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Uva BRS Carmem: potencial para produção de bioingrediente e licor

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Tese apresentada como parte dos requisitos para obtenção do título de Doutor em Engenharia e Ciência de Alimentos, junto ao Programa de Pós-Graduação em Engenharia e Ciência de Alimentos, do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista "Júlio de Mesquita Filho", Câmpus de São José do Rio Preto.

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RESUMO

A grande diversidade de cultivares de uva no Brasil possibilita a prospecção de variedades para a agroindustrialização, especialmente no contexto da agricultura familiar. Nesse sentido, este estudo teve como objetivo caracterizar detalhadamente as propriedades físico-químicas, morfológicas e funcionais da cultivar BRS Carmem, visando fomentar novas oportunidades de processamento na forma de bioingrediente em pó e licores. Foram determinadas as características físicas e químicas básicas da uva, incluindo umidade, pH, sólidos solúveis, acidez total, índice de maturação (ratio), compostos fenólicos totais, massa, tamanho e formato do cacho e da baga, distribuição percentual de polpa, casca e semente, compacidade, cor e uniformidade da cor. Além disso, foi avaliada a composição qualitativa e quantitativa de antocianinas em uvas produzidas sob dois porta-enxertos (IAC 766 e IAC 572) no mesmo ciclo produtivo. As análises de antocianinas das uvas e dos produtos desenvolvidos foram realizadas por cromatografia líquida de alta eficiência com detector de arranjo de diodo acoplado a um sistema de ionização por eletronebulização e espectrômetro de massas multidimensional (CLAE-DAD-IES-EMn). Para a obtenção do bioingrediente em pó, o suco de uva foi extraído por arraste a vapor, tecnologia amplamente difundida entre produtores rurais. Em seguida, o suco foi desidratado por secagem em leito de espuma a 80°C, resultando no pó. Os licores foram produzidos utilizando álcool de cereais e cachaça branca, em duplicata, com um período de maceração de 60 dias e adoçamento com açúcar invertido. Ambos os produtos foram caracterizados quanto às propriedades físico-químicas e à composição qualitativa e quantitativa de antocianinas. Os resultados indicaram que, apesar de diferenças significativas ($p \leq 0,05$) nas características físico-químicas das uvas, ambas apresentaram atributos adequados para processamento. O bioingrediente em pó e os licores demonstraram características compatíveis com seus respectivos nichos de mercado e atenderam às exigências da legislação vigente. A análise de antocianinas revelou que as diglicosiladas cumariladas foram as mais abundantes nas uvas (52,6–55,1%), sendo a derivada da malvidina a principal (44,8–46,8%). Perfis semelhantes foram observados no suco e no bioingrediente em pó, onde as diglicosiladas cumariladas representaram 67,4% e 69,4%, respectivamente. Os licores apresentaram propriedades físico-químicas distintas em função da base alcoólica utilizada. No entanto, a análise sensorial não revelou diferenças significativas na aceitação global e na intenção de compra entre as duas formulações, indicando que os consumidores não demonstraram preferência. Nos licores,

as antocianinas derivadas da malvidina (diglicosiladas cumariladas, diglicosiladas e glicosiladas) foram predominantes, contribuindo com 77,6–83,9% das diglicosiladas e 59,9–69,2% das monoglicosiladas. O bioingrediente em pó apresentou uma concentração total expressiva de antocianinas (2192,20 mg mv-3,5-glc·kg^{-1}), enquanto os licores registraram valores de 420,04 e 456,44 mg mv-3,5-glc·L^{-1} para as formulações com álcool de cereais e cachaça, respectivamente, após dois meses de maceração. Os achados deste estudo demonstram que o uso de tecnologias acessíveis e de baixo custo pode agregar valor à produção da uva BRS Carmem, promovendo alternativas de processamento viáveis para a agricultura familiar. Dessa forma, a valorização dessa cultivar por meio da elaboração de produtos diferenciados pode contribuir para a diversificação da agroindústria e para o fortalecimento da cadeia produtiva da uva no Brasil.

Palavras–chave: Uva brasileira. Produtos alimentícios. Compostos fenólicos. Antocianinas.

ABSTRACT

The vast diversity of grape cultivars in Brazil enables the exploration of varieties for agro-industrialization, particularly within family farming. In this context, the present study aimed to comprehensively characterize the physicochemical, morphological, and functional properties of the BRS Carmem cultivar to promote new processing opportunities in the form of powdered bioingredient and liqueurs. The basic physical and chemical characteristics of the grapes were determined, including moisture, pH, soluble solids, total acidity, ripening index (ratio), total phenolic compounds, bunch and berry mass, size and shape, percentage distribution of pulp, skin, and seeds, compactness, color, and color uniformity. Additionally, the qualitative and quantitative anthocyanin composition of grapes grown on two rootstocks (IAC 766 and IAC 572) in the same production cycle was evaluated. Anthocyanin analysis of the grapes and the developed products was performed using high-performance liquid chromatography with a diode array detector coupled to an electrospray ionization system and a multidimensional mass spectrometer (HPLC-DAD-ESI-MS^n). To obtain the powdered bioingredient, grape juice was extracted using steam distillation, a technology widely adopted by rural producers. The juice was then dehydrated through foam-mat drying at 80°C, yielding the powdered product. The liqueurs were produced using cereal alcohol and white cachaça, in duplicate, with a maceration period of 60 days and sweetened with inverted sugar. Both products were characterized in terms of their physicochemical properties and qualitative and quantitative anthocyanin composition. The results indicated that, despite significant differences ($p \leq 0.05$) in the physicochemical characteristics of the grapes, both exhibited suitable attributes for processing. The powdered bioingredient and liqueurs demonstrated properties compatible with their respective market niches and complied with current regulatory standards. Anthocyanin analysis revealed that coumaroylated diglycosylated anthocyanins were the most abundant in the grapes (52.6–55.1%), with malvidin-derived compounds being the predominant ones (44.8–46.8%). Similar profiles were observed in the juice and powdered bioingredient, where coumaroylated diglycosylated anthocyanins accounted for 67.4% and 69.4%, respectively. The liqueurs exhibited distinct physicochemical properties due to the different alcoholic bases used. However, sensory analysis showed no significant differences in overall acceptance and purchase intention between the two formulations, indicating no consumer preference. In the liqueurs, malvidin-derived anthocyanins (coumaroylated diglycosylated, diglycosylated, and

glycosylated) were predominant, contributing 77.6–83.9% of the diglycosylated and 59.9–69.2% of the monoglycosylated anthocyanins. The powdered bioingredient exhibited a high total anthocyanin concentration (2192.20 mg mv-3,5-glc·kg^{-1}), while the liqueurs contained 420.04 and 456.44 mg mv-3,5-glc·L^{-1} for the cereal alcohol and cachaça-based formulations, respectively, after two months of maceration. The findings of this study demonstrate that the use of accessible and low-cost technologies can add value to BRS Carmem grape production, promoting viable processing alternatives for family farming. Thus, enhancing the value of this cultivar through the development of differentiated products can contribute to the diversification of the agroindustry and the strengthening of the grape production chain in Brazil.

Keywords: Brazilian grape. Food products. Phenolic compounds. Anthocyanins.

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HPLC	Cromatografia Líquida de Alta Eficiência
DAD	Detector de arranjo de diodos
ESI	Ionização por eletronebulização
MS/MS	Espectrometria de massa multidimensional
dp	Delfinidina
cy	Cianidina
pt	Petunidina
pn	Peonidina
mv	Malvidina
3,5-glc	3,5-glicosídeo
3-glc	3-glicosídeo
acglc	acetilglicosídeo
cfglc	cafeilglicosídeo
cmglc	cumaroilglicosídeo
nd	não detectado
A	Licor a base de álcool de cereais
C	Licor a base de cachaça branca

SUMÁRIO

1 INTRODUÇÃO GERAL

As uvas estão entre as frutas mais consumidas e cultivadas mundialmente, destacando-se por sua versatilidade e importância econômica no setor alimentício (OIV, 2020). Seu processamento na forma de diferenciados produtos não apenas amplia o mercado de consumo, mas também oferece amplas oportunidades para a inovação e a valorização de cultivares específicas (Colombo *et al*., 2021; Lago-Vanzela *et al*., 2013; Nishiyama-Hortense *et al*., 2022; Olivati *et al*., 2022; Tavares *et al*., 2019). Além disso, as uvas e os produtos derivados são reconhecidos como fontes importantes de compostos fenólicos (CF) na dieta (Lago-Vanzela *et al*., 2011a; 2011b; Neri-Numa *et al*., 2018), cuja relevância está associada às suas propriedades antioxidantes, essenciais na proteção contra danos oxidativos e na manutenção da saúde celular (Halake; Birajdar; Lee, 2016; Leopoldini; Russo; Toscano, 2011).

No Brasil, há produção de diferentes cultivares de uvas viníferas, americanas e híbridas. Os CF presentes nessas uvas estão distribuídos em diferentes partes da baga (casca, polpa e sementes), com perfis qualitativos e quantitativos que variam em função de fatores como variedade/cultivar, condições edafoclimáticas, estádio de maturação e práticas culturais e uso de porta-enxertos (Colombo *et al*., 2021; Lago-Vanzela *et al*., 2011a; 2011b; Xia *et al*., 2010), além dos métodos de extração e de análise empregados (Alara; Abdurahman; Ukaegbu, 2021).

Entre as uvas tintas nacionais, destaca-se a cultivar BRS Carmem, cuja casca apresenta uma composição diversificada de CF (Colombo *et al*., 2020; 2021; Nishiyama-Hortense *et al*., 2023). Essa cultivar possui polpa incolor, teor de sólidos solúveis (SS) de aproximadamente 19 °Brix e sementes numa proporção de cerca de 6,2 g por 100 sementes (Assis *et al*., 2011; Camargo; Maia; Ritschel, 2008). Estudos prévios avaliaram seu uso como matéria-prima para o desenvolvimento de sucos (Da-Silva *et al*., 2019), preparado desidratado (Nishiyama *et al.*, 2015) e vinhos tintos (De-Castilhos *et al*., 2015; 2018).

Diante desse cenário, este projeto tem como objetivo caracterizar detalhadamente as propriedades físico-químicas, morfológicas e funcionais da cultivar BRS Carmem, com vistas a fomentar novas oportunidades de processamento dessa cultivar, alinhadas às demandas dos consumidores por alimentos funcionais, naturais e autênticos, ao mesmo tempo em que promove benefícios econômicos e tecnológicos para os pequenos produtores rurais (Mcdougall *et al*., 2017; Munekata *et al*., 2021).

Dentre as possibilidades de processamento, a desidratação do suco dessa cultivar para a produção de um bioingrediente em pó surge como uma estratégia promissora para reduzir perdas durante a cadeia produtiva, estender sua disponibilidade para além do período de safra, bem como viabilizar seu uso de forma versátil em regiões onde a produção de uvas é limitada, ampliando sua acessibilidade e aplicações (Premkumar; Vasudevan, 2018; Tavares *et al*., 2019). Complementarmente, a produção de licores oferece uma alternativa refinada para a extensão da vida útil da uva, diversificando a renda de pequenos agricultores e fortalecendo a multifuncionalidade da agricultura familiar, conectando-a a outros setores econômicos e sociais (Desenvolvimento Rural, 2024).

Com base nos aspectos abordados na introdução, esta fundamentação serviu de suporte para a definição dos objetivos desta tese.

2 OBJETIVO GERAL

O objetivo geral deste estudo foi caracterizar detalhadamente as propriedades físico-químicas, morfológicas e funcionais da cultivar BRS Carmem, com vistas a fomentar novas oportunidades de processamento dessa cultivar na forma de bioingrediente em pó e licores.

Os objetivos específicos estão discriminados a seguir:

- Determinar as características físico-químicas da uva BRS Carmem cultivadas sobre dois porta-enxertos;
- Produzir suco da uva BRS Carmem a partir de extração por arraste a vapor e realizar sua desidratação a partir de secagem em leito de espuma para obtenção do bioingrediente em pó;
- Padronizar o processo de elaboração e determinar as características-físico-químicas de licores de uva BRS Carmem elaborados com álcool de cereais e cachaça branca;
- Identificar e quantificar as antocianinas utilizando cromatografia líquida de alta eficiência com detector de arranjo de diodo acoplada a sistema de ionização por eletronebulização e espectrômetro de massas multidimensional (CLAE-DAD-IES-EM^n) nas uvas BRS Carmem e os produtos derivados (bioingrediente e licor).

Considerando tais objetivos, este trabalho está organizado em quatro seções descritos a seguir:

1. BRS Carmem e a produção de derivados de uva: uma revisão sobre composição fenólica e aplicações tecnológicas. Apresenta uma revisão bibliográfica que fundamenta a pesquisa, abordando a cultivar de uva brasileira BRS Carmem, os principais produtos derivados de uvas (com ênfase em sucos, produtos desidratados e licores) e os compostos fenólicos, destacando sua relevância e características.

2. Morphological, physical-chemical, and anthocyanin profiles of BRS Carmem grapes grafted on different rootstocks. Enfoca a caracterização morfológica e química da uva BRS Carmem, cultivada sobre dois porta-enxertos em um ciclo produtivo. Inclui a análise detalhada de sua composição qualitativa e quantitativa de antocianinas, fornecendo subsídios para compreender as particularidades dessa cultivar.

3. Qualitative and quantitative composition of anthocyanins in BRS Carmem grape juice powder: Potential as a natural colorant and bioactive ingredient. Detalha o processo de produção do suco da uva BRS Carmem e do produto desidratado obtido a partir desse suco. Além disso, aborda a identificação e quantificação das antocianinas presentes, destacando o potencial funcional e tecnológico desses produtos.

4. BRS Carmem grape liqueurs: influence of alcoholic base on physicochemical characteristics, anthocyanin composition, and sensory acceptance. Descreve o desenvolvimento de licor de uva utilizando a cultivar brasileira BRS Carmem, avaliando a influência de duas bases alcoólicas diferentes: álcool de cereais (A) e cachaça branca (C); os efeitos dessas fontes alcoólicas nas propriedades físico-químicas, composição de antocianinas e atributos sensoriais dos licores. Contribuindo para o conhecimento existente sobre licores à base de uva, apoiando o desenvolvimento de produtos inovadores e aumentando o valor dos recursos locais.

Essa organização busca fornecer uma abordagem sequencial e estruturada para atender aos objetivos da pesquisa, conectando a caracterização da matéria-prima ao desenvolvimento e análise de produtos.

3 BRS CARMEM E A PRODUÇÃO DE DERIVADOS DE UVA: UMA REVISÃO SOBRE COMPOSIÇÃO FENÓLICA E APLICAÇÕES TECNOLÓGICAS

3.1 PANORAMA NACIONAL DE UVAS E CULTIVARES COM POTENCIAL PARA PROCESSAMENTO

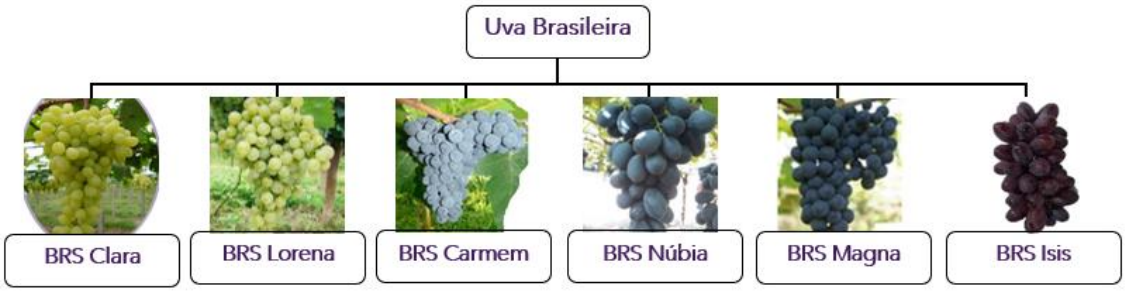
No Brasil, a viticultura ocupa vinhedos estabelecidos desde o extremo sul do país, até regiões situadas muito próximas ao Equador. A produção de uvas no Brasil em 2024 foi estimada em 1,8 milhão de toneladas, aumentos de 2,5% em relação a 2023. A safra de uva em 2024 demonstra a complexidade do setor agrícola brasileiro, marcado por contrastes entre estados e segmentos de produção (IBGE, 2024). Pode ser segmentada em dois grandes grupos: uva para consumo *in natura* e a uva para o processamento, principalmente na forma de suco de uva e vinhos de mesa e finos. A Região Sul do Brasil é a maior produtora de uvas no país, principalmente as destinadas ao processamento. Nos Estados de São Paulo, Minas Gerais, Bahia e Pernambuco a produção de uvas, em sua maior parte é voltada para o consumo *in natura* (Mello; Machado, 2020). Relativamente à estrutura produtiva e mercadológica, o setor vinícola brasileiro apresenta uma característica relativamente atípica aos países tradicionais produtores de vinhos e derivados da uva e do vinho. Em países como Itália, Espanha e França são admitidos apenas produtos originários de variedades/cultivares viníferas, enquanto no Brasil, além destes, existem produtos originários de variedades americanas e híbridas (*V. labrusca e V. bourquina*, por exemplo). Em torno de 80% do volume total de produção desta cadeia produtiva é representado por uvas e produtos derivados de uvas híbridas, o que evidencia a existência de uma dualidade estrutural no setor (Protas; Camargo; De Mello, 2002).

As características regionais distintas, com particularidades no ciclo de produção, época de colheita, cultivares, tratos culturais, tipo de produto e foco de mercado permitem a produção de uvas de diferenciadas cores, tamanhos e sabores. No Brasil, a Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) tem contribuído consideravelmente para o aumento da produção de uva ao desenvolver, por meio do melhoramento genético, novas cultivares de uvas. O melhoramento genético tem auxiliado não só na ampliação dos centros de produção e produtividade da uva no Brasil, mas também é essencial no processo de inovação do setor. Contribui para reduzir a necessidade de uso de insumos,

ampliar a geografia de produção, diversificar e incrementar a qualidade, enfim, contribui gerando novos produtos, mais competitivos no mercado globalizado. A expectativa para 2025 é que as contínuas inovações tecnológicas em manejo e estratégias comerciais possam consolidar o Brasil como referência global na produção de uvas de qualidade, ampliando ainda mais sua competitividade nos principais mercados consumidores.

Na Figura 3.1, estão apresentadas algumas cultivares de uvas viníferas e híbridas interespécies desenvolvidas pela EMBRAPA, que possuem características que as tornam relevantes tanto para o mercado de uvas de mesa quanto para o processamento. O melhoramento genético dessas cultivares teve como foco a adaptação às condições climáticas brasileiras, resistência a doenças e pragas, além de atributos sensoriais e tecnológicos que favorecem a aceitação comercial e a viabilidade econômica da produção.

Figura 3.1 - Exemplos de cultivares de uva desenvolvidas pela Embrapa.



Fonte: Autor.

Entre as cultivares brasileiras de uvas brancas destinadas ao consumo *in natura* e processamento, destacam-se a BRS Clara (*Vitis vinifera*, CNPUV 154-147 e Centennial Seedless) e a BRS Lorena (Malvasia Bianca x Seyval), respectivamente. A BRS Clara apresenta características desejáveis, como bagas de coloração amarela dourada, textura firme e alto teor de SS, tornando-se uma alternativa competitiva às variedades comerciais importadas (Camargo *et al*., 2003a; Lago-Vanzela *et al*., 2011a). Já a BRS Lorena é amplamente reconhecida pelo seu aroma moscatel, boa adaptação ao cultivo em diferentes regiões e equilíbrio entre açúcares e acidez, sendo também utilizada na elaboração de vinhos brancos finos devido ao seu perfil sensorial característico (Camargo; Guerra, 2001).

