature. The samples were extracted for 60 min and centrifuged. The methanol and acetone extracts were combined and brought to a final volume of 100 mL with distilled water for the determination of antioxidant activity, phenolic content and total monomeric anthocyanin content.

2.3.2 Antioxidant activity

The antioxidant activity was determined using the ABTS and DPPH method.

For ABTS assay, the procedure followed the method of Re, Pellegrini, Proteggente, Pannala, Yang & Rice-Evans.(1999) with few modifications. The 2,2-azinobis (ABTS) radical cation (ABTS•+) was generated by reaction of 5 mL of aqueous ABTS solution (7 mM) with 88 μL of 140 mM (2.45 mM final concentration) potassium persulfate. The mixture was kept in the dark for 16 hour before use and then diluted with ethanol to obtain an absorbance of 0.7±0.05 units at 734 nm using a spectrophotometer. The fruit and jellies extracts (30 μL) or a reference substance (Trolox) were allowed to react with 3 mL of the resulting blue-green ABTS radical solution in the dark. The decrease of absorbance at 734 nm was measured after 6 min. Ethanolic solutions of known Trolox concentrations were used for calibration. The results are expressed as micromoles of Trolox equivalents (TEs) per gram of fresh weight (μmol of TEs/g of FW).

DPPH free radical-scavenging capacity was estimated using the method of Brand-Williams Cuvelier & Berset (1995). Briefly, the solution of DPPH (600 μM) was diluted with ethanol in order to obtain an absorbance of 0.7±0.02 units at 517 nm. Fruit and jellies extracts (0,1mL) were allowed to react with 3.9 mL of DPPH radical solution for 30 min in dark and the decrease in absorbance from the resulting solution was monitored. The absorbance of the reaction mixture was measured at 517 nm. The results were expressed as EC_{50} (gram of fresh mass per gram of DPPH).

2.3.3 Total Phenolic

The total phenolic content was determined according to the adapted Folin–Ciocalteu method (Waterhouse, 2002). The extracts (0.5 mL) were mixed with 2.5 mL of Folin–Ciocalteu reagent (10%) and 2 mL of sodium carbonate solution (4%). The mixture was stirred and kept at room temperature for 2 hour in the dark. The absorbance was measured at 750 nm against a blank. Aqueous solutions of gallic acid were used for calibration. The results are expressed as g gallic acid equivalents (GAE)/100 g.

2.3.4 Total monomeric anthocyanin

The total monomeric anthocyanin content (TMAC) was estimated using the pH differential method (Wrolstad, 1976) as described by Meng et al. (2012). Briefly each fruit and jellies extract were diluted with buffers at pH 1.0 and pH 4.5 to attain the same dilution. Absorbance was measured at 510 nm and 700 nm in both pH 1.0 and pH 4.5 buffers.

The TMAC (expressed in terms of cyanidin-3-glucoside) was calculated using the following formula:

$$A = (A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5} \quad (1)$$

$$\text{TMA content} = (A \times MW \times DF \times V_e \times 1000) / (\varepsilon \times 1 \times M) \quad (2)$$

where MW is the molecular weight of cyanidin-3-glucoside (449 g mol^{-1}), DF is the dilution factor, Ve is the extract volume (mL), ε is the molar extinction coefficient of cyanidin-3-glucoside (29,600), and M is the mass of the berries extracted.

Results were expressed as mg cyanidin-3-glucoside equivalents 100g of fresh weight- fw.

2.3.5 Ascorbic Acid

The vitamin C content of each fruit pulp and jelly was determined by a colorimetric method with 2,4 – dinitrophenylhydrazine (DNPH 2.4) according to Strohecker & Henning (1967). The results are expressed as mg ascorbic acid/100 g of fresh weight.

2.4 Statistical analysis

Data were reported as mean ± standard deviation (SD) values of triplicate experiment. To correlate the antioxidant activity and bioactive compounds contents with the blackberry fruit pulps and with the jellies formulations, the data were analyzed by a principal component analysis

(PCA). The data set was arranged in a matrix of 7 lines (fresh fruit or jellies samples) and 5 columns (antioxidant activity TEAC, antioxidant activity DPPH, total phenolics, total anthocyanins and ascorbic acid). The data were standardized (correlation matrix) and the PCA was applied. The PCA score plot and loading was built from the first two principal components.

