UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de São José do Rio Preto

Poliana Moser

Secagem por atomização do suco de uva: microencapsulação das antocianinas

São José do Rio Preto
2016

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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", Campus de São José do Rio Preto.

Orientador: Profª. Drª. Vânia Regina Nicoletti Telis
Coorientador: Prof. Dr. Isidro Hermosín-Gutiérrez

São José do Rio Preto
2016

Moser, Poliana.
Secagem por atomização do suco de uva : microencapsulação das antocianinas / Poliana Moser. -- São José do Rio Preto, 2016
149 f. : il., tabs.

Orientador: Vânia Regina Nicoletti Telis
Coorientador: Isidro Hermosín-Gutiérrez
Tese (doutorado) – Universidade Estadual Paulista “Júlio de Mesquita Filho”, Instituto de Biociências, Letras e Ciências Exatas

1. Tecnologia de alimentos. 2. Suco de uva - Indústria. 3. Secagem em spray. 4. Atomização. 5. Fenóis. 6. Microencapsulação. 7. Antocianina. I. Telis, Vânia Regina Nicoletti. II. Hermosín-Gutiérrez, Isidro. III. Universidade Estadual Paulista "Júlio de Mesquita Filho". Instituto de Biociências, Letras e Ciências Exatas. IV. Título.

CDU – 634.8.077

Ficha catalográfica elaborada pela Biblioteca do IBILCE
UNESP - Câmpus de São José do Rio Preto

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25 de fevereiro de 2016

Aos meus pais, Gervásio e Irene e a minha irmã Pamela, dedico.

AGRADECIMENTOS

Em especial, à professora Dra. Vânia Regina Nicoletti Telis, pela orientação no desenvolvimento deste trabalho. Pela dedicação que demonstrou em todos os momentos, pelos ensinamentos, conselhos, confiança e pela amizade. A você, Vânia, toda a minha gratidão e carinho.

Ao professor Dr. Isidro Hermosín-Gutiérrez da Universidad de Castilla-La Mancha – UCLM pela coorientação. Meus sinceros agradecimentos pela oportunidade de realizar parte do doutorado na UCLM, pelos ensinamentos, pela ajuda com discussão dos resultados e pela amizade.

À FAPESP, pela bolsa de estudos (Processo nº 2012/09074-4) e a CAPES, pela bolsa de doutorado-sanduíche (Processo PDSE 99999.013844/2013-000).

À Profª. Drª. Izabela Dutra Alvim e à Profª. Drª. Ellen Silva Lago Vanzela pelas valiosas sugestões no exame de qualificação.

Aos membros da banca examinadora, meus sinceros agradecimentos pelas sugestões e correções.

Aos professores do DETA/UNESP pelos ensinamentos. Em especial ao professor Dr. Javier Telis Romero pela oportunidade dos trabalhos "extra-tese".

Ao Sérgio Gómez-Alonso da Universidad de Castilla-La Mancha pela paciência e dedicação ao me ensinar as análises dos compostos fenólicos e pela amizade.

Aos pesquisadores da EMBRAPA, Dr. Reginaldo Teodoro de Souza e Dr. Giuliano Elias Pereira, pelas sugestões no início do trabalho e pela ajuda na elaboração do suco de uva.

À professora Dra. Natália Soares Janzantti da Universidade Estadual Paulista "Júlio de Mesquita Filho" pela ajuda com análise das antocianinas.

À Dra. Izabela Dutra Alvim do Instituto de Tecnologia de Alimentos pela ajuda com a análise do tamanho de partículas.

À Esteban García-Romero do Instituto de la Vid y el Vino de Castilla-La Mancha pela análise dos flavan-3-óis.

À aluna de iniciação científica Thais Cristina Benatti Gallo, pela constante ajuda durante o desenvolvimento do trabalho, pela dedicação e amizade.

À Nathália de Andrade Neves, pela ajuda no laboratório e com as análises estatísticas. Agradeço principalmente por ter tornado os meus dias na Espanha mais alegres e bonitos.

Aos meus queridos amigos Juliane Viganó, Tiago Carregari Polachini, Mírian Luisa Faria Freitas e Larissa A. C. Zuanon Sardela por estarem sempre presentes e pela constante ajuda na elaboração deste trabalho.