No grupo das uvas tintas para consumo *in natura*, destacam-se a BRS Morena (*Vitis vinifera*, Marroo Seedless x Centennial Seedless), a BRS Isis (CNPUV 681-29 x

BRS Linda) e a BRS Núbia (Michele Palieri x Arkansas 2095, híbrida interespécies). A BRS Morena é uma cultivar apirênica com excelente produtividade, bagas grandes e sabor neutro, favorecendo sua aceitação comercial (Camargo *et al*., 2003b; Lago-Vanzela *et al.*, 2011a). A BRS Isis se destaca pelo alto teor de antocianinas e resistência ao rachamento, além de apresentar bagas sem sementes e com casca fina, características que atendem à demanda do mercado consumidor por uvas de fácil consumo (Ritschel *et al*., 2013). Já a BRS Núbia, embora seja uma cultivar pirênica, possui um mercado consolidado devido à sua coloração intensa, bagas de grande porte e sabor equilibrado, tornando-se uma opção diferenciada dentro do segmento de uvas de mesa (Maia *et al*., 2013; Silvestre *et al*., 2017; Dodorico, 2019). O crescimento da demanda por uvas apirênicas nos últimos anos tem favorecido a popularização de cultivares como a BRS Clara, BRS Morena e BRS Isis, que oferecem qualidade superior e melhor experiência sensorial ao consumidor.

Dentre as cultivares desenvolvidas para o processamento, a BRS Carmem (*Muscat Belly A* × *H 65.9.14*), a BRS Violeta (BRS Rúbea × IAC 1398-21) e a BRS Magna (BRS Rúbea × IAC 1398-21, Traviú) apresentam características que as tornam estratégicas para a produção de sucos e vinhos. A BRS Violeta se destaca pelo alto teor de compostos fenólicos (CF) e antocianinas, resultando em sucos e vinhos de coloração intensa e excelente estabilidade durante o armazenamento, sendo uma alternativa promissora para a indústria de bebidas (Camargo; Maia; Nachtigal, 2005; Rebello *et al*., 2013). Por sua vez, a BRS Magna é uma cultivar versátil que combina resistência ao míldio, elevado rendimento por hectare e excelente adaptação ao processamento, originando sucos de alta qualidade com bom equilíbrio entre açúcares e acidez (Ritschel *et al*., 2012; Nishiyama, 2020).

Já a BRS Carmem (Figura 3.2) trata-se de uma uva tardia, de ciclo longo, com elevada produtividade e resistência a doenças. Além dessas características, seu aroma e sabor aframboezado, muito similar ao da uva Bordô – amplamente utilizada no Brasil para a produção de sucos –, fazem dela uma alternativa promissora para ampliar o período de processamento e aprimorar a qualidade dos sucos de uva (Camargo; Maia; Ritschel, 2008). Por ser uma uva tintureira, ou seja, apresenta pigmentos antociânicos tanto na casca quanto na polpa, possui uma significativa concentração de antocianinas bem como de outros CF com propriedades bioativas (Nishiyama-Hortense et al., 2023). Há inúmeros relatos que sugerem que os CF possam apresentar efeitos biológicos nos seres humanos, quando ingeridos na dieta, reduzindo o risco de desordens cardiovasculares e neurológicas, bem como desenvolvimento de câncer e de doenças como Alzheimer e

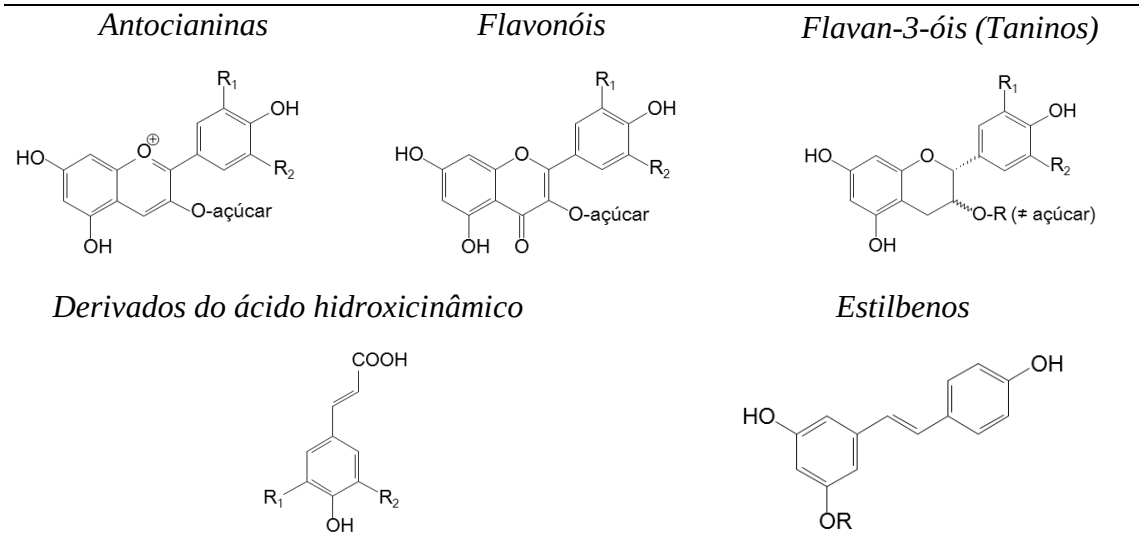
Parkinson (Chuang; Mcintosh, 2011; Georgiev; Ananga; Tsolova, 2014; Konczak; Zhang, 2004; Ribéreau-Gayon *et al*., 2006; Ruiz *et al.*, 2015; Youssef *et al*., 2015).

Figura 3.2 - Cacho da cultivar BRS Carmem.

Fonte: Autora (2022).

Todas essas uvas citadas apresentam composição fenólica complexa e dependendo da cultivar ou variedade e espécie, as uvas apresentam perfil fenólico bem como concentrações dos compostos diferenciados. Eles apresentam diversas estruturas químicas e exercem distintas funções (Belmiro; Pereira; Paim, 2017), dentre as quais: a pigmentação, a proteção contra a luz ultravioleta e a defesa contra micro-organismos patogênicos (Valiña *et al*., 2017). Dentre os compostos presentes nas uvas, bem como nos produtos derivados, é importante ressaltar as antocianinas, os flavonóis, os flavan-3-óis, os estilbenos e os derivados do ácido hidroxicinâmico (Figura 3.3). Estes compostos são classificados como flavonoides e não-flavonoides.

Figura 3.3 - Principais compostos fenólicos presentes nas uvas.



Fonte: Lago-Vanzela, 2011.

Os flavonoides são os que apresentam a estrutura química descrita como C_6-C_3-C_6 consistindo de dois anéis fenólicos (A e B), ligados por um centro pirano (contendo oxigênio) anel (C). Variações na substituição do anel C resultam nos compostos flavonóis, flavan-3-óis (flavanóis) e antocianinas (Del-Rio *et al*., 2013). Os não flavonoides são compostos tais como ácidos fenólicos, taninos hidrolisáveis e estilbenos (Jackson, 1994).

No presente estudo será dado destaque para as antocianinas, que conferem as uvas tintas e rosadas, bem como aos produtos derivados, como vinhos tintos e sucos, a coloração vermelho-violeta atrativa de diferentes tonalidades (Padilha *et al*., 2017). Como matéria-prima foco do estudo utilizaremos a uva BRS Carmem. Seu potencial tecnológico e sua composição diferenciada reforçam sua relevância para a indústria de processamento de uvas.

3.2 CARACTERÍSTICAS MORFOLÓGICAS E FÍSICO-QUÍMICAS DA UVA: BASE PARA O PROCESSAMENTO DE SUCOS

O conhecimento detalhado das características morfológicas e físico-químicas da uva é essencial para sua adequada utilização na indústria de sucos. A morfologia da fruta influencia diretamente aspectos como rendimento, qualidade sensorial e viabilidade do processamento, enquanto a composição físico-química determina atributos como teor de

açúcares, acidez, CF e perfil de pigmentação, impactando a estabilidade e aceitação do produto final (Burle; Oliveira, 2010; Callili *et al*., 2023; Martínez-Ballesta *et al*., 2021).

A caracterização morfológica é uma das primeiras etapas na avaliação da matéria-prima e consiste na análise de descritores visuais e mensuráveis que definem a estrutura dos cachos e bagas. Esses descritores, estabelecidos por organismos internacionais como a *Organisation Internationale de la Vigne et du Vin* (OIV), permitem a padronização das avaliações e a comparação entre diferentes cultivares, sendo fundamentais para a seleção de variedades com maior potencial tecnológico (OIV, 2001).

Para a caracterização morfológica da uva pode-se utilizar dezessete descritores pré-estabelecidos pela *Organisation Internationale de la Vigne et du Vin* (OIV) (2001), Mascarenhas *et al*. (2012), Weaver (1976) e Sousa (1996):

1) Massa dos cachos em gramas (g): foi determinada pela média da massa dos cachos de uva, que foram pesados em balança semi-analítica digital (Marca Gehaka BG2000, Brasil). A classificação foi realizada com base no descritor de número 502 (OIV, 2001), que contém uma escala com cinco categorias: 1- Muito baixa (até 100 g); 3- Baixa (cerca de 300 g); 5- Média (cerca de 500 g); 7- Elevada (cerca de 700 g); e, 9- Muito elevada (cerca de 900 g ou mais).

2) Comprimento dos cachos em milímetros (mm): foi mensurado pelo comprimento da extremidade (superior e inferior) do engaço central com o auxílio de um paquímetro digital (Marca King Tools, China). A classificação foi realizada com base no descritor de número 202 (OIV, 2001), que apresenta escalas com cinco categorias em milímetros: foram: 1- Muito curto (até uns 80 mm); 3- Curto (cerca de 120 mm); 5 - Médio (cerca de 160 mm); 7 - Largo (cerca de 200 mm); e 9- Muito largo (cerca de 240 mm ou mais).

3) Largura ou diâmetro dos cachos em milímetros (mm): determinado a partir da média das aferições das extremidades dos engaços laterais (“ombros”), utilizando um paquímetro digital (Marca King Tools, China). A classificação foi realizada com base no descritor de número 203 (OIV, 2001), que apresenta escalas com cinco categorias, respectivamente de: 1- Muito estreita (até uns 40 mm); 3- Estreita (cerca de 80 mm); 5- Média (cerca de 120 mm); 7- Largo (cerca de 160 mm); e 9- Muito largo (cerca de 200 mm ou mais).

4) Compacidade dos cachos: classificado a partir de observações visuais dos cachos com base no descritor estabelecido por Sousa (1996), que contém uma escala visual de cinco categorias: 1- Muito solto; 3- Solto; 5- Bem cheio; 7- Medianamente compacto; e 9- Muito compacto.

5) Formato dos cachos: classificado a partir de observações visuais dos cachos e com base no descritor estabelecido por Weaver (1976), que contém uma escala visual de seis categorias: 1- Cônico curto; 2- Cônico com ombros bem desenvolvidos; 3- Cônico; 4- Cilíndrico; 5- Cilíndrico Alado; 6- Alado com cacho duplo.

6) Presença de pruína nas cascas das bagas: classificada a partir de observações visuais das cascas das bagas com base no descritor de número 227 da OIV (2001), que contém uma escala com cinco categorias: 1- Nula ou muito baixa; 3- Baixa; 5- Média; 7- Alta; e 9- Muito alta.

7) Massa das bagas em gramas (g): correspondente a média das massas das bagas, que foi determinada com o auxílio de uma balança analítica digital (Marca Shimadzu, Brasil). A classificação realizada com base no descritor de número 503 da OIV (2001), que contém uma escala com cinco categorias: 1- Muito baixa (até 1 g); 3- Baixa (cerca de 3 g); 5- Média (cerca de 5 g); 7- Elevada (cerca de 7 g); e 9- Muito elevada (cerca de 9 g ou mais).

8) Formato das bagas: classificado a partir de observações visuais das bagas que compõem os cachos, com base no descritor estabelecido por Bioletti (SOUSA, 1996), que contém uma escala com oito categorias: 1- Esférica; 2- Achatado; 3- Elipsóide; 4- Elipsóide alongado; 5- Ovóide; 6- Oval; 7- Obovóide e 8- Alongado recurvo.

9) Comprimento das bagas em milímetros (mm): correspondente a média das medidas adquiridas a partir da determinação da distância entre o eixo polar do ápice até à base da baga. A classificação foi realizada com base no descritor de número 220 (OIV, 2001), que contém cinco categorias: 1- Muito curto (até 8 mm); 3- Curto (cerca de 13 mm); 5- Médio (cerca de 18 mm); 7- Largo (cerca de 23 mm) e 9- Muito largo (cerca de 28 mm ou mais).

10) Largura ou diâmetro das bagas em milímetros (mm): foi determinada pela média do valor medido entre a base central da baga à largura máxima. A classificação foi realizada com base no descritor de número 221 (OIV, 2001), classificado em cinco categorias: 1- Muito estreita (até 8 mm); 3- Estreita (cerca de 13 mm); 5- Média (cerca de 18 mm); 7- Larga (cerca de 23 mm); e 9- Muito larga (cerca de 28 mm ou mais).

11) Uniformidade do tamanho da baga: classificada a partir de observações visuais das bagas que compõem os cachos com base n o descritor de número 222 (OIV, 2001), que contém uma escala com duas categorias: 1- Não uniforme e 2- Uniforme.

12) Coloração da casca (epiderme) das bagas: classificada a partir de observações visuais das bagas com base no descritor de número 225 (OIV, 2001), que contém uma

escala com seis categorias: 1- Verde amarelada; 2- Rosa; 3- Vermelha; 4- Acinzentada; 5- Vermelho arrocheada escura; e 6- Azul escuro.

13) Uniformidade da cor da casca das bagas: classificada a partir de observações visuais da coloração das bagas que compõem os cachos com base no descritor de número 226 (OIV, 2001), que contém uma escala com duas categorias: 1- Não uniforme e 2- Uniforme.

14) Intensidade da pigmentação da polpa da baga: classificada a partir de observações visuais da polpa das bagas, que foram previamente cortadas ao meio com o auxílio de uma faca de aço inoxidável, com base no descritor de número 231 (OIV, 2001), que contém uma escala com cinco categorias: 1- Ausente ou muito fraca; 3- Fraca; 5- Média; 7- Forte; e 9- Muito forte.

15) Percentuais (%, m/m) de casca, polpa e semente das bagas: correspondem a média das massas das partes das uvas que foram previamente retiradas com o auxílio de uma faca inoxidável e pesadas em balança analítica (Marca Shimadzu, Brasil). O cálculo dos percentuais correspondentes foi realizado com base na massa total de cada baga analisada.

16) Esfericidade da baga, em porcentagem (%): determinada pela divisão entre o diâmetro e o comprimento da baga, multiplicada por 100, sendo o valor de 100% correspondente ao formato perfeitamente esférico (Mascarenhas *et al*., 2012). De acordo com os modelos clássicos estabelecidos por Biolletti (Sousa, 1996) as esfericidades são: 100%- Globóide; 90%- Achatado; 70%- Elipsóide; 55%- Elipsóide alongado; 72%- Ovóide; 65%- Oval; 67%- Obovóide e 55%- Curvado alongado.

17) Presença de sementes: foi determinada a partir de observações visuais da polpa das bagas, que foram previamente cortadas ao meio com o auxílio de uma faca de aço inoxidável. A classificação foi realizada com base no descritor de número 241 (OIV, 2001), que contém uma escala com três categorias: 1- Ausentes; 2- Rudimentares (desenvolvimento incompleto do embrião); e 3- Bem formadas (sementes perfeitamente desenvolvidas).

Além desses descritores morfológicos, a caracterização físico-química complementa essa análise ao fornecer informações sobre a composição da uva, incluindo parâmetros como pH, teor de sólidos solúveis (°Brix), acidez titulável e concentração de compostos bioativos, entre outros. Esses fatores são determinantes para o desenvolvimento de sucos com perfis sensoriais e nutricionais adequados, permitindo otimizar processos industriais e garantir a qualidade do produto final (Burle; Oliveira, 2010; Callili *et al.*, 2023).

Dessa forma, o estudo das características morfológicas e físico-químicas da uva não apenas contribui para um melhor entendimento da matéria-prima, mas também subsidia estratégias para aprimorar o processamento da uva e a formulação de diferenciados produtos derivados.

3.3 TRANSFORMAÇÃO DA UVA: PRODUTOS E PROCESSOS DE PRODUÇÃO

A uva é uma das frutas mais versáteis para o processamento agroindustrial, resultando em uma ampla gama de produtos com elevado valor agregado. Entre os principais produtos derivados, destacam-se as passas, obtidas pelo processo de desidratação e amplamente consumidas como snacks saudáveis e ingredientes na panificação e confeitaria (Shimizu-Marin *et al*., 2024).

O suco de uva é outro importante derivado, sendo apreciado tanto pelo seu sabor quanto pelos benefícios à saúde devido à presença de CF e antocianinas com propriedades antioxidantes (Bender *et al*., 2020; Mello; Santos, 2022; Monteiro, 2020). Além disso, o vinho continua sendo um dos produtos mais emblemáticos da uva, com uma produção que envolve desde variedades viníferas até híbridas desenvolvidas para diferentes condições edafoclimáticas (Camargo; Maia; Ritschel, 2008; De-Castilhos *et al*., 2015, 2019; Mendonça *et al*., 2016).

Além dessas aplicações tradicionais, a uva também é amplamente utilizada na produção de geleias, que preservam o sabor e os compostos bioativos da fruta, oferecendo uma alternativa para o consumo de derivados da uva em diferentes momentos do dia (Lazzarotto; Fioravanço; Taffarel, 2022; Silva, 2022), bem como em iogurtes (Silva, 2020). Além disso, os pigmentos naturais presentes nas cascas da uva, principalmente as antocianinas, têm sido explorados na produção de corantes naturais, uma alternativa promissora para substituir corantes artificiais em diversos alimentos e bebidas (Oliveira Filho, 2017). Outro produto de interesse crescente é o vinagre de uva, que apresenta não apenas atributos gastronômicos diferenciados, mas também potenciais benefícios à saúde devido à presença de CF e ácidos orgânicos que contribuem para efeitos antioxidantes e antimicrobianos (Kobayashi; Da Silva, 2020; Ticllahuanca Sayritupac, 2022).

Além dos produtos convencionais, o aproveitamento de coprodutos da uva tem sido uma estratégia cada vez mais explorada na indústria alimentícia, cosmética e farmacêutica. Subprodutos como a casca, as sementes e o bagaço da uva, ricos em

compostos bioativos, têm sido utilizados na formulação de fitoterápicos e suplementos alimentares, devido à presença de CF com efeitos antioxidantes e cardioprotetores (Cataneo *et al*., 2008). Outra aplicação inovadora está na produção de farinhas obtidas a partir do bagaço da uva, que são empregadas no desenvolvimento de alimentos funcionais e enriquecidos com fibras e compostos antioxidantes. Essas farinhas têm sido utilizadas na formulação de produtos como bolos (Huerta *et al*., 2018; Nakov *et al*., 2020; Silva *et al*., 2020) e cookies (De Melo *et al*., 2019; Poiani; Montanuci, 2019; Tonon, 2019; Abreu, 2018), promovendo um maior aproveitamento nutricional e sensorial.

Diante do exposto, observa-se que a uva e seus derivados desempenham um papel fundamental na inovação da indústria de alimentos e bebidas. O contínuo avanço na pesquisa e no desenvolvimento de novas aplicações para a uva e seus coprodutos reforça seu potencial como matéria-prima estratégica para diferentes segmentos do mercado.

No presente estudo será dado enfoque para a produção de sucos, preparados desidratados em pó a base de suco de uva e licores.