The degradation percentage of each bioactive compound and antioxidant capacity was calculated:

$$\text{Degradation (\%)} = \frac{(\text{Amount presented in the fruit} - \text{Amount presented in the jelly}) * 100}{\text{Amount presented in the fruit}} \quad (3)$$

This degradation expressed is not the real degradation of the bioactive compounds and antioxidant since it was not discounting the amount of added sugar (40%) and was not considering the jelly yield (concentration of the pulp). But as the goal is to study the jellies processing were influenced by cultivars, for more than the value of the degradation rate be overestimated, this fact will affect all cultivars in the same way.

A medium test for the percentage of apparent degradation was conducted, and to facilitate the visualization the data was plotted in a graph of the percentage of apparent degradation of the antioxidant activity and bioactive compounds depending on the cultivars of blackberry.

Data analysis was performed with SensoMaker software version 1.6 (Pinheiro, Nunes & Vietoris, 2013).

A Pearson correlation test was conducted to determine the correlation between variables. Significance levels were defined $p < 0.05$.

3. RESULTS AND DISCUSSION

The means for the antioxidant activity and bioactive compounds to the different blackberry cultivars and theirs jellies are show in Table 1. A principal component analysis (PCA) was generated to correlate the antioxidant activity and bioactive compounds with the different blackberry cultivars and theirs jellies formulations as show in Figure 2 and Figure 3, respectively.

Table 1 The antioxidant capacity (ABTS and DPPH method), total phenolic, total monomeric anthocyanin and ascorbic acid content of the different blackberry cultivars and in their jellies.

Blackberry Cultivars	**Samples**	**TEAC**	**DPPH**	**Total Phenolics**	**Total anthocyanin**	**Ascorbic Acid**
Guarani	Fruit	38.94±0.56	1933.93±74.11	852.65±62.68	135.26±3.91	87.03±7.66
	Jelly	9.51±1.64	4485.45±155.91	386.84±7.25	11.85±1.61	17.27±3.48
Brazos	Fruit	29.34±1.60	1874.54±87.56	859.34±26.55	120.89±4.20	80.54±12.35
	Jelly	18.24±2.12	3762.63±83.21	467.88±7.66	15.03±1.32	13.75±0.89
Comanche	Fruit	28.56±1.02	1972.72±74.24	1019.55±18.14	212.74±3.89	89.52±2.24
	Jelly	14.45±1.66	5020.45±43.18	414,83±5.13	16.20±1.26	16.77±1.82
Tupy	Fruit	28.72±2.47	2014.54±109.29	921.53±7.74	146.45±5.36	81.30±8.25
	Jelly	10.54±0.41	4994.24±560.45	357.98±12.21	11.19±0.29	13.72±1.84
Cherokee	Fruit	25.37±1.92	2197.28±16.59	808.54±15.48	144.78±3.91	94.08±18.81
	Jelly	15.84±3.98	4757.12±503.79	437.54±4.24	20.04±2.00	17.79±1.58
Caingangue	Fruit	20.95±1.75	2437.14±106.55	748.18±60.36	65.63±2.50	75.76±18.81
	Jelly	13.57±2.74	4861.67±793.70	365.45±3.52	16.53±0.50	20.08±1.36
Choctaw	Fruit	34.29±3.31	1998.49±169.87	943.20±17.02	193.37±3.05	65.37±2.14
	Jelly	15.58±1.67	4252.42±227.70	433.87±2.57	11.19±1.26	17.60±2.38

TEAC: Trolox equivalent antioxidant capacity (µM Trolox equiv./g fw), DPPH: 2-diphenyl-1-picryhydrazyl radical scavenging activity (EC_{50} – g f.w/ g DPPH), Total phenolic (mg GAEs/ 100g f.w.), Total anthocyanin (mg of cyanidin 3-glucoside equivalent/ 100 g of f.w.), Ascorbic acid (mg/ 100g f.w)