Aos meus amigos do Laboratório de Medidas Físicas da UNESP pelas discussões acadêmicas, pela ajuda e apoio. Agradeço também aos meus amigos da pós-graduação pelos bons momentos durante os seis anos de convivência.

À minha família, por todo o apoio, incentivo e por nunca medirem esforços para me proporcionar o melhor. Não tenho palavras para expressar a gratidão e o amor que sinto por vocês.

À todos aqueles que contribuíram, direta ou indiretamente, para a realização deste trabalho.

MUITO OBRIGADA!

RESUMO

O suco de uva possui um alto teor de antocianinas, com elevada atividade antioxidante e grande potencial de uso como corante natural. Porém, a secagem dos sucos de frutas em *spray dryer* apresenta complicações devido à pegajosidade e elevada higroscopicidade dos pós, o que ocasiona problemas durante o processamento e armazenamento do produto. Uma alternativa para facilitar a secagem é a adição de carreadores, que também desempenham o papel de encapsulantes. O objetivo desse trabalho foi a obtenção do suco de uva em pó pelo processo de atomização, utilizando misturas de maltodextrina e proteínas de soja ou de soro de leite como agentes carreadores, visando proteger as antocianinas e obter um produto com boa estabilidade em relação às alterações físicas e químicas. Inicialmente, avaliou-se a influência das condições do processo sobre o rendimento, umidade, solubilidade e cor do suco em pó. As condições selecionadas para realizar a secagem do suco foram: temperatura de entrada do ar de 140 ºC, fluxo de ar de 500 L/h, vazão de alimentação de 2 mL/min e baixa concentração de carreador. Posteriormente, foram avaliados diferentes carreadores - misturas de proteína isolada de soja e maltodextrina (SM) e misturas de proteína concentrada de soro de leite e maltodextrina (WM) - no rendimento de processo, solubilidade, retenção de antocianinas, eficiência de encapsulação (EE), cor e morfologia das microesferas. As concentrações de agente carreador (CAC, g de carreador/g sólidos solúveis do suco) utilizadas foram na faixa de 0,65 a 1,35 para SM e de 0,15 a 0,85 para WM, enquanto as proporções de proteína foram de 5,86 a 34,14 g de proteína/100 g de agente carreador. Os sucos em pó apresentaram alta solubilidade e baixa umidade. Os pós produzidos com as misturas WM resultaram em alto rendimento e alta retenção de antocianinas, enquanto as misturas de SM apresentaram alta EE. Os tratamentos que apresentaram os melhores resultados globais foram: CAC = 1,25, 10% de proteína de soja; CAC = 1,00, 5.86% de proteína de soja; CAC = 0,75, 30% de proteína de soro; e CAC = 0,85, 20% de proteína de soro, sendo codificados como 1SM, 2SM, 1WM e 2WM, respectivamente. Os sucos atomizados com os carreadores selecionados foram avaliados quanto à distribuição de tamanho das partículas, pegajosidade, isotermas de sorção de água, temperatura de transição vítrea (Tg) e estabilidade das antocianinas expostas à luz. As isotermas de sorção foram classificadas como tipo II e o modelo de GAB foi o que melhor se ajustou aos dados experimentais. A temperatura de transição vítrea diminuiu com o aumento da atividade de água, confirmando o efeito plasticizante da água. No estudo da estabilidade frente à luz, verificou-se que o tratamento 1SM

foi o que apresentou a melhor proteção das antocianinas. Finalmente, a estabilidade dos compostos fenólicos e a atividade antioxidante do suco de uva em pó armazenados nas temperaturas de 5 ºC, 25 ºC e 35 ºC durante 150 dias foi avaliada. As microesferas formuladas com 1SM, que possuía maior CAC, foram mais efetivas na proteção das antocianinas, enquanto o tratamento 1WM, que possuía menor CAC, não apresentou boa proteção. Com exceção do 1SM, os tratamentos aumentaram a sua proporção de antocianinas *p*-cumariladas com o tempo de armazenamento. Os flavonóis, os ácidos hidroxicinâmicos e os flavan-3-óis foram analisados no tempo inicial (0 dias) e no tempo final do armazenamento (150 dias). Após 150 dias, o tratamento 1SM apresentou perdas significativas de flavonóis. Todos os tratamentos tiveram perdas de ácidos hidroxicinâmicos, independente da temperatura de armazenamento, e perdas de flavan-3-óis a 35 ºC. Apesar das perdas de compostos fenólicos, o tempo e a temperatura não influenciaram a atividade antioxidante dos pós e a cor do suco de uva reconstituído, após 150 dias de armazenamento.