3.3.1 Suco de uva e preparados desidratados

O consumo de suco de uva, que já era elevado, teve um aumento significativo durante a pandemia, conforme apontado por AGAS (2020). Esse crescimento é atribuído à sua composição rica em compostos bioativos, os quais, embora distintos dos encontrados no vinho, estão associados à prevenção de doenças como estresse oxidativo, câncer, doenças cardiovasculares e neurodegenerativas (Copetti *et al*., 2018; Cosme *et al*., 2018). Segundo a legislação brasileira (Lei n° 7.678/1988, art. 5°), o suco de uva é definido como uma bebida não fermentada, obtida do mosto simples, sulfitado ou concentrado de uva fresca, sã e madura (Brasil, 2019), com requisitos físico-químicos específicos, como o teor mínimo de sólidos solúveis de 14 °Brix e acidez total mínima de 55 $mEq \cdot L^{-1}$ (Brasil, 2019).

A composição dos sucos de uva é influenciada por diversos fatores, incluindo a cultivar da uva, condições edafoclimáticas, tratos culturais, uso de porta-enxertos e técnicas de processamento (Granato *et al.*, 2015; Padilha *et al*., 2017). A variação da temperatura, as condições de maceração e o uso de preparados enzimáticos têm impacto não só nas características físico-químicas, mas também na composição fenólica, na atividade antioxidante e no rendimento do suco (Cabrera et al., 2009; Mojsov *et al*., 2011; Vagiri; Jensen, 2017). No Brasil, dois principais processos industriais para a obtenção do

suco de uva são adotados: a maceração sulfurosa da uva para extração, com e posterior dessulfitação (método Flanzy) e, a extração por aquecimento, sendo o último uma alternativa para produção sem resíduos de sulfitos (método Welch). É possível utilizar a prensagem da uva aquecida (processo "*Hot press*") ou realizar a prensagem da uva a temperatura ambiente ou até refrigerada, sendo denominado então processo "*Cold press*" (Bresolin *et al*., 2013; Marzarotto, 2010).

Além disso, no contexto rural, a produção artesanal de suco de uva também desempenha um papel importante, especialmente com a utilização de técnicas como a extração por arraste a vapor, que favorece a viabilidade econômica para pequenos produtores (Bresolin *et al*., 2013).

Nesse cenário, a desidratação do suco de uva surge como uma alternativa promissora para ampliar a vida útil do produto, reduzir custos e agregar valor à produção (Ajila *et al*., 2010; Santos *et al.*, 2012). A desidratação também apresenta uma importante função como substituto de corantes sintéticos, uma vez que o suco desidratado contém pigmentos naturais e compostos com propriedades funcionais, oferecendo uma alternativa saudável e prática para a indústria de alimentos (Moser; Souza; Telis, 2017).

Um processo de desidratação amplamente utilizado é a liofilização (Shofian *et al*., 2011; Wilkowska *et al*., 2016). Por este tipo de desidratação se obtém um efeito conservante pela redução da atividade de água sem aquecimento do alimento e, como resultado, uma melhor preservação da maioria das propriedades da matéria prima inicial tal como sabor, cor, aroma e atividade biológica (Ceballos; Giraldo; Orrego, 2012; Fernandes *et al*., 2013; Hammami; René, 1997). No entanto, os altos custos de produção decorrentes, principalmente, da lenta taxa de secagem e alto consumo energético necessário para sublimação da água (vapor d'água) da câmara (Raharitsifa; Ratti, 2010) inviabilizam o uso desta técnica por produtores rurais.

Outras técnicas mais acessíveis, como a secagem em leito de espuma (*foam-mat drying*), têm se mostrado alternativas viáveis para pequenos produtores (Pereira, 2008; Tavares *et al*., 2017). O processo de secagem em leito de espuma é realizado em três etapas: primeiramente, a amostra líquida ou pastosa é transformada em espuma estável, se necessário, por meio da adição de aditivos (Pereira, 2008) como, por exemplo, a clara de ovo, a albumina (Kadam *et al*., 2012), gomas (Breda; Sanjinez-Argandoña; Correia, 2012), glicerol monoestearato (Falade; Okocha, 2012), maltodextrina (Gabas *et al*., 2007; Mosquera; Moraga; Martínez-Navarrete, 2012) e carboximetilcelulose (Kadam *et al*., 2011) e, agitação em equipamentos apropriados; em seguida, a espuma obtida é

acomodada na forma de fina camada (espessura em torno de 2 a 5 mm) em uma superfície plana para posterior desidratação em secador com circulação de ar forçada a uma temperatura pré-estabelecida; e, a terceira e última etapa, consiste na desintegração da espuma da superfície em que foi desidratada, com o auxílio de uma espátula, obtendo-se o produto na forma de escamas ou pó poroso de fácil reidratação (Kadam; Patil; Kaushik, 2010; Tavares *et al.*, 2017; Thuwapanichayanan; Prachayawarakorn; Soponronnarit, 2012). Resultados de estudo mostram que o curto tempo de secagem por leito de espuma é benéfico para a retenção de compostos bioativos, se destacando como uma tecnologia adequada para a produção de pós alimentícios com alta retenção dos compostos de interesse (Reis; Moraes; Masson, 2021; Tavares *et al*., 2019).

Vários purês, polpas e sucos já foram desidratados por secagem em leito de espuma, tal como manga (Kumar *et al*., 2022a; Kumar; Kandasamy; Chakraborty, 2022), purê de abóbora (Panato; Muller, 2022), polpa de abacate (Santos *et al*., 2022), polpa mista de jambolão e acerola (De Matos *et al*., 2022), polpa de açaí (Colaço *et al.*, 2022), pitaya (Macedo *et al.*, 2021), banana vermelha (Kumar *et al*., 2022b), suco de laranja (Nemati; Motamedzadegan; Milani, 2022), beterraba vermelha e marmelo (Hajiaghaei; Sharifi, 2022), polpa de melão (Salahi; Mohebbi; Taghizadeh, 2017), jambolão (Tavares, 2017; Tavares *et al*., 2020), polpa de beterraba (Ng; Sulaiman, 2018), figo (Varhan; Elmas; Koç, 2019), pêssego (Brar *et al*., 2020) e suco de uva (Nishiyama *et al.*, 2015; Tavares *et al*., 2019). Essa técnica permite a obtenção de produtos desidratados com boa retenção de compostos bioativos, tornando-se uma solução eficiente e de baixo custo para a indústria de alimentos (Reis; Moraes; Masson, 2021; Tavares *et al.*, 2019).

Especificamente para a uva BRS Carmem, de acordo com Da-Silva *et al.* (2019), os sucos produzidos desta cultivar apresentam teores de CF elevados e uma boa atividade antioxidante. De acordo com os autores, os altos teores de CF e atributos de cor apresentados por essa cultivar as tornam uma boa alternativa para serem utilizadas na produção de sucos de uva de alta qualidade, individualmente ou em combinação com outras cultivares, portando-lhes cor, aroma e sabor. Deve-se destacar também que Ribeiro (2015) avaliou os coprodutos gerados do processamento de suco de BRS Carmem (cascas e sementes) e relatou que ainda havia presença de compostos com propriedades bioativas como ácido ascórbico, proteínas e CF, o que evidencia seu potencial para uso de forma mais sustentável. De forma geral, os coprodutos gerados do processamento da uva na forma de sucos e vinhos retêm ainda grande parte dos CF, cerca de 20-30% nas cascas e 60-70% nas sementes (Gupta; Alam, 2014; Souza *et al*., 2015).

Após produção de suco, Nishiyama *et al.* (2015) demonstraram ser possível produzir um produto em pó a base do suco da uva BRS Carmem para uso como ingrediente em diferentes formulações alimentícias. Informações adicionais são necessárias para avaliar a qualidade dos produtos desenvolvidos e sua viabilidade. Já De-Castilhos *et al*. (2016, 2019) exploraram as potencialidades desta cultivar na produção de vinhos e destacaram a sua contribuição para o aroma frutado dos vinhos produzidos.

Neste contexto, pode-se inferir que a produção de suco de uva e seus derivados desidratados oferece uma vasta gama de oportunidades para a indústria alimentícia, tanto em termos de inovação tecnológica quanto de sustentabilidade. A combinação de técnicas de processamento adequadas, como a secagem em leito de espuma, e a utilização de matérias-primas como o suco de uva, são essenciais para o desenvolvimento de produtos de valor agregado, com alta retenção de compostos bioativos e benefícios funcionais. Além disso, essas alternativas tecnológicas apresentam grande potencial para serem implementadas por pequenos produtores rurais, proporcionando uma maior inclusão econômica e o aproveitamento dos excedentes de produção, com impactos positivos tanto para o setor agrícola quanto para o consumidor final.

3.3.2 Licor

A produção de suco de uva e seus preparados desidratados oferece uma série de oportunidades para a indústria alimentícia, e, de forma similar, o licor tem se destacado como um segmento crescente no mercado de bebidas, especialmente quando produzido a partir de frutas nativas ou de cultivo local. Trata-se de uma prática antiga que tem se mostrado cada vez mais viável, principalmente para pequenos produtores e agricultores familiares. Assim como na produção de sucos desidratados, a fabricação de licores possibilita o aproveitamento das frutas em períodos de alta produção, proporcionando uma alternativa interessante para agregar valor aos produtos agrícolas e diversificar as fontes de renda. Segundo Vieira *et al.* (2010), o licor é o produto obtido pela mistura de álcool, água, açúcar e substâncias que lhe fornecem aroma e sabor, em medidas adequadas, sem que haja fermentação durante sua elaboração. Por ser considerada bebida alcoólica digestiva, os licores são geralmente consumidos após as refeições, tais como, o jantar (SEBRAE, 2012).

A definição de licor segunda a legislação brasileira (Brasil, 2024) é a bebida com graduação alcoólica de quinze a cinquenta e quatro por cento em volume, a vinte graus

Celsius, com percentual de açúcar superior a trinta gramas por litro, com a seguinte composição: i) elaborada com: álcool etílico potável de origem agrícola; destilado alcoólico simples de origem agrícola; bebida alcoólica; ou mistura de um ou mais produtos citados; ii) adicionada: de extrato ou substância de origem vegetal; de extrato ou ingredientes de origem animal, tal mel e doce de leite; ou da mistura de um ou mais produtos citados; e iii) opcionalmente de substância: aromatizante; saborizante; corante; outro aditivo; ou mistura de um ou mais produtos citados.

O licor pode ser denominado de seco, fino ou doce, creme, escarchado ou cristalizado, sendo: licor seco, a bebida que contém mais de trinta gramas por litro e no máximo cem gramas por litro de açúcares; licor fino ou doce, a bebida que contém mais de cem gramas por litro e no máximo trezentos e cinquenta gramas por litro de açúcares; licor creme, a bebida que contém mais de trezentos e cinquenta gramas por litro de açúcares; ou licor escarchado ou cristalizado, a bebida saturada de açúcares parcialmente cristalizados (Brasil, 2024).

É produzido pela maceração, método simples de infusão de álcool, que consiste em mergulhar os ingredientes na bebida escolhida. O álcool absorve lentamente os compostos aromáticos dos ingredientes, resultando em bebidas com características únicas e marcantes. O seu processamento exige tecnologia simples, que é facilmente reprodutível e se adequa a realidade da agricultura familiar, incluindo na etapa de comercialização que não exige custos com a cadeia do frio. Com isso, a elaboração de licores torna-se uma forma viável de agregar valor aos frutos, possibilitando o aumento na renda de pequenos agricultores (Barros *et al*., 2008; Oliveira *et al*., 2015).

Assim como os sucos desidratados, os licores oferecem a possibilidade de utilizar as propriedades sensoriais e funcionais das frutas, com o benefício adicional de uma maior durabilidade e viabilidade para comercialização ao longo do ano (Teixeira, 2010).

No setor de bebidas alcoólicas, tal como nos vinhos, os consumidores estão mais dispostos a pagar por produtos que enfatizam o "local" (Eustice; Mccole; Rutty, 2019; Kustos, *et al*., 2019) e palavras como regionalidade e tipicidade geográfica tornaram-se cada vez mais populares em todo o mundo (Slaghenaufi, *et al*., 2019). Neste contexto, alguns estudos têm se concentrado nas características químicas reconhecíveis e únicas relacionadas à tipicidade geográfica de uvas e vinhos (Merkytė, *et al*., 2020). A produção artesanal de licores, que frequentemente utiliza o método de maceração, representa uma alternativa acessível e de baixo custo, especialmente para pequenos produtores rurais. Esse processo simples de infusão de álcool permite extrair compostos bioativos, como os

fenólicos, das frutas utilizadas, e pode ser facilmente adaptado para a realidade da agricultura familiar. Além disso, o licor elaborado de maneira artesanal pode competir com produtos industrializados, beneficiando-se da valorização das frutas locais e nativas, como a jabuticaba, maracujá, goiaba e outras frutas de importância regional como as uvas (Almeida *et al.*, 2012; Oliveira *et al.*, 2015; SEBRAE, 2012).

Neste contexto, uma grande variedade de frutas tem sido utilizada no processamento de licores, a exemplos da tangerina (Almeida *et al*., 2012), açaí (Oliveira; Santos, 2011), maracujá (Dias *et al.*, 2011), camu-camu (Vieira *et al.*, 2010), graviola (Oliveira *et al*., 2015), damasco (Akamatsu *et al*., 2019), goiaba (Almeida; Gherardi, 2018), jabuticaba (Almeida; Gherardi, 2019), laranja (Cantos-Macías; Fernando; Cassoma-Sassupe, 2020), banana (Jesus Filho *et al*., 2018), entre outras.

Nota-se que são inúmeras as possibilidades para melhor uso das cultivares de uvas brasileiras, incluindo a BRS Carmem. O grande desafio é encontrar alternativas de processamento que permitam reconfigurar a produção e o consumo dos alimentos de forma que possam atender às necessidades de todos, ao mesmo tempo em que salvaguarda a saúde planetária.

3.4 CONSIDERAÇÕES GERAIS

A uva BRS Carmem tem se destacado como uma matéria-prima promissora para a produção de diferentes derivados, devido à sua composição fenólica rica e potencial tecnológico. A elaboração de sucos, preparados desidratados em pó à base de suco e licores não apenas permite o aproveitamento integral da fruta, mas também contribui para a agregação de valor e diversificação de produtos no setor vitivinícola. Além disso, a exploração dessas aplicações favorece a valorização da produção local, beneficiando pequenos agricultores e incentivando o desenvolvimento de produtos inovadores com apelo funcional e sensorial. Assim, o aprofundamento dos estudos sobre os CF presentes nesses produtos é essencial para maximizar seus benefícios e ampliar seu potencial de mercado.

4 MORPHOLOGICAL, PHYSICALCHEMICAL, AND ANTHOCYANIN PROFILES OF BRS CARMEM GRAPES GRAFTED ON DIFFERENT ROOTSTOCKS

ABSTRACT

Due to the great variety and cultivars of grapes in Brazil, the prospecting of cultivars that can be used as raw material for the production of different products based on social technologies is a way of encouraging agro-industrialization, mainly within the scope of family farming. Therefore, the present work aimed to determine the basic physical and chemical characteristics of the BRS Carmem grape (moisture, hydrogen potential, soluble solids and total acidity, ripeness index, as well as characteristics such as, for example, bunch and berry mass, size and shape of the bunch and berry, percentage of pulp, skin and seed, compactness, color and color uniformity) and also the anthocyanin composition, using high-performance liquid chromatography coupled with diode array detection and electrospray ionization mass spectrometry (HPLC-DAD-ESI-MS/MS^n) of the BRS Carmem grape [Muscat Belly A x H 65.9.14 (BRS Rúbea)] on two different rootstocks (IAC 572 and IAC 766). Student's t test was used ($p \leq 0.05$) to evaluate differences in rootstocks. Both rootstocks presented high values of total phenolic compounds (621.08 and 538.04). Twenty-two anthocyanins were detected in the samples and the anthocyanins derived from mv (3,5-glc, 3-cfglc-5-glc and 3-cmglc-5-glc) had a higher molar percentage than the others. Regarding the determination of the concentration of total anthocyanins (mg mv-3.5-glc·kg fruit^{-1}), the rootstocks presented similar values, IAC 572 (977.90) and IAC 766 (916.00). Although some significant differences were observed in the physical, chemical and anthocyanic characteristics of the grapes, both rootstocks present suitable characteristics for use as raw material in the development of derived products.

Keywords: Brazilian hybrid grape. Rootstocks. Anthocyanin composition. HPLC-DAD-ESI-MS/MS

4.1 INTRODUCTION

Grapes are among the most consumed and cultivated fruits worldwide (OIV, 2020), holding significant cultural and economic value. Their transformation into value-added products presents extensive opportunities for the food sector. Beyond their popularity and versatility, grapes are a rich source of phenolic compounds (PC), which contribute to their recognized health benefits and appeal to diverse consumer preferences. (Colombo *et al*., 2021; Gao *et al.*, 2022; Neri-Numa *et al*., 2018; Nishiyama-Hortense *et al*., 2022). However, the quality characteristics of grapes vary widely depending on genetic, environmental, and cultivation factors, highlighting the importance of characterizing specific cultivars.

The Brazilian BRS Carmem cultivar, developed from the hybridization of Muscat Belly A x H 65.9.14 (BRS Rúbea), stands out for its late harvest potential, which allows for extending the processing period, intense violet skin, and raspberry-like aroma and flavor (Camargo; Maia; Ritschel, 2008). With a high soluble solids (SS) content (about 19 °Brix) and notable resistance to fungal diseases, this cultivar represents a promising raw material for processing into differentiated products, including grape juice, wines, and other derivatives (Assis *et al*., 2011; Camargo; Maia; Ritschel, 2008). In addition, the BRS Carmem grape has a diverse composition of PC (Colombo *et al.*, 2020, 2021). Despite these attributes, comprehensive studies exploring its morphological and chemical characteristics across different growing conditions and rootstocks remain scarce.

Cultivation practices, environmental conditions, and the use of different rootstocks are known to significantly influence grape composition and quality (Tecchio *et al*., 2022; Silva *et al*., 2019; Domingues Neto *et al*., 2022). Rootstocks, in particular, can affect nutrient absorption, plant vigor, and disease resistance, leading to variations in fruit morphology, phenolic content, and anthocyanin profiles. These findings highlight the importance of characterizing grapes cultivated on different rootstocks to enrich the existing literature and advance research in the field to optimize the production and market positioning of the BRS Carmem grape, especially given the growing demand for natural and functional foods (Mcdougall *et al*., 2017; Munekata *et al*., 2021).

The use of grafted rootstocks is a common practice in the cultivation of BRS Carmem in Brazil, as they can adapt to adverse soil conditions. Among the most widely used in the country are ‘IAC 572’ ((*Vitis riparia* × *Vitis rupestris*) × *Vitis caribaea*) and ‘IAC 766’ (*Vitis caribaea* × *Vitis cinerea*). ‘IAC 572’ exhibits high vigor, excellent root development, and

adaptability to both sandy and clayey soils. In contrast, ‘IAC 766’ stands out for its high drought resistance and good adaptation to low-fertility soils, making it particularly suitable for warmer climates. This rootstock also provides a balanced vine vigor and enhances nutrient uptake, contributing to grape productivity and quality. Proper rootstock selection plays a crucial role in the success of grapevine cultivation, as it requires compatibility and affinity between the rootstock and the scion cultivar (Callili *et al.*, 2023; Leão, 2021).

Given the above, this study aimed to evaluate the morphological, physicochemical, and anthocyanin composition of BRS Carmem grapes cultivated on two rootstocks (IAC 766 and IAC 572). Morphological parameters such as bunches and berry dimensions, mass, and color uniformity were assessed using international descriptors for grape classification. Chemical characteristics, including pH, SS, total acidity (TA), ratio (SS/TA), water activity (aw), and total phenolic content (TPC), were analyzed to provide insights into the grape's quality and potential applications.

Finally, anthocyanins were identified and quantified using high-performance liquid chromatography coupled with diode array detection and electrospray ionization mass spectrometry (HPLC-DAD-ESI-MS/MS^n), offering a detailed profile of the bioactive compounds in this cultivar.