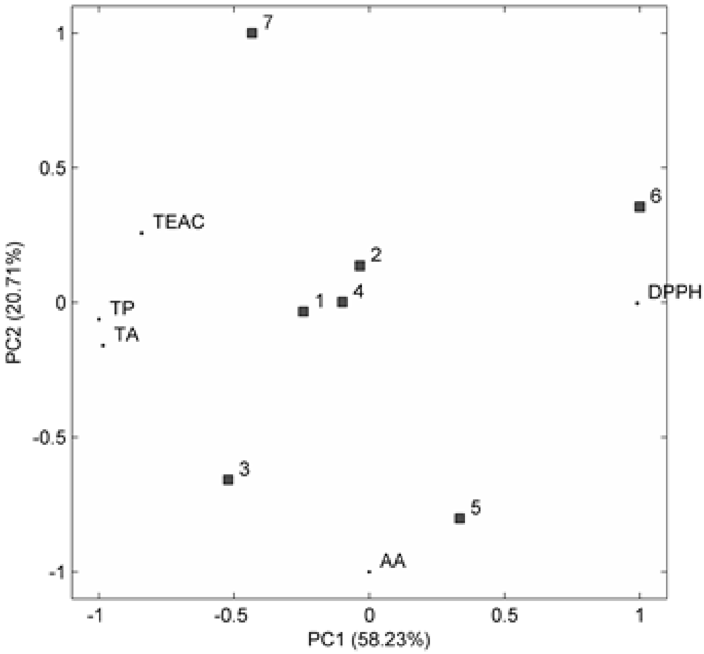

Figure 2 Principal Component Analysis (PCA) antioxidant activity and bioactive compounds of different blackberry samples.

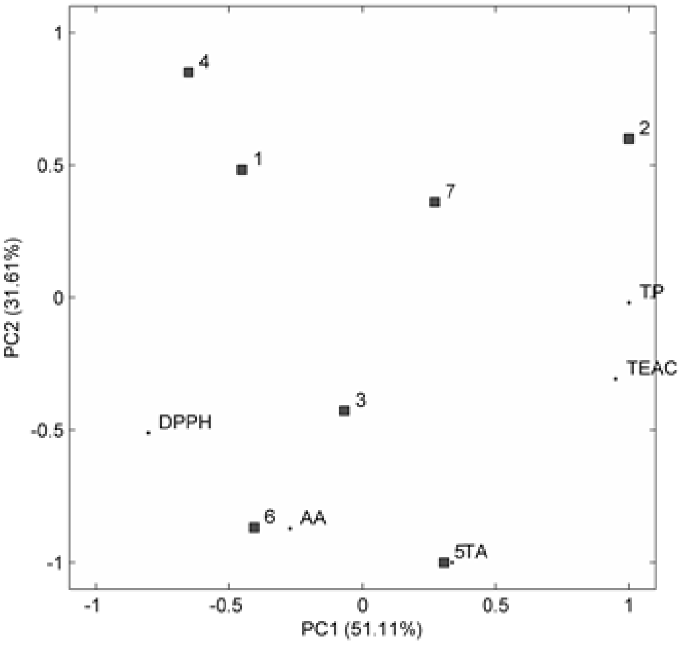

Figure 3 Principal Component Analysis (PCA) antioxidant activity and bioactive compounds of different blackberry jellies

Abreviations: TEAC: Trolox equivalent antioxidant capacity, DPPH: 2-diphenyl-1-picryhydrazyl radical scavenging activity, AA: Ascorbic acid, TP: Total phenolic, TA: Total anthocyanin. *Blackberry Cultivars:*C1- Guarani, C 2 –Brazos, C3- Comanche, C4 –Tupy, C5- Cherokee, C6 – Caingangue, C7 –Choctaw.