Palavras-chave: Atomização. Proteínas. Maltodextrina. Compostos fenólicos. Estabilidade.

ABSTRACT

Grape juice has a high content of anthocyanins, with high antioxidant capacity and great potential to be used as a natural dye. Nevertheless, drying of fruit juices by atomization presents complications due to stickiness and high hygroscopicity of powders, which cause problems during processing and storage of the product. An alternative to facilitate drying is the addition of carriers that also play the role of encapsulants. The aim of this work was to produce grape juice powders by spray drying, using blends of maltodextrin and soy or whey protein as carrier agents, in order to protect the anthocyanins and to obtain a stable product with respect to physical and chemical changes. In a first step, the influence of process conditions on yield, moisture, solubility and color of powdered juice was evaluated. The conditions selected to perform drying of juice were: inlet-air temperature of 140 °C, air flow of 500 L/h, feed flow rate of 2 mL/min and low concentration of carrier. Subsequently, the use ofdifferent carriers - blends of soy protein isolate with maltodextrin (SM) and whey protein concentrated of milk with maltodextrin (WM) – was assessed on yield, solubility, anthocyanin retention, encapsulation efficiency (EE), color and morphology of the microspheres. The carrier agent concentration (CAC, g of carrier/g of soluble solids of the juice) used was in the range of 0.65 – 1.35 to SM, and of 0.15 – 0.85 to WM, whereas the protein ratio (R, g protein/100 g carrier agent) varied between 5.86 - 34.14. The grape juice powders presented high solubility and low water content. WM blends resulted in higher yields and higher anthocyanin retention than SM blends, whereas SM blends leaded to higher encapsulation efficiency. Treatments that presented the best overall results were: CAC = 1.25, 10 % soy protein; CAC = 1.00, 5.86 % soy protein; CAC = 0.75, 30 % whey protein; and CAC = 0.85, 20 % whey protein, which were coded as 1SM, 2SM, 1WM e 2WM, respectively. The powdered juices with the selected carrier formulations were evaluated for particle size distribution, water sorption isotherms, glass transition temperature (Tg), stickiness and light stability of anthocyanins. The sorption isotherms were classified as type II and the GAB model was well fitted to experimental data. The glass transition temperature decreased with the increase in water content, confirming the plasticizing effect of water. Regarding light stability, 1SM treatment showed the best anthocyanin protection. Finally, the stability of phenolic compounds and antioxidant activity of powdered grape juice - stored at 5, 25 and 35 ºC up to 150 days - were evaluated. The microspheres formulated with 1SM, which had the largest CAC, were more effective in the preservation of anthocyanins, whereas 1WM, which had the lowest CAC, did not

provide good protection. With the exception of 1SM, the treatments increased the proportion of p-coumaroylated anthocyanins over storage time. Flavonols, hydroxycinnamic acid derivatives (HCAD) and flavan-3-ol were analyzed at initial (0 day) and final storage time (150 days). After 150 days, 1SM powder decreased flavonols. All treatments decreased HCAD, independently of the storage temperature, and lost flavan-3-ol at 35 ºC. Despite of all the aforementioned losses in the studied phenolic compounds, time and temperature did not influence on the antioxidant activity of the powder and reconstituted grape juice color, after 150 days of storage.

Keywords: Spray drying. Proteins. Maltodextrin. Phenolic compounds. Stability.

LISTA DE FIGURAS

CAPÍTULO 1

CAPÍTULO 2

CAPÍTULO 3

CAPÍTULO 4

CAPÍTULO 5

APÊNDICE

LISTA DE TABELAS

CAPÍTULO 2

CAPÍTULO 3

CAPÍTULO 4

CAPÍTULO 5

SUMÁRIO

INTRODUÇÃO GERAL

A preocupação com a qualidade de vida tem provocado mudanças nos hábitos alimentares das pessoas, levando-as à procura de alimentos que lhes garantam melhor saúde e bem estar. O consumo de uvas e seus derivados, como os sucos e vinhos tintos, trazem diversos benefícios para a saúde.