4.2 MATERIAL AND METHODS

Representative batches (± 130 kg) of the BRS Carmem grape (Muscat Belly x BRS Rúbea), growth under two differents rootstocks (IAC 766 and IAC 572) using the trellis training system, were purchased at the IAC Experimental Station - Centro de Seringueira e Sistemas Agroflorestais, Votuporanga, SP, Brazil (20°20′S, 49°58′W, at 525 m above sea level).

4.2.1 Chemicals

Analytical-grade chemicals with a purity of >99% and ultrapure water (Milli-Q system, Merck-Millipore, Darmstadt, Germany) were utilized throughout the experiments. LC-MS grade solvents (acetonitrile, formic acid, and methanol) were procured for anthocyanin analysis from Fisher Scientific (Madrid, Spain). Chemical standards, including malvidin (mv) 3-

glucoside (mv-3-glc) and mv-3,5-diglucoside (mv-3,5-glc), were sourced from Phytolab (Vestenbergsgreuth, Germany).

4.2.2 Morphological characterization

The morphological characterization properties were evaluated by measuring their dimensions (mass, length, and width) with a digital caliper, and the mass parameters were determined using an analytical balance. For the morphological characterization of each batch of BRS Carmem grapes (lots 1 and 2, with IAC 766 and IAC 572 rootstocks), twelve bunches were selected according to the predominant shape, size, and compactness.

To characterize the berries, ten representative berries were selected from each of the twelve bunches, totaling one hundred and twenty berries per rootstock. Seventeen pre-established descriptors (bunches mass, length and width; berries mass, length and width; percentage of peel, pulp and seed; bunches shape and compactness; presence of epicuticular wax in the berry skin; shape of the berries; sphericity of the berries; skin color of the berries; uniformity of the size berries; uniformity of the skin color of the berries; pulp pigmentation intensity; presence of seeds) were used by Mascarenhas *et al*. (2012), the Organization Internationale de la Vigne et du Vin (OIV) (2001), Souza (1996), and Weaver (1976).

4.2.3 Physicochemical characterization

For the physicochemical characterization of the grapes, 3 kg of berries collected from each lot were manually homogenized and separated into small portions of 100 g to carry out the analyses in triplicate. According to the methodologies described by AOAC (2005), the following determinations were carried out: moisture content by the thermogravimetric method in a vacuum oven at 70°C (TE-395, Tecnal, Brasil); pH, directly in pH meter (Tec 5, Tecnal, Brasil); SS by refractometry (Q767b, Quimis, Brasil) with results expressed in °Brix at 25°C; TA, by potentiometric volumetry with results expressed in g of tartaric acid·100 g of sample^{-1}; and *ratio*. Furthermore, Aw was also determined at 25°C using an electric hygrometer (Axair Ltd., Novasina, Aw Sprint, Switzerland).

The TPC was determined using the Folin-Ciocalteu reagent (Ribereau-Gayon; Stonestreet, 1965). The absorbance was determined at 765 nm after 30 min of reaction, and the

results were calculated using a standard curve and expressed as mg equivalent of gallic acid·100 g grape^{-1}.

4.2.4 Identification and quantification of anthocyanins present in BRS Carmem grapes

The characterization of the qualitative and quantitative composition of the anthocyanins present in the BRS Carmem grape was carried out, aiming to evaluate its potential as a raw material for the development of derived products. The extraction was performed as described by Lago-Vanzela *et al.* (2011a). The separation, identification, and quantification of the anthocyanins present in the sample were carried out according to previously described methods (Rebello *et al*., 2013).

The extract was filtered using a Chromafil PET 20/25 polyester membrane (0.20 μm) (Macherey-Nagel, Germany) and injected (10 μl) directly into a Zorbax Eclipse XDB-C18 reversed-phase column (2.1 mm×150 mm; 3.5 μm particle size, Agilent Technologies, USA), maintained at 40 °C. The Agilent 1100 Series system HPLC (Agilent Technologies, USA, and Macherey-Nagel, Germany) equipped with a Diode Array Detector (DAD; G1315B) and an LC/MSD Trap VL (G2445C VL) electrospray ionization mass spectrometry (ESI-MS/MS) system was used in positive ionization coupled to an Agilent ChemStation (version B.01.03) data-processing unit. The mass spectra data was analyzed using the Agilent LC/MS Trap software (version 5.3). An ion trap ESI/MS-MS was used in positive ionization mode and all the standards were used for identification and quantitation through calibration curves covering the expected concentration ranges.

The identification was mainly based on spectroscopic data (UV–Vis and MS/MS) for authentic standards or data from previous reports (Lago-Vanzela *et al*., 2011a, Rebello *et al*., 2013). For quantitation, DAD-chromatograms were extracted at 520 nm. The quantification of each anthocyanin was carried out as the equivalent of the most representative compounds: mv-3,5-glc, for anthocyanidin-3,5-glc, and mv-3-glc, for anthocyanidin-3-glc. The anthocyanins identified in the sample were thus presented qualitatively in the form of molar ratio (%), normalized to the total content. The sum of all compounds of the same type was quantitatively reported as total anthocyanins concentrations, with results expressed as mg equivalents of mv-3-glc·kg^{-1} and mv-3,5-glc·kg^{-1}.

4.2.5 Statistical analysis

To evaluate significant differences between the results obtained for BRS Carmem grapes from different rootstocks, Student's t-test was used to compare the means. A significant level of 5% was considered for both analyses. All statistical analyses were performed using the Statistica 7.0 statistical program (StatSoft Inc., USA).

4.3 RESULTS AND DISCUSSION

4.3.1 Morphological characterization

The morphological characteristics of the BRS Carmem grape, grown on the IAC 766 and IAC 572 rootstocks, are presented in Table 4.1. There was no significant difference ($p \leq 0.05$) between the rootstocks for the mass of the bunches, which, according to descriptor nº 502 (OIV, 2001), were categorized as low mass (around 300 g). According to Camargo, Maia, and Ritschel (2008), the BRS Carmem vine produces medium bunches mass around 200 g. However, lower values were determined in the present study and in others available in the literature, such as that of Assis *et al*. (2011), who reported a mass of BRS Carmem bunches of 90 g and a length of 15 cm, while Sato et al. (2021) reported an average bunch mass of 133 g (Figure 4.1).

Furthermore, although they presented significantly different mean length and width values ($p \leq 0.05$), they were categorized similarly: about length, descriptor nº 202, the rootstocks are of the short type (around 120 mm) while the diameter, descriptor nº 203, of both rootstocks showed narrow clusters (around 80 mm).

BRS Carmem bunches from the two rootstocks evaluated were categorized as cylindrical shapes (Weaver, 1976). Regarding the compactness of the bunches of both rootstocks, it is possible to classify them as moderately compact, according to Souza (1996) (Figure 4.2). The compactness of the bunches is a genetic characteristic resulting from the high fertilization of the flowers and the length of the pedicel. This agronomic characteristic of the grape is essential, as bunches that have high cluster compactness may experience phytosanitary problems during cultivation, mainly due to insect attacks and the proliferation of larvae, such as the problems faced with the grape moth (Cryptoblabes gnidiella). The caterpillars are found inside the bunches, feeding on the stem and berries, causing injuries to the berries and leakage

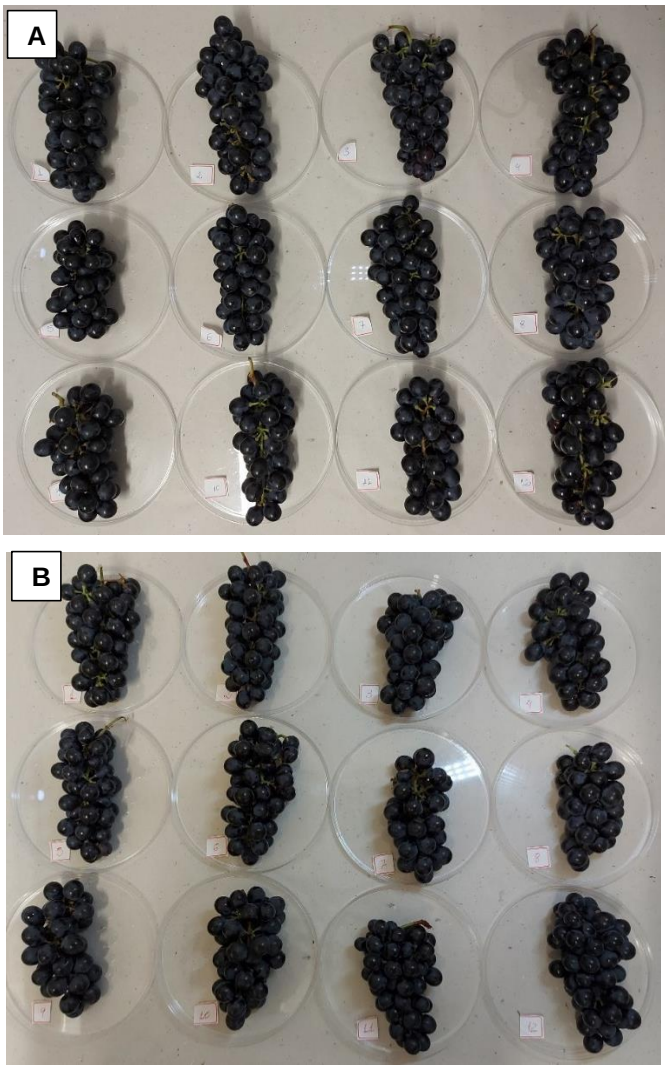
of the juice, favoring the proliferation of pathogens that make their use for processing unfeasible or compromise the quality of the derived products (Souza *et al.,* 2010). Furthermore, the high compactness of the bunches is also an unfavorable element, making them more sensitive to acid and gray rot (Brighenti *et al*., 2021).

Table 4.1 - Morphological characterization of BRS Carmem grape bunches and berries from different rootstocks according to the pre-established descriptors and categories. Results expressed numerically according to the category framed or as mean ± standard deviation.

Parameters	**Rootstock**	
	IAC 572	**IAC 766**
Bunches Mass (g)	$131.62^{a} \pm 15.15$	$137.84^{a} \pm 16.14$
Bunches length (mm)	$128.20^{b} \pm 9.10$	$137.44^{a} \pm 12.33$
Bunches width (mm)	$63.44^{b} \pm 6.68$	$68.92^{a} \pm 4.51$
Bunches shape	Cylindrical	Cylindrical
Bunches compactness	Moderately compact	Moderately compact
Presence of epicuticular wax in the berry skin	High	High
Berries Mass (g)	$3.08^{a} \pm 0.34$	$3.10^{a} \pm 0.40$
Berries length (mm)	$18.11^{b} \pm 0.89$	$18.58^{a} \pm 0.75$
Berries width (mm)	$16.19^{b} \pm 0.70$	$16.63^{a} \pm 0.65$
Peel percentage (%)	$38.27^{a} \pm 0.16$	$35.21^{b} \pm 0.16$
Pulp percentage (%)	$55.47^{b} \pm 0.18$	$58.69^{a} \pm 0.16$
Seed percentage (%)	$6.27^{a} \pm 0.01$	$6.09^{b} \pm 0.02$
Shape of the berries	Globose	Globose
Sphericity of the berries (%)	Flattened	Flattened
Skin color of the berries	Blue black	Blue black
Uniformity of the size berries	Uniform	Uniform
Uniformity of the skin color of the berries	Uniform	Uniform
Pulp pigmentation intensity	Medium	Medium
Presence of seeds	Perfectly developed	Perfectly developed

n=12 to bunches, n=120 to berries. a and b are letters that differ in the same line, which indicates the difference between grapes from IAC 572 and IAC 766 by test t Student ($p \leq 0.05$).

Figure 4.1 - Bunches of BRS Carmem grapes analyzed: (A) IAC 766 and (B) IAC. 572.



Source: Author, 2022.

Figure 4.2 - Bunches of BRS Carmem grapes with illustration of bunch compactness established by Bioletti (SOUZA, 1996).

IAC 766 — Moderately compact — IAC 572

Source: Author, 2022.

Another important characteristic of the grape berry is the presence of epicuticular wax in the berry skin (pruin). Pruin is a natural wax that covers the grape berry and according to the Technical Regulation on identity and quality for the classification of fine table grapes (Normative Instruction, 2002), its absence in the berries can be classified as a slight defect. In the present study, pruin in the berry skins was detected in both rootstocks, being classified as high (Figure 4.3), according to descriptor nº 227 (OIV, 2001).

Figure 4.3 - Pruína on BRS Carmem grape berries.

Source: Author, 2022.

The mass of the berries for both rootstocks was very close, without significant differences (3.1 g), which, according to the classification of descriptor nº 503 (OIV, 2001), are considered of low mass (about 3 g). Furthermore, the berries can be classified as uniform (descriptor nº 222) (OIV, 2001). A result close to that obtained in the present study was reported by Assis *et al.* (2011), in which the mass of BRS Carmem berries presented a value of 3.3 g. In relation to the length and width of the berries, according to descriptors 220 and 221 of the OIV (2001), it is possible to classify it as medium size for both descriptors.

Regarding the percentage of skin, pulp, and seeds, it was observed that the grapes from the two rootstocks presented significantly different values ($p \leq 0.05$), with those grown under the IAC 766 rootstock showing a higher percentage of pulp while the grapes grown under the IAC 572 rootstock had a higher percentage of skin. Nishiyama (2020), after determining the proportions of skin, pulp, and seeds for two production cycles of the BRS Carmem grape, the authors reported 67 and 74% pulp, 29 and 21% skin, and 4 and 6% seeds, with no statistical difference ($p \leq 0.05$) between the samples. The same authors reported for another Brazilian red cultivar, namely, BRS Magna, from two different harvests, 67 and 77% pulp, 30 and 18% peel,

and approximately 6% seeds, with only a statistical difference ($p \leq 0.05$) in the proportion of seeds. The results show that it is possible to find large variations in the percentages of pulp and skin of grapes from the same cultivar.

In the present study, it can be highlighted that the BRS Carmem grape had thick skin, and the solid co-products generated from its processing have the potential for use as ingredients in other products since it is rich in fiber and compounds with bioactive properties. Machado (2018), for example, developed cereal bars using grape skin flour. A proportion close to that of grape skin was reported by Lago-Vanzela *et al.* (2011b) when studying the Bordô grape, presenting values of 30% skin, 67% pulp, and approximately 4% seeds.

According to the standards established by Souza (1996), based on visual and comparative observations between the IAC 766 and IAC 572 rootstocks of the BRS Carmem grape berries, it is possible to state that there was no variation between the globose shape and the sphericity of the berries the classification found is flattened (Figure 4.4).

Figure 4.4 - BRS Carmem grape berries purchased with the illustration of the berry shape described by Biolletti (SOUZA, 1996).

IAC 766 IAC 572 Globose

Source: Author, 2022.

The standards established in descriptors nº 225 and nº 226 (OIV, 2001) for color and color uniformity, respectively, the berries from both rootstocks can be characterized as having a uniform, dark blue color. For descriptor nº 222 (OIV, 2001) for uniformity of the size berries, classified based on visual observations, the berries present uniformity. For the intensity of pigmentation of the pulp and presence of seeds, respectively, descriptors nº 231 and nº 241 (OIV, 2001), the BRS Carmem grape berries present medium intensity and presence of well-formed seeds.

4.3.2 Physicochemical characterization

Table 4.2 presents the results of the physicochemical characteristics of the grapes grown under the different rootstocks (IAC 572 and IAC 766).

Table 4.2 - Physicochemical characterization of BRS Carmem grape from different rootstocks.

Parameters	**Rootstock**	
	IAC 572	**IAC 766**
Moisture content (%)	78.65 ± 1.17[a]	79.13 ± 1.52[a]
Aw	0.98 ± 0.00[a]	0.98 ± 0.00[a]
pH	3.44 ± 0.06[a]	3.51 ± 0.06[a]
SS (°Brix)	16.25 ± 0.25[a]	15.58 ± 0.38[a]
TA	1.06 ± 0.02[a]	0.92 ± 0.02[b]
ratio (SS/TA)	15.38 ± 0.18[b]	16.94[a] ± 0.57[a]
TPC	621.08 ± 62.20[a]	538.04 ± 48.50[a]

[1]Abbreviations: aw. water activity; SS. soluble solids; TA. total acidity; TPC. total phenolic compounds. *n*=4. a and b are letters that differ in the same line, which indicates the difference between IAC 572 and IAC 766 by test t Student ($p \leq 0.05$).

Both rootstocks presented moisture content ranging from 78.65 to 79.13%. In a study by Ribeiro (2015), the moisture content of BRS Carmem was 84%. According to the Food Composition Table (TACO, 2011), the percentage of moisture content for fresh grapes is approximately 85% for the Itália cultivar and 86.1% for the Rubi grape. For aw, the value was 0.98 for both rootstocks.

In the present study, the pH value of the grapes ranged from 3.44 to 3.51, values higher than those found by Sato *et al.* (2021) and Assis *et al.* (2011), namely 3.17 and 3.3 respectively. However, the values found are within the range reported by Bender (2021) after evaluating four harvests of this cultivar (2.96 to 3.64) and by Da-Silva *et al.* (2019) (3.44 and 3.57), after evaluating this cultivar produced under the IAC 766 and IAC 572 rootstocks. Mota *et al.* (2006) reported that pH affects the stability of anthocyanins, directly influencing the grape coloring matter content.

The SS content of the grapes varied between 15.58 and 16.25 °Brix, with no significant difference ($p \leq 0.05$) between them. Assis *et al.* (2011) reported a value of 13.7 °Brix for the same cultivar produced in the North of Paraná, while Paiva (2018) and Da-Silva *et al.* (2019) reported values close to 15 ºBrix, for BRS Carmem produced in the region of São Manuel, São

Paulo, and in Votuporanga, northwest region of the State of São Paulo, respectively. On the other hand, Sato *et al.* (2021), after analyzing two production cycles of the BRS Carmem grape under the IAC 572 rootstock, from Palotina, Paraná, reported a SS content of 18.0 ºBrix. Camargo, Maia and Ritchel (2008), in Serra Gaúcha, reported an average value of 19.0 °Brix for the same cultivar, while Bender (2021), after evaluating four consecutive production cycles, reported SS values varying between 15.12 and 18.90 °Brix. These differences occur due to variations in the climatic conditions of each productive region (Warmling, 2017).

TA values ranged from 0.92 to 1.06 g tartaric acid·100 g^{-1}, results close to those reported by Assis *et al.* (2011) and by Sato *et al.* (2021) for the same cultivar, with values of 0.9 and 0.97 g tartaric acid·100 g^{-1}, and higher than those reported by Da-Silva *et al*. (2019), which were 0.88 and 0.68 tartaric acid·100 g^{-1} for grapes grown on IAC 766 and IAC 572 rootstocks, respectively.

The results related to SS and AT were also used to calculate the Ratio (SS/AT) or maturity index, which is a more representative relationship than the isolated measurement of SS or AT, and acts as an excellent parameter to evaluate the quality of fruits, including grapes (Bleinroth, 1993; Maia *et al*., 2013). The *ratio* ranged from 16.94 to 15.38, values lower than those presented in the study of Pedro Júnior, Hernandes, and Silva (2020), with different cultivars and harvests showed an average variation in the *ratio* between 25.0 and 33.7 and found that the lowest average value was in the winter harvest. Freitas *et al.* (2010) evaluated the ratio in Concord and Rúbea grape juice samples and found averages of 12.64 to 17.64.

Knowledge of grape maturation rates is essential for defining the ideal harvest point, considering the moment in which the physical-chemical characteristics are ideal for producing quality wines. The maturation phase, which covers the period from color change to harvest, may present fluctuation in sugar and acid levels caused by rain or periods of drought (Mota *et al*., 2006). Then the balance between AT and SS is extremely important for the quality of the grape flavor and producers often make changes to the production cycle to harvest in drier months, aiming to increase SS levels (Mota *et al*., 2006). The higher this value, the better the quality of the raw material.