In Figure 2 and Table 1, it is noticed that the blackberry cultivars Guarani, Brazos, Comanche, Tupy and Choctow characterized for having the highest antioxidant activity, ie, these cultivars showed higher TEAC antioxidant activity ranging from 28.56 (Comanche) to 38.94 µM Trolox equiv. / g fw (Guarani) and lower values of DPPH, ranging from 1874.54 (Brazos) to 2014.54 EC_{50} - g fw / g DPPH (Tupy). These cultivars also showed the greatest total phenolic content, ranging from 852.65 (Guarani) to 1019.55 mg GAEs/100 g of fw (Comanche) and the highest levels of anthocyanins, ranging from 120.89 (Brazos) to 212.74 mg of cyanidin 3-glucoside equivalent / 100 g of fw (Comanche). The Caingangue cultivar presented the lower antioxidant activity by DPPH method (2437.14 EC_{50} - g fw / g DPPH) and Cherokee and Comanche cultivars, were characterized by high content of vitamin C (94.08 and 89.52 mg/100g fw, respectively) (Figure 2 and Table 1).

Regarding jellies obtained from different cultivars of blackberry, perceives in Figure 3 and Table 1 that the jelly obtained from cultivar Brazos, has the highest antioxidant activity (18.24 µM Trolox equiv./g fw e 1874.54 EC_{50} – g f.w/g DPPH). It can be seen that the loss of antioxidant capacity is not similar for all cultivars, ie, because of the cultivars that had been highlighted showing the highest antioxidant activity, just jelly prepared with Brazos cultivar stood. The Guarani cultivar, for example, had presented the highest antioxidant activity by TEAC method (38.94 µM Trolox equiv./g fw), however, after processing, the jelly made with this cultivar had the lowest antioxidant activity (9.51 µM Trolox equiv./g fw).

The Brazos cultivar jelly stood also for having the highest total phenolic content (467.88 mg GAEs/100g f.w.) (Figure 3 and Table 1). The same fact discussed earlier is verified for phenol content, this is because the cultivar Brazos had the lowest content of phenols from the group of cultivars with higher levels of this compound (Guarani, Brazos, Comanche, Tupy, Choctaw). As for anthocyanin, the jelly highlighted showing the highest levels (20.04 mg of cyanidin 3-glucoside equivalent/ 100 g of f.w.) was prepared with the cultivar Cherokee, not one more time corroborating with expected.

Regarding the content of vitamin C, jelly prepared with cultivar Caingangue stood presenting the highest content (20.04 mg/100 g fw) and not to cultivate Cherokee, which had presented the highest values for this nutrient.

In Table 2 are expressed the percentage of average degradation of antioxidant activity and each bioactive compound with the jelly processing, and the medium test. The medium test confirms what had been seen in the principal component analysis (Figures 2 and 3), ie, except for the antioxidant activity DPPH, blackberry cultivars showed losses for other bioactive compounds significantly different ($p < 0.05$) to the jelly processing. For better visualization, in Figure 4 is expressed the significantly degradation percentage average of bioactive compounds and antioxidant activity of the decay of different blackberry cultivars due to processing in the form of jelly.

Table 2 Average degradation of bioactive compounds and decay of antioxidant activity of different cultivars of blackberry due to processing in the form of jelly

Blackberry cultivars	TEAC	DPPH	TP	TA	AA
Guarani	76.60^{a}	56.88	54.48^{bc}	91.20^{a}	80.17^{ab}
Brazos	37.90^{b}	50.16	47.96^{d}	87.54^{b}	82.78^{a}
Comanche	49.26^{ab}	60.71	59.30^{ab}	92.39^{a}	81.26^{a}
Tupy	63.11^{ab}	59.26	61.15^{a}	92.35^{a}	83.15^{a}
Cherokee	37.25^{b}	53.46	45.88^{d}	86.17^{b}	80.82^{a}
Caingangue	34.21^{b}	48.85	50.93^{cd}	74.77^{c}	73.37^{bc}
Choctaw	54.05^{ab}	52.92	50.99^{bc}	94.21^{a}	73.10^{c}

In a column, means with no common superscripts are significantly different ($p < 0.05$).
Abreviations: TEAC: Trolox equivalent antioxidant capacity, DPPH: 2-diphenyl-1-picryhydrazyl radical scavenging activity, AA: Ascorbic acid, TP: Total phenolic, TA: Total anthocyanin.