A uva BRS Violeta é uma cultivar hibrida, obtida a partir do cruzamento de "BRS Rúbea x IAC 1398-21", desenvolvida pela Embrapa Uva e Vinho. Essa cultivar contém altos níveis de antocianinas e outros compostos fenólicos, sendo uma alternativa para produzir sucos com coloração intensa e, além disso, ricos em antioxidante (CAMARGO; MAIA; NACHTIGAL, 2005; LAGO-VANZELA et al., 2014). Estudo composicional mostrou que a uva BRS Violeta possui grande quantidade de antocianinas, 3930 mg/kg (REBELLO et al., 2013).

O elevado teor de antocianinas tem aumentado o destaque dado à uva, por ser considerada uma importante fonte de pigmentos naturais, podendo reduzir o uso de corantes sintéticos na indústria alimentícia. Além disso, as antocianinas possuem um importante papel na prevenção de doenças crônicas, como câncer, doenças cardiovasculares e neurodegenerativas (FANG; BHANDARI, 2010; DEL RIO et al., 2013). Por outro lado, o uso das antocianinas como aditivo ainda é bastante restrito, devido à sua baixa estabilidade ao oxigênio, temperatura, luz, pH, ácidos orgânicos, entre outros (SAGAR; SURESH KUMAR, 2010).

O Brasil é um grande produtor e exportador de frutas *in natura*, porém, uma grande quantidade dessa produção é perdida durante o seu manuseio e transporte. Uma alternativa para minimizar as perdas é agregar valor ao produto, através do processo de secagem. Além disso, a elaboração do suco de frutas em pó melhora a conservação do produto, facilita o seu transporte e armazenamento, e resulta num produto de grande conveniência tanto para a indústria quanto para o consumidor final.

A secagem por atomização é muito utilizada na elaboração de sucos de frutas em pó. Ele é um processo econômico e flexível, realizado em um equipamento de fácil manipulação. O processo consiste na atomização de um líquido em uma corrente de ar quente para obter um pó, caracterizando-se por um curto tempo de secagem. Dessa forma, essa técnica permite a secagem de produtos sensíveis ao calor, sem afetar demasiadamente sua qualidade (RÉ, 1998). Porém, durante a secagem de sucos de frutas, o processo de secagem é dificultado devido ao comportamento pegajoso que as frutas apresentam quando submetidas a variações de umidade e

temperatura. A pegajosidade e a elevada higroscopicidade ocasionam problemas de manipulação, pois causam aglomeração e aderência do material nas paredes do secador, reduzindo o rendimento do processo e causando danos à qualidade do produto durante seu armazenamento.

É de grande importância para a indústria melhorar o desempenho do processo de secagem por atomização, a fim de obter produtos com melhores características sensoriais e nutricionais, bem como aumentar o rendimento. Uma alternativa para evitar os problemas de manipulação e processamento é adicionar agentes carreadores, que melhoram a estabilidade do material durante a secagem e armazenamento e também desempenham a função de encapsulantes.

A microencapsulação é uma técnica que consiste na incorporação da substância de interesse (núcleo) em um sistema de revestimento (material de parede ou agente carreador). O material de parede age como um filme protetor, isolando a substância a fim de estabilizar, proteger e permitir o controle de sua liberação (CAVALCANTI; SANTOS; MEIRELES, 2011; MADENE et al., 2006; RÉ, 1998). O encapsulante ideal deve apresentar baixa viscosidade, ser de fácil manipulação, possuir baixa higroscopicidade, não ser reativo com o material a ser encapsulado, ter habilidade de selar e manter a substância de interesse dentro da estrutura da cápsula, ser solúvel, apresentar boa disponibilidade e baixo custo (GHARSALLAOUI et al., 2007).

Neste contexto, estudos de secagem por atomização utilizando maltodextrina (CANO-CHAUCA et al., 2005; GABAS et al., 2007; MARTINELLI; GABAS; TELIS-ROMERO, 2007; TONON; BRABET; HUBINGER, 2009; ROBERT et al., 2010; TELIS; MARTÍNEZ-NAVARRETE, 2010), proteína de soja (ROBERT et al., 2010; RASCÓN et al., 2011) e proteína de soro de leite (ADHIKARI et al., 2009; BASTOS et al., 2012; FANG; BHANDARI, 2012) foram realizados. Porém a literatura não apresenta estudos sobre o uso de misturas de maltodextrina e proteínas de soro ou soja na secagem e microencapsulação das antocianinas do suco de uva.