There was no significant difference regarding the TPC of the two rootstocks. The values found in this study were higher than those observed by Nishiyama (2016), 422.9 mg equivalent of gallic acid·100 g $grape^{-1}$ for the same grape, as well as in relation to the Bordô grape (113.0) found by Lago-Vanzela *et al*. (2011b) and, in comparison with other wine grapes (73.1 – 348.6), studied by Katalinic *et al.* (2010). Da-Silva *et al.* (2022) studied several Brazilian

grapes, including BRS Carmem under the same rootstock, and the TPC values obtained for the grape skin were 2.54 $mg \cdot kg^{-1}$ equivalent to gallic acid.

4.3.3 Identification and quantification of anthocyanins from BRS Carmem Grape

The qualitative and quantitative profile of anthocyanins in BRS Carmem grape rootstocks was determined. Twenty-two anthocyanins were detected in the samples. Anthocyanins derived from delphinidin (dp), cyanidin (cy), petunidin (pt), peonidin (pn), and mv are present, both in monoglycosylated and diglycosylated forms. For some anthocyanins, acylated forms (acetylated, caffeylated, and coumarylated) were detected. The anthocyanins with the highest molar percentages in the rootstocks were the anthocyanins derived from mv (3,5-glc, 3-cfglc-5-glc, and 3-cmglc-5-glc). Eleven anthocyanins showed significant differences between the rootstocks: dp-3,5-glc, dp-3-cmglc-5-glc, cy-3-glc, pt-3.5-diglc, pt-3-cfglc-5-glc, pt-3-cmglc, pn-3.5-diglc, pn-3-cmglc-5-glc, mv-3-glc, mv-3-cfglc-5-gl and mv-3-cmglc-5-glc (Table 4.3).

In a study by Nishiyama-Hortense *et al.* (2023), where the qualitative profile of anthocyanins from this same grape was analyzed in two harvests, 26 compounds were found for the grape under the IAC 572 rootstock and 31 compounds for the grape under the IAC 766 rootstock, as well as being derived from the same five main anthocyanins found in this study. For the two rootstocks studied, the main anthocyanins were derived from mv, different from what was found for the IAC 572 rootstock in a survey by Nishiyama-Hortense *et al*. (2023), where they were derived from dp. The *p*-coumaryl derivatives of dp, pt, and mv presented a higher molar percentage in relation to the other derivatives identified in the grape. The anthocyanins that appeared least in the rootstocks were dp and cy.

The predominance of anthocyanins derived from mv has already been observed for Bordô grape skins (Lago-Vanzela *et al.* 2011a) and for the hybrid grape Máximo (Fraige; Pereira-Filho; Carrilho, 2014), corroborating what was identified in the two carriers. grafts studied. Da-Silva *et al.* (2018) reported higher concentrations of mv derivatives for BRS Carmem grown on the same rootstocks used in this study.

Table 4.3 - Anthocyanin profile of BRS Carmem grape from rootstocks IAC 572 and IAC 766 by HPLC-DAD-ESI-MS/MS (positive ionization mode): mass spectrum data, molar ratio, and total anthocyanin concentration (as equivalents of mv-3-glc and mv-3,5-glc). Given as mean ± standard deviation (*n*=2).

Assignation	**Fragmentation (m/z)**	**Molar ratios (%)**	
		IAC 572	**IAC 766**
dp-3,5-glc	627, 467, 303	0.36 ± 0.02 [b]	0.44 ± 0.01 [a]
dp-3-glc	465, 303	3.00 ± 0.03 [a]	2.65 ± 0.14 [a]
dp-3-cmglc-5-glc	773, 611, 303	3.13 ± 0.21 [a]	3.91 ± 0.06 [b]
dp-3-cmglc	611, 303	13.34 ± 0.59 [a]	15.16 ± 0.66 [a]
cy-3-glc	449, 287	1.66 ± 0.01 [a]	1.42 ± 0.06 [b]
cy-3-cmglc-5-glc	757, 595, 287	0.43 ± 0.01 [a]	0.32 ± 0.04 [a]
cy-3-acglc	491, 287	1.52 ± 0.25 [a]	0.84 ± 0.66 [a]
pt-3.5-diglc	641, 479, 317	1.13 ± 0.03 [b]	1.29 ± 0.04 [a]
pt-3-glc	479, 317	5.47 ± 0.07 [a]	5.05 ± 0.71 [a]
pt-3-acglc	521, 317	1.25 ± 0.07 [a]	1.46 ± 0.02 [a]
pt-3-cfglc-5-glc	803, 641, 317	0.72 ± 0.05 [b]	0.90 ± 0.01[a]
pt-3-cmglc	609, 317	17.30 ± 0.04 [a]	19.36 ± 0.39 [b]
pt-3-cmglc-5-glc	787, 625, 317	0.72 ± 0.03 [a]	0.71 ± 0.05 [a]
pn-3.5-diglc	625, 463, 301	3.52 ± 0.12 [a]	2.72[b] ± 0.07
pn-3-glc	463, 301	5.78 ± 0.35 [a]	5.34 ± 0.03 [a]
pn-3-acglc-5glc	667, 505, 301	0.29 ± 0.09 [a]	0.19 ± 0.06 [a]
pn-3-cmglc-5-glc	771, 609, 301	3.58 ± 0.04 [a]	3.36 ± 0.01 [b]
pn-3-cmglc	609, 301	2.68 ± 0.05 [a]	2.41 ± 0.10 [a]
pn-3-cfglc-5-glc	787, 625, 301	1.19 ± 0.02 [a]	0.88 ± 0.11 [a]
mv-3.5-diglc	655, 493, 331	21.72 ± 0.01 [a]	21.19[a] ± 0.35
mv-3-glc	493, 331	16.63 ± 0.36 [a]	14.60 ± 0.33 [b]
mv-3-acglc-5-glc	697, 535, 331	0.49 ± 0.07 [a]	0.48 ± 0.21 [a]
mv-3-acglc	535, 331	0.88 ± 0.29 [a]	1.08 ± 0.22 [a]
mv-3-cfglc-5-glc	817, 655, 331	17.98 ± 0.04 [a]	16.83 ± 0.17 [b]
mv-3-cfglc	655, 331	4.10 ± 0.34 [a]	3.43 ± 0.90 [a]
mv-3-cmglc-5-glc	801, 639, 331	44.75 ± 0.13 [b]	46.78 ± 0.40 [a]
mv-3-cmglc	639, 331	26.38 ± 0.01 [a]	27.21 ± 1.03 [a]
Total Anthocyanins concentration (mg mv-3-glc·kg^{-1})		743.19 ± 127.94 [a]	696.15 ± 119.84 [a]
Total Anthocyanins concentration (mg mv-3.5-glc·kg^{-1})		977.90 ± 68.22 [a]	916.00 ± 63.90[a]

[1]Abbreviations: dp. delphinidin; cy. cyanidin; pt. petunidine; pn. peonidin; mv. malvidin; 3.5-glc. 3.5-glucoside; 3-glc. 3-glycoside; acglc. 6″-acetylglucoside; cfglc. 6″-caffeyl glucoside; cmglc. 6″-p-coumaryl-glucoside; nd. not detected. a. b: different letters in the same column for each anthocyanin indicate that there is a significant difference ($p \leq 0.05$) between the mol % determined in the IAC 572 and IAC 766 rootstocks. By the Anova one-way test Tukey ($p \leq 0.05$).

For the quantitative profile of anthocyanins (Table 3), the IAC 572 rootstock provides a higher concentration, but does not differ significantly. The small difference between the total anthocyanin concentrations (mg mv-3.5-glc·kg fruit^{-1}) determined in the IAC 572 (977.90) and

IAC 766 (916.00) grapes may be related to the different rootstocks. Nishiyama-Hortense *et al.* (2023) and Silva *et al*. (2022) also found higher values for the same grape under the IAC 572 rootstock (1650.19 mg mv-3.5-glc·kg fruit^{-1}) in feed on the 766 rootstock (1631.41) and 572 (373.4 mg malvidin-3,5-glc·Kg^{-1}) and 766 (342.9) respectively. It is known that grape anthocyanins are synthesized via the flavonoid biosynthetic pathway, however, it is not yet well understood how the rootstock affects the biosynthesis and content of these compounds (Xi *et al*., 2016). In addition to the coloring properties of anthocyanins, the interest in these compounds is due to their health benefits, demonstrating prevention of cardiovascular, neurodegenerative, lung diseases, diabetes and cancer (Gomes *et al*., 2022; Rodrigues *et al*., 2024).

In wines produced with BRS Carmem, mv-3-glc was identified and quantified. On the other hand, the acylated forms of anthocyanidins 3-glc were not found. The derivatives 3-cmglc-5-glc were also detected in wines. MS/MS spectra showed the detection of mv-3-cfglc-5-glc, which was also identified in BRS Carmem wines (De-Castilhos *et al*., 2015). In a study by Tavares *et al*. (2019) in which they evaluated the BRS Violeta grape, the authors reported that the anthocyanins with the highest molar ratios were the diglycosylated derivatives (3,5-glc) of the three tri-substituted anthocyanidins with ring B (dp, pt, and mv). The most abundant anthocyanins in the Bordô grape were also from the group of 3,5-glycoside derivatives, with high concentrations of mv 3,5-glc and its *p*-coumaroyl derivative (Lago-Vanzela *et al*., 2011a).

The trend of higher anthocyanin concentration in the IAC 572 rootstock, already observed in other studies, suggests that the interaction between the scion cultivar and the rootstock may influence the accumulation of these compounds, even though the underlying mechanisms are not yet fully understood. In addition to the essential role of anthocyanins in the coloration of grapes and derived products, their functional importance stands out, with potential health benefits, including antioxidant properties and a role in the prevention of various diseases. The presence of these anthocyanins in wines produced with BRS Carmem reinforces their enological and functional potential.

4.4 CONCLUSION

Based on the results obtained, both rootstocks, IAC 572 and IAC 766, exhibited suitable physicochemical characteristics for the production of BRS Carmem grapes, with no significant differences in parameters such as soluble solids content (SS) and total phenolic content (TPC).

Moisture and water activity (aw) were similar between the two, while pH and total acidity (TA) values remained within the reported range for the cultivar, indicating stability in the grape's chemical composition. Although SS content and the SS/TA ratio are fundamental for determining grape ripeness and sensory quality, the values found in this study suggest a good balance between sugar and acidity, enhancing the enological potential of the cultivar. Additionally, the high phenolic content reinforces the importance of this grape as a source of bioactive compounds, regardless of the rootstock used. Therefore, both rootstocks proved to be suitable for cultivating BRS Carmem, with characteristics that can be adjusted according to production needs and the edaphoclimatic conditions of the region. The choice between them may consider other agronomic factors, such as soil and climate adaptation, as well as the grower's preferences regarding vineyard management and the final production goal.

Furthermore, it was possible to qualitatively and quantitatively characterize the anthocyanin profile of BRS Carmem grapes cultivated under the IAC 572 and IAC 766 rootstocks. The detection of 22 anthocyanins, including monoglycosylated, diglycosylated, and acylated forms, corroborates previous studies that analyzed this cultivar. The predominance of malvidin (mv) derivatives, especially mv-3,5-glc, mv-3-cfglc-5-glc, and mv-3-cmglc-5-glc, aligns with research conducted on other hybrid and vinifera cultivars, reinforcing the characteristic profile of this grape. Quantitatively, the analyzed grapes showed no significant difference, yet both contained a high concentration of anthocyanins. Therefore, both rootstocks proved to be suitable for cultivating BRS Carmem, with a well-defined anthocyanin profile concentrated in malvidin derivatives. The choice between rootstocks may consider not only anthocyanin content but also other agronomic and enological factors, aiming to optimize the quality of the grapes and the final products.

5 QUALITATIVE AND QUANTITATIVE COMPOSITION OF ANTHOCYANINS IN BRS CARMEM GRAPE JUICE POWDER: POTENTIAL AS A NATURAL COLORANT AND BIOACTIVE INGREDIENT

ABSTRACT

An important way to incorporate phenolic compounds into the diet is through the consumption of grapes and their derived products. Due to the wide variety of grape cultivars in Brazil, the prospecting of cultivars that can be used as raw material for the production of colorants and bioingredients through social technologies is a means to encourage agro-industrialization, particularly within the scope of family farming. Thus, the present study aimed to develop a dehydrated product based on Brazilian grape juice BRS Carmem and to perform a qualitative and quantitative analysis of anthocyanins. Using the IAC 766 rootstock of the BRS Carmem cultivar, grape juice was obtained with a steam extraction pan, and this juice was used to produce the powdered preparation through foam mat drying at 80°C. High-performance liquid chromatography with diode array and multi-dimensional mass spectrometry detectors (HPLC-DAD-MS^n) was used to determine the qualitative and quantitative changes in phenolic compounds present in the grape, juice, and powder preparation. The BRS Carmem grape presented a total anthocyanin concentration of 1670.70 ± 158.60 mg mv-3,5-glc·kg-1, on a wet basis, or 309.08 ± 29.34 mg mv-3,5-glc·kg-1, on a dry basis. After grape processing, products with concentrations of 943.33 ± 45.04 mg mv-3-glc·L-1 juice and 2192.20 ± 211.53 mg mv-3-diglc·kg-1 powder were developed, on a wet basis, respectively for the juice and powder. The anthocyanins with the highest molar ratios in the derived products were diglycosylated coumaroylated anthocyanins (3-cmglc-5-glc derivatives) derived from dp, pt, and mv. These anthocyanins exhibit greater stability compared to most anthocyanins typically found in grapes, which encourages the use of this preparation as a colorant in different food products.

Keywords: Grape. Anthocyanins. Bioingredient. HPLC-DAD-MS/MS.

5.1 INTRODUCTION

Grape juice is a rich source of phenolic compounds such as anthocyanins, flavonols, phenolic acids, flavan-3-ols, proanthocyanidins, and stilbenes (Guler, 2023; Rodriguez-Lopez *et al*., 2022; Sabra; Netticadan; Wijekoon, 2021; Zhou *et al*., 2022). This rich variety of compounds with bioactive properties is associated with various potentially beneficial effects on health, including reducing the risk of cancer, cardiovascular and neurodegenerative diseases, as well as antimicrobial and anti-inflammatory activity (Copetti *et al*., 2018; Cosme *et al*., 2018; García-Martínez *et a*l., 2021; Kandylis, 2021; Kersh *et al*., 2023).

The BRS Carmem grape is a hybrid cultivar resulting from the crossing of Muscat Belly A x H 65.9.14 (BRS Rúbea). Previous studies have demonstrated its well-balanced sugar and acid content (Silva *et al.*, 2018) and its complex phenolic profile, which is evident even in its immature stage (Nishiyama-Hortense *et al*., 2023). These characteristics underscore its potential for juice production, which is enhanced by its intense purple color, which may be attributed to a high concentration of anthocyanins (Da-Silva *et al*., 2019). However, different techniques used for juice processing involve varying temperatures, maceration conditions, and the use and type of enzymes, which influence not only the physicochemical composition of the produced juice but also its antioxidant capacity, yield, and phenolic composition (Guler, 2023; Ide *et al*., 2021; Kersh *et al*., 2023).

In rural environments, juice production typically involves steam extraction using an extractor pan. During this process, water is introduced into the juice as steam interacts with the grapes. This extraction method serves as a visible alternative for small-scale farmers, enabling economically feasible production on modest properties and facilitating the utilization of excess directly on-site (Bresolin; Gularte; Manfroi, 2013; Ide *et al*., 2021; Lopes *et al.*, 2016). As a second alternative for the rural producer, the dehydration of grape juice stands out in the present study (Moser; Souza; Telis, 2017; Tavares *et al*., 2019). The food industry is increasingly producing powdered fruits and juices, as they reduce costs with packaging, transportation, storage, and conservation, in addition to increasing the shelf life of products compared to fresh fruit (Silva *et al*., 2024). These products can be used as bioingredients and food colorants (Moser; Souza; Telis, 2017).

Freeze-drying is usually considered the optimal technology for preserving the nutritional and functional qualities of fruit juices (Antal, 2024; Wilkowska *et al.*, 2016). However, its high production costs, driven by slow drying rates and high energy consumption

required for water sublimation from the chamber (Kumar *et al*., 2023), often render this technique financially impractical for rural producers.

As an alternative method, the foam mat drying technique presents a viable option for grape juice dehydration due to its simplicity and low initial investment requirements (Islam *et al*., 2024). This technique involves a three-stage process: first, the liquid or paste is transformed into a stable foam (Hardy; Jideani, 2017), if necessary, by incorporating additives such as egg white albumin (Islam *et al*., 2024), gums (Paiva *et al*., 2023), maltodextrin (Islam *et al*., 2024; Silva et al., 2024; Tavares *et al*., 2019; Tavares *et al*., 2017) or methylcellulose and carboxymethylcellulose (Kumar *et al*., 2023) and stirring in appropriate equipment. Second, the foam is spread into a thin layer on a flat surface and dehydrated in a dryer with forced air circulation at a pre-established temperature. The final stage consists of breaking down the dried foam from the surface using a spatula, obtaining the product in the form of flakes or porous powder that rehydrates easily (Hardy; Jideani, 2017; Kumar *et al.*, 2023).

This research aims to develop a BRS Carmem juice powder using foam mat drying and analyze anthocyanins' qualitative and quantitative composition using high-performance liquid chromatography coupled with diode array detection and multidimensional mass spectrometry (HPLC-DAD-MS^n).

5.2 MATERIALS AND METHODS

5.2.1 Chemicals

During the analysis, chromatographic-grade solvents (>99 %), analytical-grade chemical standards (>95 %), and ultrapure water (Milli-Q system) were used. The chemical standards used were: malvidin (mv) 3-glucoside (3-glc) and 3,5-diglucoside (3,5-glc), and peonidin (pn) 3,5-glc, from Phytolab (Vestenbergsgreuth, Germany); cyanidin (cy) 3-glc and cy-3,5-glc from Extrasynthese (Genay, France).

5.2.2 Production of juice and powder product

At an ideal ripening stage, a representative batch of the BRS Carmem grape was purchased at the IAC Experimental Station - Rubber Tree Center and Agroforestry Systems, Votuporanga, SP, Brazil. The grapes were planted under IAC 766 rootstock, using the trellis

training system. In the Fruit and Vegetable Processing Laboratory, at the Sao Paulo State University “Júlio de Mesquita Filho”, Campus of São José do Rio Preto (UNESP/IBILCE), those grapes that showed some type of imperfection such as cracks or rot were discarded.

After selection, the grape berries were washed with potable water, sanitized using a chlorinated solution (0.005% v/v active chlorine), dried with paper towels, homogenized manually, deposited in plastic boxes, labeled, and stored under cooling (7° C) or frozen (-18°C), depending on the end of its use.

To obtain the juices, 2 kg of BRS Carmem grapes underwent steam extraction using an extractor pan (Suga Sucos, Bento Gonçalves, Brazil), held at a minimum temperature of 85 °C for 2.5 h. The extracted juices were immediately transferred into sterilized glass bottles with screw-on plastic lids, cooled in running water, and stored frozen until further analysis and processing (Figure 3.1).

Figure 3.1 - Artisanal process of obtaining grape juice.

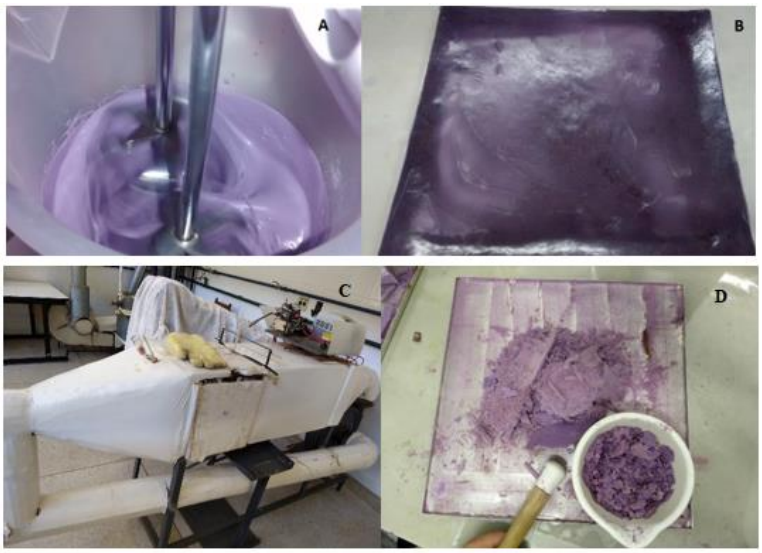
Legend: a - perforated upper container; b - container with conical opening; c - lower container; d - perforated upper container with grape berries. Source: Santos, 2016.