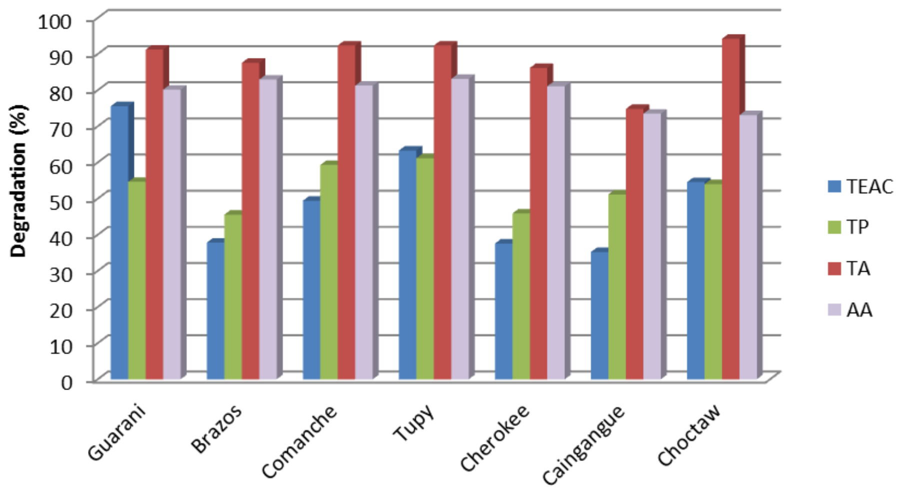

Figure 4 Average degradation of bioactive compounds and decay of antioxidant activity of different cultivars of blackberry due to processing in the form of jelly

Abreviations: TEAC: Trolox equivalent antioxidant capacity, DPPH: 2-diphenyl-1-picryhydrazyl radical scavenging activity, AA: Ascorbic acid, TP: Total phenolic, TA: Total anthocyanin.

In relation to the total phenolic content, levels of degradation reached 61.15% (Table 2 and Figure 4). Wu, Frei, Kennedy & Zhao (2010) reported losses of up to 67% degradation of phenolics in the processing of jelly blackberry. During processing strawberry into jams, Kovačević, Levaj & Dragović-Uselac (2009) and Levaj, Kovačević, Bituh & Dragovic-Uzelać, (2012) found levels of total phonelic degradation to 63% and 70%, respectively. Already Šavikin, Zdunić, Janković, Tasić, Menković, Stević & Đorđević (2009) found that jam processing of berries fruits decreased the total phenolics content for less than 50%. Brazos and

Cherokee cultivars highlighted by presenting the smallest loss with processing (Table 2 and Figure 4). The Brazos cultivar, although not to grow richer in phenolic compounds (Table 1) resulted in a jam with the highest level of this bioactive compound in order to have presented lower levels of degradation with processing (Table 2 and Figure 4). According Zafrilla, Ferreres & Tomás-Barberán (2001) processing and storage can have marked effects on the phenolic content of fruits that might affect their health-promoting properties. Although the degree of degradation of phenolic compounds have been high, all samples of jams, following the polyphenol classification proposed by Vasco et al (2008), can be categorized as having an average phenol content, and may be considered a good source of phenols.

The anthocyanin was more thermosensitive compound, reaching levels greater than 90% of biodegradation (Table 2 and Figure 4). The Caingangue cultivar was that stood out with the smallest losses in processing (Table 2 and Figure 4). Although this cultivar has presented a much lower content of anthocyanins comparing to the others blackberry cultivars (Table 1), due to less degradation, the jelly had obtained one of the highest levels of anthocyanins, being only lower of jelly prepared with cultivar Cherokee, which presented almost double that of anthocyanin in the fruit (Table 1).

As the red/black color of berries belongs to the group of anthocyanins, the colour of the product depends on these natural pigments and their degradation products (Francis, 1985). Anthocyanins degradation is affected by temperature, pH, oxygen, sugar content, ascorbic acid and metals (Withy, Nguyen, Wrolstad & Heatherbell (1993). Mota (2006a), Mota (2006b), Wu, Frei, Kennedy & Zhao (2010) and Gancel, Feneuil, Acosta, Pérez & Vaillant (2011) found that the cooling and heating of blackberry pulp causes considerable anthocyanin losses, and this degradation may vary from 8.8% to 80%. Already Šavikin et al. (2009) in studies on the effect of processing of berries in jellies, found levels of degradation of anthocyanins up to 85%.