To develop the BRS Carmem juice powder using the foam bed drying method, a stable foam was created using 75.5% grape juice and the emulsifying additives Emustab® (2.0 %), stabilizer Super Liga Neutra® (SLN, 7.5%) and the anti-humectant Maltodextrin - DE 10 (15%). The selection of the best formulation was guided by a study conducted by Nishiyama *et al.* (2015) based on analyses of the percentage of expansion, density, and stability of the BRS Carmem juice foams. To obtain the foam, the formulation was subjected to constant stirring using a domestic mixer (Walita®) at minimum speed for 1 min and then at maximum speed for 8 min.

The foams were placed in circular stainless steel trays (radius 150 mm and height 5 mm) and placed in a tray dryer with forced air circulation (speed 1 m/s) at 80°C, until

equilibrium humidity. The samples were dried at the Drying Plant of the Department of Food Engineering and Technology at IBILCE/UNESP. The dehydrated product was removed from the trays using plastic spatulas and ground into powder using a mortar. The powdered products were promptly vacuum-packed in laminated packaging with light and oxygen barriers and stored in a desiccator until analysis (Figure 3.2).

Figure 3.2 - Production of foam containing the juice (A), foam placed on the tray (B) for the subsequent drying stage (C) and final product being macerated (D).



Source: Author, 2022.

5.2.3 Determination of qualitative and quantitative profiles of anthocyanins

The composition of anthocyanins present in the BRS Carmem grape, juice and powder was determined using HPLC-DAD-ESI-MS^n. A representative portion of the batch was separated, and a portion was used to extract the compounds of interest, as described by Rebello *et al.* (2013). In the case of whole grapes (with seeds), 100 g of berries were weighed in triplicate and homogenized using an ultra turrax (Heidolph DIAX 900, Merck, Kenilworth, USA), with 100 mL of a methanol:formic acid solution (97: 3, v/v). The mixture was exposed to an ultrasound bath (Q5.9/40, Eco-Sonics) for 10 min and centrifuged (CR-G111, Hitachi, Japan) at 9400 *g* and 5°C for 10 min. The supernatants obtained were reserved under refrigeration (± 7 °C), and the precipitates were subjected to two more consecutive extractions with 50 mL of a solution containing methanol, water, and formic acid (50:48.5:1.5, v/v/ v), following the procedure described previously, to ensure exhaustive extraction of phenolic

compounds of the grape. The supernatants from the centrifugations of each repetition were combined, resulting in a single sample. No prior extraction was required for the juice samples.

The powder extraction was conducted by homogenizing 0.5 g of the product in 5 mL of 0.1N HCl, which was sonicated for 10 min in an ultrasound bath (Q5.9/40, Eco-Sonics) and centrifuged at 9400 *g* and 5°C for 10 min (CR-G111, Hitachi, Japan). The supernatant was collected, and the precipitate was subjected to another extraction step, totaling two extractions. The supernatants obtained were combined into a single extract used in the analysis. Each sample (grape and powder) was extracted in triplicate, resulting in three extracts corresponding to each repetition (Tavares *et al*., 2017). To remove excess sugars and acids present in the powder, as well as the juice, aliquots of the samples (5 mL) were subjected to solid phase extraction in a SPE-C18 cartridge (Waters®, Sep -Pak Plus, Milford, USA, coated with 820 mg of absorbent), according to the methodology described by Rebello *et al.* (2013). The partially purified extracts were then rotoevaporated (Hei-VAP Precision, Heidolph, Germany), diluted with 0.1N HCl and filtered (0.20 μm, polyester membrane, Chromafil PET 20/25, Macherey-Nagel, Düren, Germany) directly into a chromatographic vial.

To carry out chromatographic analyses, 10 μL aliquots were injected into a chromatographic system consisting of an Agilent 1100 Series chromatograph (Agilent, Germany) equipped with a diode array detector (DAD) (Model G1315B, Agilent, Germany) coupled to a trap analyzer of ions (LC/MSD Trap VL, G2445C VL) by electronebulization ionization system (ESI-MS^n). During the analysis, the reversed-phase C18 chromatographic column (Zorbax Eclipse XDB, 2.1 × 150 mm, 3.5 μm particle, Agilent) was maintained at 40 °C. The solvents used in the mobile phase were: A, acetonitrile/water/formic acid (3:88.5:8.5, v/v/v); and B, acetonitrile/water/formic acid (50:41.5:8.5, v/v/v). The linear gradient was: zero min, 94% A and 6% B; 10 min, 70% A and 30% B; 30 min, 50% A and 50% B; 34 min, 100% B; 36 min, 100% B; 42 min, 96% A and 4% B; and, 50 min, 96% A and 4% B.

The ESI-MS^n ion trap analyzer was used in positive mode according to the parameters described by Rebello *et al.* (2013): drying gas, N2, 8 L/min; drying temperature, 325°C; nebulizer, N2, 50 psi; ionization and fragmentation parameters were optimized through direct infusion of appropriate standard solutions (mv-3-glc and mv-3,5-glc in positive ionization mode); scan range, 50-1200 m/z. The identification was mainly based on UV-vis and MS/MS spectroscopic data obtained from original standards or previous discoveries (Castillo-Munoz et al., 2009; Lago-Vanzela *et al*., 2011a; 2011b; Rebello *et al*., 2013). The analyses were carried out in triplicate. The compounds obtained in the DAD-chromatograms were extracted at 520

nm for quantification. Commercially available standard curves of mv-3-glc and mv-3,5-glc were used to quantify all anthocyanins 3-glc and 3,5-glc, respectively.

5.2.4 Statistical analysis

All results were expressed as mean ± standard deviation. To compare the data obtained for the different samples (juice and powder), significant differences were determined using analysis of variance (ANOVA) followed by Tukey's mean test ($p \leq 0.05$). The software used for statistical analysis was Statistica® 7.0 (Statsoft, Oklahoma, Tulsa, USA).

5.3 RESULTS AND DISCUSSIONS

The BRS Carmem grape presented a total anthocyanin concentration of 1670.70 ± 158.60 mg mv-3,5-glc·kg^{-1}, on a wet basis, or 309.08 ± 29.34 mg mv-3,5-glc·kg^{-1}, on a dry basis (Table 5.1). On a wet basis, Nishiyama-Hortense *et al*. 2023, after analyzing two harvests of BRS Carmem grape, reported concentrations between 1922 – 2528 mg mv-3,5-glc·kg^{-1}, values higher than those determined in the present study. After grape processing, products with concentrations of 943.33 ± 45.04 mg mv-3-glc·L^{-1} juice and 2192.20 ± 211.53 mg mv-3-diglc·kg^{-1} powder were developed, on a wet basis, respectively for the juice and powder. By transforming these values into a dry basis and expressing them in kg of grapes, it is possible to determine the retention of anthocyanins in these compounds compared to the grapes used as raw material. In the juice and powder produced, average anthocyanin retentions of 53 and 40% were achieved, respectively.

Considering the degradations that occurred during the process, it is extremely important to determine the qualitative profile of these developed products, and the results are expressed in Table 5.1. Twenty-six anthocyanins were detected in samples of juice and powdered products. Anthocyanins derived from all the most commonly found anthocyanidins in grapes were identified, namely dp, cy, pt, pn, and mv, in their both monoglycosylated (3-glc) and diglycosylated (3,5-glc) form. All anthocyanins were also found in some of their acylated forms (acetylated and coumarylated). The anthocyanins with the highest molar percentages in grapes and derived products (juice and powdered dehydrated product) were coumarylated diglycosylated anthocyanins (3-cmglc-5-glc derivatives) derived from dp, pt, and mv.

Table 5.1 - Anthocyanins derived from delphinidin, cyanidin, petunidin, peonidin, and malvidin in BRS Carmem juice and powder by HPLC-DAD-MS^n (positive ionization mode), molar ratio. Given as mean (*n*=2).

Assignation	Fragmentation (m/z)	Molar ratios (%)	
		Juice	Powder
Anthocyanin 3,5-diglucoside			
dp-3,5-glc	627,465;303	0.61±0.03a	0.66±0.01a
pt-3,5-glc	641,479;317	1.25±0.01b	1.39±0.01a
pn-3,5-glc	625,463;301	1.79±0.02b	2.24±0.03a
mv-3,5-glc	655,493;331	15.11±.06b	17.16±0.05a
pt-3-acglc-5-glc	683,521;317	0.23±.02a	0.12±0.02b
pn-3-acglc-5-glc	667,505;301	0.23±0.00a	0.12±0.01b
mv-3-acglc-5-glc	697,535;331	2.44±0.00a	1.57±0.12a
dp-3-cmglc-5-glc	773,611;303	5.82±0.00a	5.21±0.02b
cy-3-cmglc-5-glc	757,595;287	0.66±0.02a	0.71±0.00a
pt-3-cmglc-5-glc	787,625;317	4.20±0.03a	2.07±0.05b
cis-mv-3-cmglc-5-glc	801,639;331	6.41±0.05b	8.96±0.08a
pn-3-cmglc-5-glc	771,609;307	0.91±0.00b	3.57±0.04a
trans-mv-3-cmglc-5-glc	801,639;331	49.43±0.16a	48.82±0.13a
Anthocyanin 3-glucoside			
dp-3-glc	465;303	0.50±0.00a	0.39±0.00b
cy-3-glc	449;287	0.23±0.00a	0.22±0.00a
pt-3-glc	479;317	0.20±0.03b	0.50±0.03a
pn-3-glc	463;301	0.48±0.00a	0.32±0.02b
mv-3-glc	493;331	0.59±0.00a	0.71±0.02a
dp-3-acglc	507;303	0.17±.01a	0.11±0.01b
pt-3-acglc	521;317	0.14±0.00a	0.05±0.00b
pn-3-acglc	505;301	0.13±0.01	NA
mv-3-acglc	535;331	2.73±0.00a	0.16±0.02b
dp-3-cmglc	611;303	1.98±0.05a	1.69±0.01b
pt-3-cmglc	625;317	1.31±0.01a	1.12±0.00b
pn-3-cmglc	609;301	0.25±0.00a	0.21±0.01a
mv-3-cmglc	655;331	2.22±0.02a	1.92±0.01b
mg mv-3-glc·L^{-1} juice		632.34±30.19	-
mg mv-3-glc·kg^{-1} powder		-	1469.50±141.79
mg mv-3-glc·kg^{-1} dry grape		110.08±5.26a	83.73±8.08a
mg mv-3,5-glc·L^{-1} juice		943.33±45.04	-
mg mv-3,5-glc·kg^{-1} powder		-	2192.20±211.53
mg mv-3,5-glc·kg^{-1} dry grape		164.22±7.84a	124.91±12.05a

Abbreviations: dp, delphinidin; cy, cyanidin; pt, petunidine; pn, peonidin; mv, malvidin; 3,5-glc, 3,5-glucoside; 3-glc, 3-glycoside; acglc, 6″-acetylglucoside; cfglc, 6″-caffeyl glucoside; nd, not detected; a, b: different letters on the same line for each anthocyanin indicate that there is a significant difference ($p \leq 0.05$) between the mol % determined in the grape, juice and dehydrated product, by the Anova one-way Tukey test ($p \leq 0.05$).

The acetyl group was found in monoglycosylated and diglycosylated derivatives of petunidin, peonidin, and malvidin, and monoglycosylated derivatives of delphinidin. The p-coumaric derivatives were more abundant and are present in all anthocyanins. They were also found in *cis* and *trans* mv-3-cmglc-5-glc linkages. Significant differences ($p \leq 0.05$) in AT profiles were revealed for most anthocyanin compounds after grape processing in juice and powder preparation. After processing the juice into powder there was a significant reduction in anthocyanins derived from pt (3-acglc-5-glc, 3-cmglc-5-glc, 3-acglc e 3-cmglc), pn (3-acglc-5-glc e 3-glc), dp (3-cmglc-5-glc, 3-glc, 3-acglc e 3-cmglc) e mv (3-acglc e 3-cmglc). On the other hand, the molar percentage of pt (3,5-glc e 3-glc), pn (3,5-glc e 3-cmglc-5-glc) e mv (3,5-glc *cis* 3-cmglc-5-glc) increased.

Nishiyama-Hortense *et al*. (2023) analyzed two harvests (IAC-572 and IAC-766) of BRS Carmem grapes. In one harvest, the main anthocyanins were derived from dp; in the other, they were derived from mv. The *p*-caffeinated anthocyanins were found in both harvests in the monoglycosylated form (3-cfglc) and the *p*-caffeinated diglycoside anthocyanin derivatives (3-cfglc-5-glc). No *p*-caffeinated derivatives were found in this study's juice and powder. Variations in the composition of the same grape variety are due to climatic parameters or even the rootstock used.

Anthocyanins is class of essential phenolic compounds that are fundamentally responsible for the red, purple, and black color of grapes and wines. The most abundant anthocyanins in grape juices were noted as cy-3-glc, dp-3-glc, and mv-3-glc (Kim *et al*., 2017). Higher concentrations of anthocyanins were found in a study of Nale *et al*. (2024), with three varieties of grape juice, maximum anthocyanin was recorded in Pusa Navrang (459.10 mg of cy-3-glc·100 mL^{-1} juice) followed by Manjari Medika (432.0). Meziane *et al*. (2023) evaluated eight different varieties of grape juice (*Vitis vinifera* L.), with black grapes standing out: Gros noir (1353.6 mg cy-3-glc·kg^{-1} of extract), Cinsault (412.21 mg·kg^{-1}), Merlot (731.11 mg·kg^{-1}), and Syrah (1455.9 mg·kg^{-1}). Climatic conditions, such as temperature, sunlight, relative humidity, and precipitation, affect fruit ripening and, therefore, the synthesis of anthocyanins in grape skin (Gil-Muñoz *et al*., 2017). This variation in grape ripening conditions is probably the factor most responsible for the variability of the results observed in the different studies.

In a study by Tavares *et al*. (2019) in which they evaluated fruit, juice, and powder from the BRS Violeta grape, the authors reported that the anthocyanins with the highest molar ratios were the diglycosylated derivatives (3,5-glc) of the three tri-substituted anthocyanidins with ring B (dp, pt, and mv). The most abundant anthocyanins in the Bordô grape were also from

the group of 3,5-glucoside derivatives, with high concentrations of mv-3,5-glc and its *p*-coumaroyl derivative (Lago-Vanzela *et al.*, 2011b).

In the present study, the molar percentage of diglycosylated anthocyaninds was 87%, 70% and 93.2% juice and powder, respectively. Study by Tavares *et al*. (2019), after obtaining BRS Violeta grape juice and subsequent production of the powdered preparation by drying on a foam bed, they found an increase in the molar percentages of diglycosylated anthocyanins from 76 to 87% of the grape for the juice and from 87 to 88 .5-90% from juice to powder products. In contrast, there were significant reductions in the molar ratios of monoglycosylated anthocyanins (24 to 13% and 13 to 9.5-11%, respectively). In work with jambolan juice powder, a higher molar proportion of diglycosylated anthocyanins (99.47 to 99.62%) was obtained (Tavares *et al.,* 2020).

The lower retention determined for the powdered product was expected since the juice used to develop this product was subjected to dehydration, which inevitably leads to the anthocyanins' thermal and oxidative degradation. On the other hand, the inherent concentration effect of the technology used led to the development of a product with a high concentration of anthocyanins, demonstrating the potential of this cultivar for the development of artisanal juices and bioingredients.

Study results show that the short drying time using a foam mat drying is beneficial for the retention of bioactive compounds, standing out as a suitable technology for the production of food powders with high bioactive retention (Reis; Moraes; Masson, 2021; Tavares *et al*., 2019). Various purees, pulps and juices have already been dehydrated by drying on a foam bed such as red beet and quince (Hajiaghaei; Sharifi, 2022), pumpkin puree (Panato; Muller, 2022), fig (Varhan; Elmas; Koç, 2019), red banana (Kumar *et al.,* 2022b), beet pulp (Ng; Sulaiman, 2018), pitaya (Macedo *et al.,* 2021), mixed jambolan and acerola pulp (De Matos *et al*., 2022), mango (Kumar *et al*., 2022a; Kumar; Kandasamy; Chakraborty, 2022), avocado pulp (Santos *et al*., 2022), orange juice (Nemati; Motamedzadegan; Milani, 2022), peach (Brar *et al*., 2020), melon pulp (Salahi; Mohebbi; Taghizadeh, 2017). Colaço *et al*. (2022) reported that Açaí pulp presented a total anthocyanins content of 345.42 mg cy-3-glc·100g^{-1} dry basis. Tavares *et al.* (2017) found for jambolan a total anthocyanin concentration between 1551.63 to 2297.90 mg of mv-3,5-glc·kg^{-1} powder and Tavares *et al.* (2019), for grape juice, a total anthocyanin concentration between 3797.77 to 5491.20 mg equivalents of mv-3,5-glc·kg^{-1} powder, values higher than those found in the present study.

Some foam bed drying conditions have been shown to influence the anthocyanin content of plant-based foods. An increase in the concentration of the foaming agent causes a decrease in the anthocyanin concentration. Furthermore, lower drying times of the foam bed are favorable to the preservation of anthocyanins. Therefore, when the drying temperature of the foam bed is slightly increased to reduce the drying time without destroying anthocyanins, an increase in the drying temperature of the foam mat is favorable to the preservation of anthocyanin. On the other hand, a severe increase in the foam drying temperature causes the thermal destruction of anthocyanins (Reis; De Moraes; Masson, 2021).

5.4 CONCLUSIONS

This study successfully developed a dehydrated product based on Brazilian BRS Carmem grape juice, demonstrating the presence of stable anthocyanins with high potential for use as food colorants, given that average anthocyanin retentions of 53 and 40% were achieved in the juice and powder produced, respectively. Qualitative and quantitative analysis performed with HPLC-DAD-MS^n revealed that the predominant anthocyanins in the derived products were diglycosylated and coumaroylated anthocyanins (3-cmglc-5-glc derivatives) from dp, pt, and mv, compounds known for their superior stability compared to most anthocyanins found in grapes. This stability profile suggests that the powdered product obtained could serve as a viable and effective alternative for applications in food products, promoting the use of Brazilian grapes as a natural source of durable food colorants. The short drying time using foam belt drying stands out as a suitable technology for the production of food powders with high bioactive retention.

6 BRS CARMEM GRAPE LIQUEURS: INFLUENCE OF ALCOHOLIC BASE ON PHYSICOCHEMICAL CHARACTERISTICS, ANTHOCYANIN COMPOSITION, AND SENSORY ACCEPTANCE

ABSTRACT

Producing grape liqueurs is a promising approach to diversifying fruit-derived beverages and adding value to local raw materials. This study evaluated the impact of cereal alcohol (A) and white cachaça (C) on the physicochemical composition, anthocyanin profile, and sensory attributes of liqueurs made with the Brazilian cultivar BRS Carmem. Both products met regulatory requirements (alcohol content >15% v/v and sugar >100 $g \cdot L^{-1}$). The alcoholic base significantly influenced most physicochemical parameters but not the anthocyanin profile. A resulted in higher extraction of organic acids (0.39 vs. 0.33 g tartaric acid·100 g^{-1}) and phenolic compounds (607.45 vs. 457.64 mg gallic acid·100 g^{-1}). HPLC-DAD-ESI-MS/MS analysis showed a predominance of diglycosylated anthocyanins (98%), with total concentrations of 420.04 $mg \cdot L^{-1}$ for CA and 456.44 $mg \cdot L^{-1}$ for C. Both liqueurs were well accepted (overall impression: A = 7.1, C = 7.2) with good purchase intent. Significant differences were observed for appearance and color (preferred in A) and aroma (preferred in C). These attributes correlated strongly with the overall impression, but flavor and alcohol content were the key factors influencing purchase decisions. The findings demonstrate that the choice of alcoholic base affects the composition and sensory acceptance of grape liqueurs, highlighting the importance of selecting the appropriate alcohol source to optimize bioactive compound extraction and consumer preference, enhancing the product's quality and market potential.

Keywords: Brazilian grape, social technology, anthocyanins, HPLC-DAD-ESI-MS/MS.

6.1 INTRODUCTION

The global consumption of alcoholic beverages remains significant, particularly in emerging markets, where younger, expanding middle classes, increasing urbanization, and a rising interest in premium and innovative alcoholic beverages (IWSR, 2024) are driving growth. With a diverse range of offerings catering to different consumer preferences, the International Wine and Spirit Research highlights promising markets of scotch, sparkling wine, and liqueurs (IWSR, 2024). Consumers are also increasingly attracted to products that emphasize the origin of their ingredients and the history behind their production, especially those linked to family farming. Considering the simplicity of production, the liqueur market presents an opportunity to blend tradition and modernity by fostering innovation and promoting the sustainable use of local resources. Moreover, contemporary consumers are prioritizing healthier and more sustainable choices, favoring beverages that strike a balance between moderation, responsibility, and sensory appeal (Caldeira *et al*., 2018; Neves *et al*., 2022; Oliveira *et al*., 2015; Silva *et al*., 2021).

With a balanced sweetness profile, intense purple color (with pigments in the skin and pulp), and distinct fruity notes (Camargo, 2008), the BRS Carmem grape (Muscat Belly A x BRS Rúbea) offers significant potential liqueur production, which requires ingredients with well-defined and distinctive flavors. Furthermore, BRS Carmem shows good adaptability to various climatic and soil conditions, supporting its cultivation in different regions of Brazil, as well as a complex and interesting concentration of bioactive compounds, such as phenolic compounds, even when immature (Nishiyama-Hortense *et al.,* 2023). Therefore, the use of this cultivar in such products can represent an opportunity to explore and value local fruits, aligning with the growing demand for products that emphasize the origin and tradition of ingredients.

However, although the Brazilian Agricultural Research Corporation (Embrapa) developed this cultivar as an alternative for processing (Camargo, 2008), research on its potential for liqueur production is scarce. In general, grape-based beverages are predominantly represented by wines and juices, which have long-standing market acceptance and well-established production processes. As an example, the phenolic composition of juices and wines from the BRS Carmem grape has been evaluated in previous studies (Da Silva *et al*., 2018; Da Silva *et al*., 2019; De Castilhos *et al*., 2019); however, in the current literature there are no reports on BRS Carmem liqueur. This is of utmost importance, as phenolic compounds may be closely related to sensory characteristics and, particularly, to the color of the final product.

Phenolic compounds are typically mainly extracted during the maceration phase of production and can be influenced by numerous factors such as time, type and concentration of alcohol, temperature, and the ratio of fruit to extracting solution (Addis *et al*., 2024). Among these factors, the choice of alcoholic source is particularly relevant, as it directly influences the extraction of bioactive compounds and the sensory characteristics of the liqueur. Dechkunchorn *et al.* (2023) demonstrated that a 60% ethanol solution led to a higher total phenolic content in the final product compared to a 40% solution, underscoring the impact of alcohol concentration on compound extraction. Similarly, Neves *et al.* (2022) reported that the use of cachaça instead of cereal alcohol in jabuticaba liqueurs modified the volatile and anthocyanin profiles, indicating that the composition of the alcoholic matrix can significantly affect both the chemical composition and the color of the product.

It is also noteworthy that, despite their potential to diversify the grape-based product portfolio, the acceptance of these liqueurs may be influenced by consumer familiarity with the sensory profiles of wines and juices. Thus, understanding consumer perception and the factors influencing the acceptance of grape liqueurs becomes essential, considering both sensory attributes and market insertion challenges. In this context, this study aimed to develop a grape liqueur using the Brazilian cultivar BRS Carmem, evaluating the influence of two different alcoholic bases: cereal alcohol (A) and white cachaça (C). The effects of these alcohol sources on the physicochemical properties, anthocyanin composition, and sensory attributes of the liqueurs were assessed, contributing to the existing knowledge of grape-based liqueurs, supporting the development of innovative products, and enhancing the value of local resources.

6.2 MATERIALS AND METHODS

6.2.1 Reagents and standards

All solutions were made of deionized water (18.2 MΩ cm, Milli-Q, Millipore, Bedford, MA, USA). All solvents were HPLC-grade, and all chemicals were analytical grade (99%). The standards used for methanol determination was methyl-4-pentanol-2 and for anthocyanin analyses were the diglucosides (3,5-glc) derivatives of malvidin (mv) and cyanidin (cy), from Sigma-Aldrich (Spain), and peonidin (pn) from Phytolab (Germany) and the monoglucosides (3-glc) derivatives of pn and cy, purchased from Sigma-Aldrich (Spain), and mv (Extrasynthese, France).

6.2.2 Grapes and ingredients for liqueur production

The BRS Carmem grape variety was acquired from the Advanced Center for Research on Rubber Trees and Agroforestry Systems, of the Agronomic Institute (IAC), located in the municipality of Votuporanga, State of São Paulo, Brazil (20°20′S and 49°58′W, altitude from 525 m above sea level). Grapes were cultivated on the IAC 572 rootstock and trained using the trellis system. After harvest, the grapes were transported to the Fruit and Vegetable Processing Laboratory of the Department of Food Engineering and Technology at the São Paulo State University, Institute of Biosciences, Humanities and Exact Sciences, São José do Rio Preto, Brazil. Each bunch had its berries detached, discarding those with imperfections or signs of rot. The berries were then washed with potable water and sanitized by immersion in a chlorinated solution (0.005% v/v active chlorine) for 20 min. After sanitization, the grapes were rinsed with potable water, placed on paper towels, and left on a previously sanitized stainless steel countertop with 70% alcohol to remove excess water. Subsequently, they were stored in food-grade plastic bags and kept under freezing (-18°C) until the start of analyses or processing, respectively.

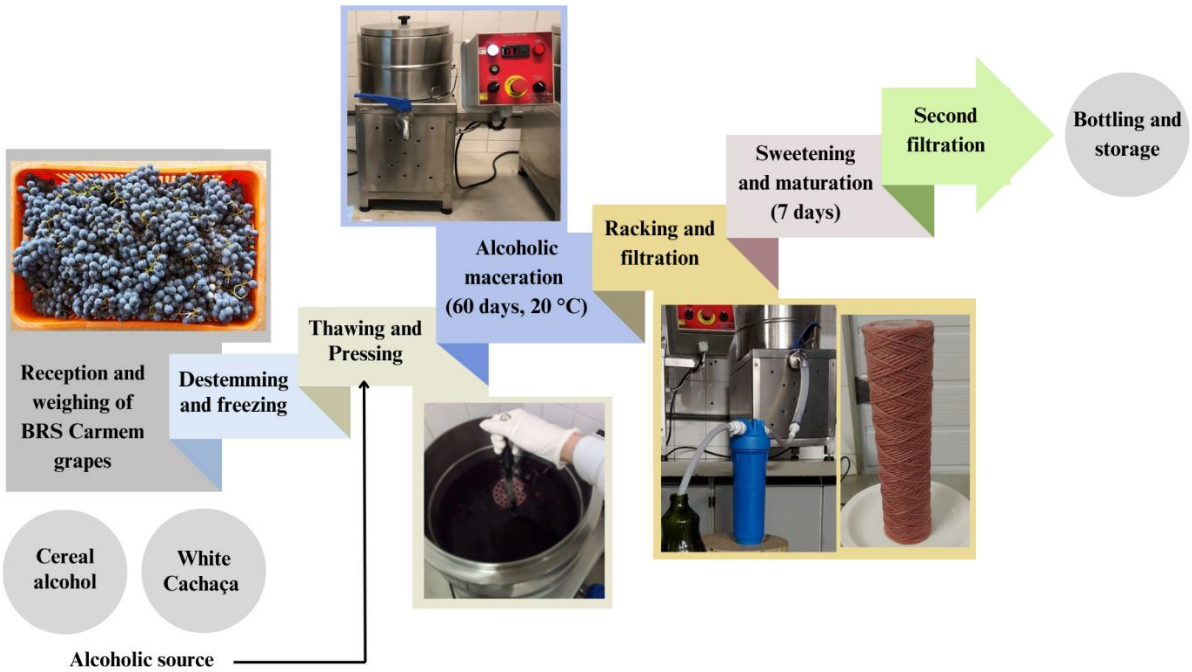
For the liqueur processing, C (~42°), sourced from the JP distillery in Itupeva, São Paulo, and A (~94°ABV), obtained from the local market in São José do Rio Preto, São Paulo, were used as alcoholic bases. Inverted sugar from the local market was used to sweeten the liqueurs. Before production, the A was diluted with potable water to match the alcohol content of the C.

6.2.3 Liqueurs production

The production of the liqueurs using the two alcoholic sources, A and C, followed a formulation adapted from EMBRAPA (Abella *et al*., 2007) and was conducted in accordance with Good Manufacturing Practices (Brazil, 2004). The flowchart of the process can be observed in Figure 6.1. The previously frozen grapes were added to 10 L stainless steel tanks (INOXTECH, Brazil) under controlled temperature (20 ± 3 °C) with the respective alcoholic source in a 1:0.5 (w/w) ratio. For this step, the A was pre-diluted with potable water to obtain a hydroalcoholic solution with the same alcohol percentage as C (~42°). The production was performed in duplicate, with two tanks containing grapes and source A, and the other two

containing grapes and source C. After thawing, the grapes were pressed using a stainless steel utensil and left to macerate.

Figure 6.1 - Flowchart of the liqueur production process from BRS Carmem grapes.



The mixture remained in maceration for 60 days, in contact with the solid matrix. After this period, the alcoholic extracts were filtered through a wound filter (5 x 2 1/2" with polypropylene thread and core) and inverted sugar was added at a concentration of 20 %.

The proportion of inverted sugar was determined to meet the standards established for liqueurs by national legislation (between 100 and 350 $g \cdot L^{-1}$ for fine liqueurs, Brazil, 2018) and other international regulations, including the Code of Federal Regulations (CFR) of the United States (minimum of 2.5% sugar by volume) and the European Parliament and the Council of the European Union (100 $g \cdot L^{-1}$). Subsequently, the liqueurs were kept in a dark place at room temperature to be maturated for an additional seven days, underwent a second filtration, and were bottled in 1 L glass containers with pre-sterilized caps for further analysis.

6.2.4 Physicochemical characterization of the liqueur

The yield of the liqueurs was determined according to Equation 1.

$$Y = \frac{Initial\ mass\ (g)}{Final\ mass\ (g)} * 100 \qquad (1)$$

To evaluate the compliance of the produced liqueurs with the identity and quality standards established by national legislation (Brazil, 2018), the analyses of alcohol content, total sugar determination, methanol content, and metallic contaminants were performed. The alcohol content (% by volume) was determined according to the methodology described by the Adolfo Lutz Institute (IAL, 2008). To estimate the actual alcohol content of the finished liqueurs, aliquots (100 mL) of each liqueur were subjected to distillation in a fractional distiller with a heating mantle (102E, Fisatom, Brazil), and the alcohol content of the distillate was determined using a densitometer (Density Meter DMA 4500M, Anton Parr, Austria), which converts the liquid density into alcohol content based on reference tables or the internal equations of the equipment. The determination of total sugars in the liqueurs was performed according to the Lane-Eynon methodology (IAL, 2008).

The determination of methanol content in the liqueurs was performed by gas chromatography with flame ionization detection (GC-FID) (Gas Chromatograph, Clarus 480, PerkinElmer, USA), using the sample distillate spiked with an internal standard (methyl-4-pentanol-2). The separation was carried out in a Carbowax 400 column at 5% and Hallcomid M.18 OL at 1%, coated on Chromosorb W (60–80 mesh) inside a stainless-steel column, 7.5 m in length and 1/8" in diameter. The chromatographic support (Chromosorb W) was pre-activated in an oven at 750–800 °C for 4 h, as recommended by the International Organisation of Vine and Wine (OIV, 1990).

The determination of metallic contaminants (lead and copper) was performed by atomic absorption spectrometry, following the methodologies described in the Standard Methods for the Examination of Water and Wastewater (Methods 3120B and 3111B, respectively) (Lipps; Braun-Howland; Baxter, 2023).

For the physicochemical characterization of the produced liqueurs, duplicate analyses were performed for the following parameters: soluble solids (SS), pH (IAL, 2008), and total acidity (TA) (AOAC, 2005). Additionally, the total phenolic compounds (TPC) (Ribereau-Gayon & Stonestreet, 1965) and chromaticity parameters (McMellan; Lind; Kime, 1995) were determined. A benchtop spectrophotometer (ColorFlex45/0 model, Hunterlab, USA) was used for the determination, with D65 illuminant settings and a 10° observer angle. The Universal Software version 4.10 was used to determine the absolute values of *L** (lightness, ranging from 0 to 100, from black to white), *a** (variation between green and red), and *b** (variation between

blue and yellow). The color specification system used was CIELAB, which utilizes the CIE *L**, *a**, *b** scale, defined by the Commission Internationale de l'Éclairage (CIE). Using the absolute values (*a** and *b**), chromaticity (*C**) was calculated in cylindrical coordinates through Equation 2, and the hue angle (h°) was determined using Equations 3, 4, or 5, depending on the values of *a** and *b** (Francis, 2005; Pathare; Opara; Al-Said, 2013).

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (2)$$

$$h° = \tan^{-1}(b^*/a^*), \text{ where } +a^* \text{ and } +b^* \quad (3)$$

$$h° = 180 + \tan^{-1}(b^*/a^*), \text{ where } -a^* \text{ and } +b^* \text{ or } -a^* \text{ and } -b^* \quad (4)$$

$$h° = 360 + \tan^{-1}(b^*/a^*), \text{ where } +a^* \text{ and } -b^* \quad (5)$$

6.2.5 Identification and quantification of anthocyanins

The composition of anthocyanins in BRS Carmem grape liqueurs was determined by high-performance liquid chromatography coupled with a diode array detector and mass spectrometry with electrospray ionization (HPLC-DAD-ESI-MS^n), without the need for prior extraction. To remove excess sugars and acids present in the liqueurs, aliquots of 4 mL of the samples were subjected to solid-phase extraction (SPE) using SPE-C18 cartridges (Sep-Pak Plus, Waters®, Milford, USA), containing 820 mg of adsorbent phase, according to the methodology described by Rebello *et al*. (2013). The partially purified liqueurs were then concentrated by rotary evaporation (Hei-VAP Precision, Heidolph, Germany), diluted in 0.1 N HCl, and filtered through polyester membranes (0.20 μm, Chromafil PET 20/25, Macherey-Nagel, Düren, Germany) before injection into the chromatographic system.

For the chromatographic analyses, 10 μL aliquots were injected into a chromatographic system consisting of an Agilent 1100 series chromatograph (Agilent, Germany), equipped with a diode array detector (DAD) (Model G1315B, Agilent, Germany) and coupled to an ion trap analyzer (LC/MSD Trap VL, G2445C VL) through an electrospray ionization system (ESI-MSn). During the analysis, a C18 reverse-phase chromatographic column (Zorbax Eclipse XDB, 2.1 × 150 mm, 3.5 μm particle, Agilent) was maintained at a temperature of 40 °C.

The mobile phase consisted of the following solvents: (A) acetonitrile/water/formic acid (3:88.5:8.5, v/v/v) and (B) acetonitrile/water/formic acid (50:41.5:8.5, v/v/v). The linear gradient program was as follows: 0 minutes, 94% A and 6% B; 10 minutes, 70% A and 30%

B; 30 minutes, 50% A and 50% B; 34 minutes, 100% B; 36 minutes, 100% B; 42 minutes, 96% A and 4% B; and 50 minutes, 96% A, and 4% B.

The ESI-MS^n ion trap analyzer was operated in positive mode, following the parameters established by Rebello et al. (2013): drying gas (N2) at 8 $L \cdot min^{-1}$, drying temperature of 325 °C, and nebulizer (N2) at 50 psi. Ionization and fragmentation settings were optimized through direct infusion of appropriate standard solutions (mv-3-glc and mv-3.5-glc in positive ionization mode), with a scanning range of 50–1200 m/z. The identification of compounds was based primarily on UV-Vis and MS/MS spectroscopic data obtained from analytical standards or previous studies (Castillo-Muñoz *et al*., 2009; Lago-Vanzela *et al*., 2011a; 2011b; Rebello *et al*., 2013). All analyses were conducted in quadruplicate.

For quantification, the compounds extracted from the DAD chromatograms were analyzed at 520 nm. Standard curves of mv-3-glc and mv-3.5-glc were used for the quantification of all 3-glc and 3.5-glc anthocyanins, respectively. The anthocyanins identified in the samples were presented qualitatively as the molar ratio (%), normalized to the total content. The sum of all compounds of the same type was quantified as the total anthocyanin concentration, expressed in mg equivalents of mv-3-glc·kg^{-1} and mv-3.5-glc·kg^{-1}.

6.2.6 Sensory analysis

The characterization of the liqueurs was performed through affective sensory analysis, including acceptance tests and purchase intention, with the goal of assessing the influence of the alcoholic source used in the formulation (A or C). The study was approved by the Ethics and Research Committee of the Institute of Biological Sciences, Letters, and Exact Sciences (IBILCE) of the São Paulo State University "Júlio de Mesquita Filho," Unesp, São José do Rio Preto Campus, Brazil, under CAAE nº 78781324.5.0000.5466. All experimental activities were conducted in the sensory analysis laboratory located on the aforementioned campus, in individual booths previously sanitized and under direct white lighting.

Before participating in the sensory analysis, the evaluators provided written informed consent and filled out a characterization form with information regarding their preference and frequency of liqueur consumption. Only participants considered potential consumers were included in the analysis. These individuals were defined as those aged 18 years or older, regardless of gender, who indicated that they liked liqueurs or consumed them at least once every six months.

For the sensory tests, the samples were presented in transparent acrylic cups (40 mL), previously coded with random three-digit numbers, containing 15 mL of each liqueur (A and C). Samples of both formulations were offered in a monadic and balanced manner (Macfee; Bratchell, 1989). Consumers were asked to evaluate the samples based on the degree of liking for eight attributes: Appearance, color, consistency, aroma, flavor, sweetness, alcohol content, and overall impression, using a balanced 9-point hedonic scale: 9 - extremely liked; 8 - liked very much; 7 - moderately liked; 6 - slightly liked; 5 - neither liked nor disliked; 4 - slightly disliked; 3 - moderately disliked; 2 - disliked very much; 1 - extremely disliked. Finally, consumers were asked about their purchase intention using a 5-point balanced scale (1 - would definitely not buy; 5 - would definitely buy).

6.2.7 Statistical Analysis

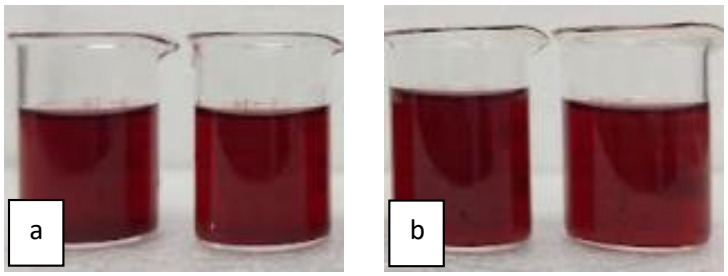
All results were expressed as mean ± standard deviation. To compare the data obtained for the liqueurs, significant differences were determined using two-way analysis of variance (Two-Way ANOVA), followed by Tukey's multiple comparison test ($p \leq 0.05$) and Pearson correlation, considering $r > 0.5$ as a strong correlation. The software used for all statistical analyses was Statistica® 7.0 (Statsoft, Oklahoma, Tulsa, USA).