The high loss of phenolic compounds, especially anthocyanin, may be explained by the acceleration in condensation and polymerization reactions of phenolic compounds after cellular disruption and increase in fruit temperature combined with oxygen exposure (Häkkinen, Kärenlampi, Mykkänen & Torronen 2000). Some authors reported that the anthocyanin degradation in processed berry products as a result of indirect oxidation by phenolic quinones generated by polyphenol oxidase and peroxidase (Kader, Rovel, Girardin & Metche 1997; Wesche-Ebeling & Montgomery, 1990; Skrede, Wrolstad & Durst 2000). In general, several factors are believed to affect the stability of anthocyanins in fruits and vegetables during preparation, processing, and storage, for jam and jellies making, the main effects are due to cooking temperature, pH and sugar concentration (Rhim 2002; Kim & Padilla-Zakour, 2004).

Since anthocyanins are the predominant phenolic compounds in blackberries, it was expected that total phenolic content showed a similar trend as anthocyanins. However, as verified by Kovačević, Levaj & Dragović-Uselac (2009) and Wu, Frei, Kennedy & Zhao (2010), total phenolics were more stable during processing in comparison with total anthocyanins, probably due to polymerization of monomeric anthocyanins, which produced polymers detected as phenolics (Hager, Howard, Liyanage, Lay & Prior, 2008a; Hager, Howard, & Prior, 2008b).

Levels of vitamin C degradation were high, achieving more than 80% (Table 2 and Figure 4). Segundo Rawson, Patras, Tiwari, Noci, Koutchma & Brunton (2011), vitamins are among the most sensitive food components in fruits to be affected by heat treatment. Caingangue and Choctow cultivars stood out for the slightest degradation of vitamin C with processing (Table 2 and Figure 4). The same fact observed for total phenolics and anthocyanins is repeated for the vitamin C content, ie, although the growing richer in vitamin C has been cultivating Cherokee, the jelly was made with the Caigangue cultivar that showed the highest levels of this vitamin (Table 1 and Figures 2 and 3).

Jellies showed antioxidant activity losses of up to 76.60% for TEAC and 60.71% DPPH (Table 2 and Figure 4). Kim & Padilla-Zakour (2004); Kovačević, Levaj & Dragović-Uselac (2009); Wu, Frei, Kennedy & Zhao (2010) and Gancel, Feneuil, Acosta, Pérez & Vaillant (2011) also found that the heat processing promotes considerable losses in the antioxidant capacity of berries. It is clear in Table 2 and Figure 4 that Brazos, Cherokee and Caingangue cultivars showed the smallest decrease in the antioxidant capacity TEAC. The Brazos cultivar jelly presented the highest antioxidant activity, although not the blackberry cultivar stands in relation to other cultivars (Table 1).

The Pearson’s correlation coefficients between antioxidant activity, total phenolic contents, total anthocyanin and ascorbic acid levels are presented in Table 3.

Table 3 Pearson's correlation coefficients (R) between antioxidant capacity parameters (TEAC e DPPH), total phenolic, total anthocyanin and ascorbic acid contents in different blackberry cultivars and in their respectively jellies.

	Correlation coefficient (R)			
Parameters	**TEAC**	**DPPH**	**TF**	**TA**
TEAC	-	-	-	-
DPPH	-0.90*	-	-	-
TF	0.90*	-0.96*	-	-
TA	0.87*	-0.89*	0.96*	-
AA	0.84*	-0.95*	0.94*	0.89*

Abreviations: TEAC: Trolox equivalent antioxidant capacity, DPPH: 2-diphenyl-1-picryhydrazyl radical scavenging activity, AA: Ascorbic acid, TP: Total phenolic, TA: Total anthocyanin.