6.3 RESULTS AND DISCUSSION

6.3.1 Physicochemical characteristics

The produced liqueurs (Figure 6.2) had an average yield of approximately 65%. This result is lower than that reported by Oliveira *et al*. (2015), who obtained yields ranging from 79.29% to 85.25% for different soursop liqueur formulations. However, the authors used the fruit pulp, which, unlike the present study, does not generate co-products containing peels and seeds, such as grape pomace. Although yield information is crucial for assessing the feasibility of processing, considering the potential commercialization of the product, little has been reported in the literature.

Figure 6.2 - Grape liqueurs of BRS Carmem produced with (a) cereal alcohol (b) white cachaça.



In Table 6.1, the results of the physicochemical characterization of both liqueurs are presented. The SS, expressed in °Brix, showed significant differences between the analyzed samples. Previous studies report values similar to those obtained in the present work. Oliveira and Santos (2011) observed an average value of 38.6 °Brix in açai liqueur, while Teixeira *et al.* (2005) found values ranging from 36.0 to 40.7 °Brix in banana liqueur. In a study on grape liqueur, Laureano *et al.* (2001) reported a soluble solids content of 37.4 °Brix. However, other research indicates lower values, such as acerola pulp liqueur (25.5 °Brix) (Penha *et al*., 2001), camu-camu (33.0 °Brix) (Vieira *et al*., 2010), jabuticaba (15.0 °Brix) (Geöczer, 2007), and beetroot (21.4 °Brix) (Marques, 2015). The pH and TA parameters of the evaluated liqueurs also showed statistically significant differences ($p \leq 0.05$). Higher acidity and, consequently, lower pH values were found in the cereal alcohol.

Table 6.1 - Physicochemical characterization of BRS Carmem grape liqueurs produced with different alcoholic sources.

Parameter	**Cereal alcohol**	**White Cachaça**
SS (ºBrix)	23.25 ± 0.01*	19.50 ± 0.01
pH	2.77 ± 0.06	2.87 ± 0.02*
AT (g ácido tartárico/100 g)	0.39 ± 0.06*	0.33 ± 0.04
CFT (mg ácido gálico/100 g)	607.45 ± 27.83*	457.64 ± 11.86
*L**	26.51 ± 0.63	30.64 ± 0.25*
*a**	15.04 ± 0.51*	11.05 ± 0.77
*b**	25.23 ± 0.91*	21.90 ± 0.72
*C**	29.38 ± 0.52*	24.55 ± 0.31
h^o	59.16 ± 1.77	63.18 ± 2.34*

SS: Soluble solids, AT: total acidity, total phenolic compounds. * In the same row indicate a significant difference by the Student's t-test ($p \leq 0.05$) between samples.

The alcoholic concentration of the beverages allowed for the extraction of phenolic compounds from the grape into the liqueur, with the total phenolic content (TPC) showing a significant difference between the samples ($p \leq 0.05$). The most efficient extraction of phenolic compounds was promoted by the A. Concerning different fruits for liqueur production, Leonarski *et al*. (2021) reported that liqueurs made from jabuticaba had the highest concentrations of TPC (84.15 mg GAE·100 g^{-1}), followed by guabiroba (34.91), and blackberry (13.80). The TPC concentrations in the liqueurs produced in this study are much higher than those reported in the cited studies. It is known that the amount of TPC depends on the type of raw material, the degree of fruit ripeness, soil type, climate, cultivation conditions, solar exposure, post-harvest storage, as well as genetic characteristics, natural selection, or plant breeding programs (Corrêa Antunes; Duarte Filho, De Souza, 2003; Fortes *et al*., 2011; Kuskoski *et al*., 2006; Malacrida; Motta, 2005; Pereira *et al*., 2012).

Additionally, it should be emphasized that the phenolic compounds present in fruits directly influence the color of the produced liqueurs. Color is a psychological attribute often decisive in selecting comparable food products (Folta; Klee, 2016). Red, in particular, is

associated with ripeness, superior flavor, and other pleasant characteristics of plant-based products, including liqueurs (Schmitzer; Mikulic-Petkovsek; Stampar, 2019).

The color characteristics of the grape liqueurs were significantly affected ($p \leq 0.05$) by the alcoholic source. In the liqueurs produced with A, the *a** parameter was higher, and they also exhibited a lower *L** value, suggesting a dark red color. The *a** parameter defines the balance between green and red tones (Pathare; Opara; Al-Said, 2013), and a reduction in red tones was observed in the liqueur obtained with C. A higher *b** value was also observed in the longer maceration time of the liqueurs, which was also reflected in the h° parameter. Although all h° values were within the orange hue range, the lower value was closer to the red hue. It is, however, of interest to conduct sensory analysis to confirm whether the differences found are perceived and affect the acceptance of the liqueurs by consumers.

The results obtained for the liqueur characteristics, in terms of compliance with the national identity and quality standards, are presented in Table 6.2.

Table 6.2 - Characteristics of BRS Carmem grape liqueurs produced with different alcoholic sources according to identity and quality standards* (mean values ± standard deviation).

Parameters	**Cereal alcohol**	**White Cachaça**
Alcohol content, % v/v at 20 °C	21.59 ± 1.49	20.14 ± 0.28
Sugar content, $g \cdot L^{-1}$	177.3 ± 69.6	140.0 ± 21.8
Methyl alcohol, $mg \cdot 100\ mL^{-1}$ of anydrous alcohol	30.53 ± 1.53	27.49 ± 1.37
Cooper ($mg \cdot kg^{-1}$)	<0,3	<0,3
Lead ($mg \cdot L^{-1}$)	<0,1	<0,1

*Brazil, 2018.

It is observed that the alcohol content and total sugar content of the produced liqueurs comply with current Brazilian legislation (Brazil, 2024), which establishes an alcohol content between 15% and 54% at 20 °C, as well as a sugar content ranging from 100 $g \cdot L^{-1}$ to 350 $g \cdot L^{-1}$, classifying them as sweet or fine liqueurs. These parameters also align with the Code of Federal Regulations (CFR) of the United States, which establishes a minimum of 2.5% sugar by volume for liqueurs and cordials (CFR, 2022), and with the European Parliament and the Council of the European Union, which sets a minimum total sugar content of 100 $g \cdot L^{-1}$ and an alcohol content of at least 15% by volume (EU, 2019). According to Vieira *et al.* (2010), most industrial fruit liqueurs have a labeled alcohol content between 18% and 25% (v/v), with a consumer

market preference for sweet liqueurs. The contaminant levels were also below the limits established by national regulations, which set thresholds of 200 mg·100 mL^{-1} for methyl alcohol, 5 mg·kg^{-1} for copper, and 0.2 mg·L^{-1} for lead.

6.3.2 Identification and quantification of anthocyanins

To evaluate the liqueurs' potential as a source of anthocyanins, a qualitative and quantitative characterization of these compounds was conducted. A total of 13 anthocyanins were identified (Table 6.3), derived from the main anthocyanidins commonly found in grapes: delphinidin (dp), cyanidin (cy), petunidin (pt), peonidin (pn), and malvidin (mv), in both their monoglycosylated (3-glc) and diglycosylated (3,5-glc) forms. Additionally, some anthocyanins were detected in their acylated forms, specifically caffeoylated and *p*-coumaroylated derivatives. Color is one of the main quality characteristics of liqueurs, determined by the quantity, type, and relative proportions of anthocyanins present. Several factors directly influence the final coloration of the liqueur, including the structure and concentration of these molecules, storage temperature, pH, oxygen presence, and other compounds such as co-pigments, ascorbic acid, sugars, and their degradation products. The color stability during storage is associated with the initial concentration and type of anthocyanin, with grapes being widely recognized for their diversity of these substances.

In both liqueurs, a predominance of diglycosylated anthocyanins was observed, accounting for 97.4% to 97.91% of the total. Nishiyama-Hortense *et al*. (2023) also reported the predominance of these anthocyanins in BRS Carmem grapes from different harvests, but with a (3,5-diglc/3-glc) ratio ranging from 2.22 to 5.64, which is significantly lower than the values reported for the liqueurs in the present study. This even higher predominance may be attributed to the greater stability conferred by the presence of two sugar molecules bound to anthocyanidins. This suggests that, during the maturation period, although anthocyanins were extracted, degradation reactions may have also occurred due to oxygen exposure.

Table 6.3 - Anthocyanin profile in BRS Carmem liqueurs obtained from different alcohol sources analyzed by HPLC-DAD-ESI-MS/MS (positive ionization mode). Data expressed as Mean ± standard deviation (*n*=4).

Anthocyanin	**Molecular íon; product (*m/z*)**	**Molar ratio**	
		Cereal alcohol	**Cachaça**
Diglycosylated (3,5-glc)			
dp-3-cmglc-5-glc	773, 611, 303	0.62 ± 0.16	0.50 ± 0.21
cy-3,5-glc	611, 449, 287	0.40 ± 0.02	0.40 ± 0.03
cy-3-cmglc-5-glc	757, 595, 287	0.34 ± 0.09	0.34 ± 0.03
pt-3,5-glc	641, 479, 317	0.60 ± 0.12	0.51 ± 0.14
pn-3,5-glc	625, 463, 301	8.17 ± 0.85	8.52 ±1.08
pn-3-cfglc-5-glc	787, 625, 301	1.25 ± 0.13	1.16 ± 0.03
pn-3-cmglc-5-glc	771, 609, 301	3.60 ± 0.21	3.44 ± 0.15
mv-3,5-glc	655, 493, 331	36.03 ± 2.91	38.36 ± 2.74
mv-3-cfglc-5-glc	817, 655, 331	12.88 ± 0.27	12.79 ±0.21
mv-3-cmglc-5-glc	801, 639, 331	33.81 ± 2.86	31.89 ± 3.02
Monoglycosylated			
pn-3-glc	463, 301	0.71 ± 0.06	0.70 ± 0.05
mv-3-glc	493, 331	1.27 ± 0.15	1.13 ± 0.13
mv-3-cmglc	639, 331	0.32 ± 0.08	0.25 ± 0.09
Ratio (3,5-glc/3-glc)		43.39 ± 5.26	47.34 ± 5.60
Total anthocyanin (mg mv-3-glc·L^{-1})		212.76 ± 25,72	225.95 ± 57.24
Total anthocyanin (mg mv-3.5-diglc·L^{-1})		420.04 ±16,39	456.44 ±45.96

Nishiyama-Hortense *et al.* (2023) also reported the predominance of anthocyanins derived from delphinidin, which does not corroborate the findings of the present study. In both liqueurs, anthocyanins derived from malvidin were the most abundant, with the diglycosylated form being the predominant one (36.03% - 38.36%), followed by the diglycosylated coumarylated form (31.89% – 33.81%). Among the monoglycosylated anthocyanins, which were present in lower amounts, malvidin-3-glc showed the highest percentages (1.13% - 1.27%). After malvidin, peonidin-derived anthocyanins were the most abundant (13.73% – 13.82%). Both peonidin and malvidin are methylated anthocyanins, and the presence of a methoxyl group contributes to the structural stability of these molecules, making them more resistant to degradation processes compared to non-methylated anthocyanins. Neves *et al.* (2022) also observed this stability of peonidin in jabuticaba liqueurs produced with cereal alcohol and cachaça.

6.3.3 Sensory acceptance

A total of 111 liqueur consumers participated in the study, 50 males and 61 females, ranging in age from 18 to 68 years. The results obtained from the data analysis are presented in Table 6.4.

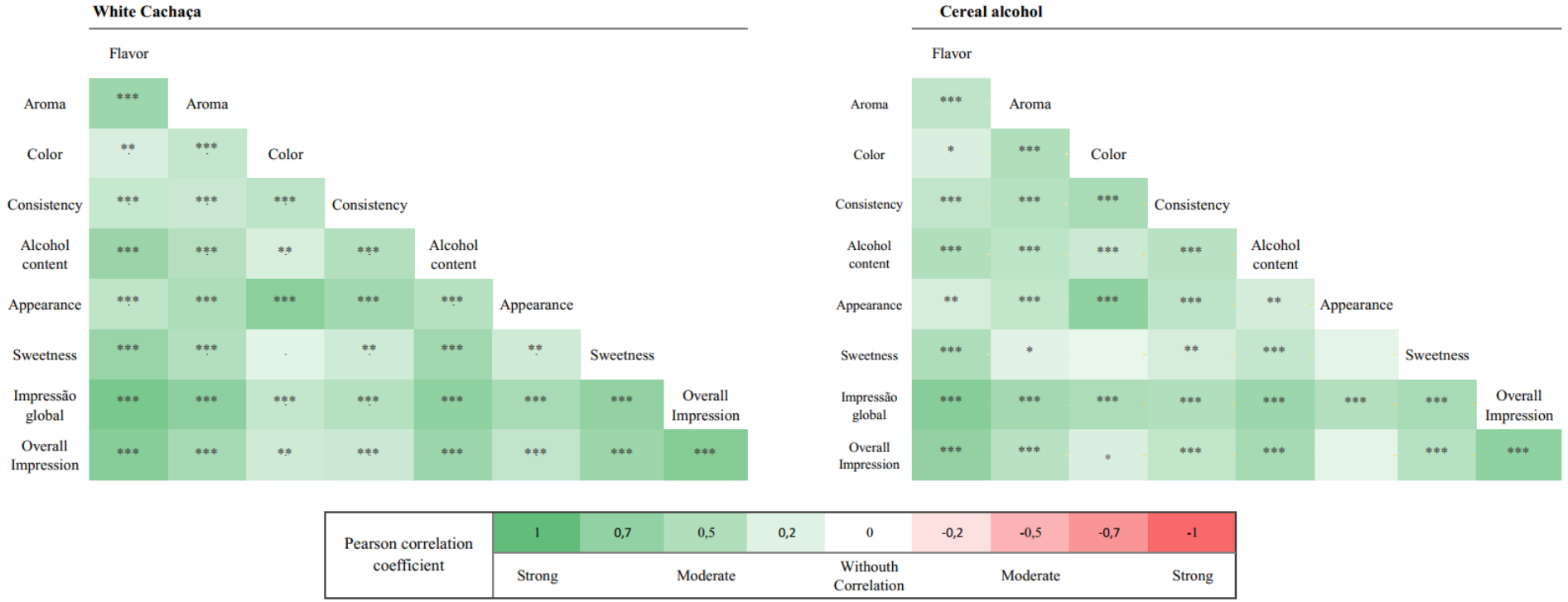
Table 6.4 - Results for each attribute obtained in the acceptance analysis of BRS Carmem grape liqueur with cereal alcohol and white cachaça.

Attribute	Cereal alcohol	White Cachaça
Appearance	7.6 ± 1.4*	7.1 ± 1.6
Color	7.5 ± 1.6*	6.9 ± 1.7
Consistency	7.3 ± 1.6	7.4 ± 1.4
Aroma	6.2 ± 1.8	6.7 ± 1.7*
Flavor	7.1 ± 1.4	7.2 ± 1.6
Sweetness	7.06 ± 1.6	7.09 ± 1.6
Alcohol Content	6.8 ± 1.8	6.9 ± 1.9
Overall impression	7.1 ± 1.3	7.2 ± 1.4

*Indicate a significant difference according to the Student's t-test ($p \leq 0.05$).

The sensory analysis of the liqueurs revealed the evaluation of consumers regarding different attributes and their influence on the final preference. No statistically significant differences ($p > 0.05$) were observed in the attributes: consistency, flavor, sweetness, alcohol content, and overall impression. However, the overall impression indicated that both liqueurs were well accepted by the evaluators, which may suggest that both alcohol sources were well-received. Regarding the aroma, the formulation containing white cachaça showed a higher preference among consumers ($p \leq 0.05$). The purchase intention rate was 63.03% for the formulation with C and 56.75% for the formulation with A, considering a 1 to 5 scale. Pearson's correlation analysis (Figure 6.3) indicated that, for both formulations, the attributes that correlated with each other were appearance and color, while the overall impression showed a significant correlation with flavor, alcohol content, and purchase intention.

Figure 6.3 - Pearson correlation presented as a heatmap for the sensory attributes of BRS Carmem grape liqueurs produced with cereal alcohol and white cachaça.*: $p \leq 0.05$; **: $p < 0.01$; ***: $p < 0.001$



This result may be associated with the presence of characteristic aromatic compounds resulting from the production process of cachaça. For the attributes of appearance and color, the formulation made with cereal alcohol had higher acceptance, possibly due to the transparency of the alcohol, which enhances the visual perception of the final color of the product.

Rodrigues *et al*. (2017) reported that curiosity about the taste of a new product is an important factor that leads consumers to try a new beverage. These authors also concluded that the price of the alcoholic beverage was not a relevant factor for consumers to try these new beverages. The results obtained in this study corroborate those of Neves *et al*. (2022), where cereal alcohol and white cachaça were used to formulate jabuticaba liqueurs, and the sensory analysis showed that the two liqueurs obtained were considered similar by consumers.

6.4 CONCLUSION

The two types of liqueurs produced showed physicochemical properties with significant differences caused by the alcoholic source used (cachaça or cereal alcohol). However, even with the physicochemical differences, the sensory analysis showed no difference in overall acceptance and purchase intention between the two liqueurs, indicating no preference among the consumers. The cereal alcohol-based liqueur stood out in appearance and color, while the cachaça-based liqueur excelled in aroma. Both liqueurs presented an interesting anthocyanin profile, with complex anthocyanins, and the data obtained may contribute to expanding the scientific literature on anthocyanin composition in more innovative alcoholic beverages. It can be concluded that, despite the difference in alcohol source, both grape liqueurs represent an excellent alternative for utilizing excess production and providing additional income for producers.

7 CONSIDERAÇÕES FINAIS

Foi possível realizar a caracterização morfológica e química das uvas BRS Carmem, bem como determinar e caracterizar as antocianinas na uva, no produto desidratado à base de suco de uva e nos licores. A uva sob o porta-enxerto 572 apresentou maiores razões molares de antocianinas e seus derivados e concentração total de antocianinas. As antocianinas cumariladas apareceram com maior frequência nos dois porta-enxertos estudados. O produto desidratado à base de suco de uva brasileira BRS Carmem, apresentou antocianinas estáveis com alto potencial para uso como corantes alimentícios.

As antocianinas predominantes nos produtos derivados são compostos conhecidos por sua estabilidade superior em comparação à maioria das antocianinas encontradas em uvas. Os licores estão dentro dos padrões da legislação e contêm elevadas quantidades de antocianinas derivadas da malvidina, sendo glicosilada (3-glc), diglicosilada (3,5-glc) e diglicosiladas cumariladas (3-cmglc-5-glc). Sendo assim uma ótima alternativa de tecnologia social, fomentando a produção local.

Pelo estudo das propriedades físico-químicas, observou-se que o perfil e níveis significativos de antocianinas, encontrados na uva e em seus produtos, torna esta cultivar adequada como matéria-prima para desenvolvimento de produtos ou bioingredientes. O produto em pó obtido pode servir como uma alternativa viável e eficaz para aplicações em produtos alimentícios, promovendo o uso de uvas brasileiras como fonte natural de corantes alimentícios duráveis. O licor de uva mostra-se uma alternativa potencial à utilização do excedente de produção de uvas e os dados obtidos, podendo auxiliar no estudo de compostos fenólicos em bebidas alcoólicas não tradicionais.

Entende-se que fatores e processos podem levar a alterações na bioatividade das antocianinas, sendo necessário novos estudos que abrangem outras tecnologias de produção, visando fomentar novas oportunidades de processamento dessa cultivar e dos produtos desenvolvidos neste trabalho.

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