According to Table 3, all bioactive compounds and antioxidant activity showed high correlation. As expected, the TEAC and DPPH antioxidant activity showed high negative correlation with each other and content of anthocyanins and phenolics showed high positive correlation between them (Table 3). The total antioxidant activity – TEAC was high and positively correlated and the antioxidant activity – DPPH was high and negatively correlated with ascorbic acid, phenolics content and total anthocyanin. These results suggest that the ascorbic acid and phenolic compounds, such as phenolic acids, tannic acid, proanthocyanidin and anthocyanin may be the most important contributors to the antioxidant activity in the blackberry.

According to Patras, Brunton, Pieve & Butler (2009), polyphenols, ascorbic acid and anthocyanins are key antioxidant groups. Several studies actually demonstrate the high positive correlation between the antioxidant activity of fruit with their total phenolic content and vitamin C (Roesler, Malta, Carrasco, Holanda, Sousa & Pastore, 2007; Rufino, Fernandes, Alves & Brito 2010; Contrerás-Calderón, Jaimes, Hernández & Villanova, 2010; Ramful, Tarnus, Aruoma, Bourdan & Bahorun, 2011, Almeida, Souza, Arriaga, Prado, Magalhães, Mais & Lemos, 2011; Souza, Pereira, Queiroz, Borges & Carneiro, 2012). Thus, the decrease of the jellies antioxidant capacity may be attributable to the destruction of active antioxidant compounds such as vitamin C, phenolics and anthocyanins by the heating process during jelly preparation (Kim & Padilla-Zakour, 2004).

In general, it is noticed that the blackberry cultivars richer in antioxidant activity and bioactive compounds have higher levels of degradation with processing (Table 1, Table 2 and Figure 4). Thus, the blackberry cultivars that had the lowest nutrient losses, although not the most nutritionally rich have, in general, lead to jellies richer in antioxidant activity and bioactive compounds. Levaj, Kovačević, Bituh & Dragovic-Uzelać. (2012) in studies with different cultivars of strawberries subjected to processing in the form of jam, showed that cultivars with higher levels of anthocyanins approved the greater degradation during processing.

The Brazos cultivar stood out as being the one that has the lowest loss of antioxidant capacity and total phenolic giving rise to a jelly with higher phenolic content and antioxidant activity. So how to cultivate Caingangue for having less degradation of anthocyanins and vitamin C, which also produces a jelly richer these bioactive compounds.

The influence of processing and cultivation of berries in the degradation of bioactive compounds and reduced antioxidant capacity of different berries has been the subject of several studies showing that the thermal processing of berries such as strawberries, blackberries and cherry significantly reduces the levels of phenolic, anthocyanins, flavonoids, vitamin C and antioxidant activity, and that this degradation is similar for the different cultivars when subjected to the same processing technology (Kim & Padilla-Zakour, 2004; Wicklund, Rosenfeld, Martinsen, Sundfor, Lea, Bruun, Blomhoff & Haffner, 2005; Kovačević, Levaj & Dragović-Uselac, 2009; Patras Brunton, Pieve & Butler, 2009; Šavikin, Zdunić, Janković, Tasić, Menković, Stević & Đorđević, 2009; Wu, Frei, Kennedy & Zhao, 2010; Arancibia-Avila, Namiesnik, Toledo, Werner, Martinez-Ayala, Rocha-Guzmán, Gallegos-Infante & Gorinstein, 2012; Levaj, Kovačević, Bituh & Dragovic-Uzelać, 2012).

It can be noticed that the antioxidant activity and the content of bioactive compounds of blackberry jam are connected with the loss that each cultivar presents during processing and the initial content of phenolics, anthocyanins, vitamin C and antioxidant activity. Thus, to obtain a blackberry jelly more nutritionally rich attention must be paid not only to the quantity of the bioactive compounds and antioxidant initial raw material, but also the effect of processing these health promoting compounds.

4. CONCLUSION

It is concluded that occurs degradation of the bioactive compounds and reduction of antioxidant activity due to the jam processing and the degradation of these compounds was significantly different between the different blackberry cultivars. The Brazos and Caingangue cultivars stand out as suffering the smallest losses in processing, resulting in jellies richer in bioactive compounds and higher antioxidant capacity. All blackberry jellies, although heavy losses in processing, are a good source of phenolic compounds.

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