Considerando-se que a uva apresenta uma grande quantidade de antocianinas e, consequentemente, uma elevada atividade antioxidante quando comparada a outras frutas, e levando-se em conta o fato de que as antocianinas são instáveis, a microencapsulação do suco de uva pode representar uma técnica promissora no sentido de aumentar a estabilidade desses compostos.

Dessa forma, estudar a influência de agentes carreadores na secagem do suco de uva por atomização torna-se importante para facilitar este processo; além disso, o uso de carreadores apresenta o potencial de proteger as antocianinas, garantindo um produto estável, com alto poder antioxidante e que poderá ser utilizado como corante natural.

OBJETIVO GERAL

Com base nas considerações apresentadas na Introdução Geral, este trabalho teve como objetivo geral a obtenção de suco de uva em pó por meio de secagem por atomização utilizando misturas de maltodextrina e proteína isolada de soja (SM) e misturas de maltodextrina com proteína concentrada de soro de leite (WM) como agentes carreadores, visando proteger as antocianinas e obter um produto com boa estabilidade em relação as alterações físicas e químicas. Para atingir esse objetivo geral, os trabalhos foram conduzidos de forma a atingir uma série de objetivos específicos, descritos a seguir.

Objetivos específicos

- Estudar a influência da concentração de maltodextrina, temperatura do ar de secagem, vazão de alimentação e fluxo de ar durante o processo de secagem do suco de uva por atomização, avaliando o teor de umidade, o rendimento do processo, a cor e a solubilidade dos pós;

- Estudar a influência da concentração dos agentes carreadores e da proporção de proteína em relação à quantidade total de carreador no rendimento e nas propriedades físico-químicas (umidade, solubilidade, retenção e eficiência de encapsulação das antocianinas, cor, tamanho e morfologia das microesferas) do suco de uva em pó obtido por processo de atomização;

- Determinar as isotermas de sorção, pegajosidade e temperaturas de transição vítrea (T_g), buscando correlacioná-las com a eficiência de encapsulação e estabilidade das antocianinas.

- Avaliar a estabilidade das antocianinas do suco de uva produzido com os diferentes agentes carreadores ao longo do armazenamento com exposição à luz;

- Avaliar a estabilidade das antocianinas, flavonóis, ácidos hidroxicinâmicos e flavan-3-óis, assim como a atividade antioxidante e coloração do suco de uva produzido com os diferentes agentes carreadores, ao longo do armazenamento em diferentes temperaturas.

ORGANIZAÇÃO DO TRABALHO EM CAPÍTULOS

O presente trabalho encontra-se estruturado da seguinte forma:

Introdução e objetivos

Capítulo 1: Revisão bibliográfica: são apresentados os principais fundamentos teóricos que abrangem este estudo, incluindo informações referentes ao suco de uva e os benefícios de seus compostos fenólicos, considerações sobre a secagem em *spray dryer* e as dificuldades encontradas ao secar sucos de frutas, a microencapsulação, e a importância do uso dos agentes carreadores, além da estabilidade dos alimentos em pó.

Capítulo 2: Testes preliminares: neste capítulo avaliaram-se os parâmetros de secagem do suco de uva em *spray dryer*. Também foram realizados testes para determinar as concentrações mínimas de agentes carreadores (misturas de proteínas e maltodextrina) e a proporção de proteína na mistura, necessários para a secagem do suco de uva.

Capítulo 3: Spray drying of grape juice from hybrid cv. BRS Violeta: microencapsulation of anthocyanins using protein/maltodextrin blends as drying aids: neste capítulo avaliou-se a influência da concentração de agentes carreadores (misturas de maltodextrina com proteína de soja ou proteína de soro de leite) e da proporção de proteína nos sistemas na secagem do suco de uva e na microencapsulação das antocianinas. Nos sucos em pó foi avaliado o rendimento, umidade, solubilidade, retenção de antocianinas e cor. Já as micropartículas foram avaliadas em relação à eficiência de encapsulação, tamanho e morfologia.

Capítulo 4: Water sorption, glass transition temperature and stability of anthocyanins in grape juice microencapsulated in blends of protein and maltodextrin: neste capítulo as quatro melhores formulações do suco de uva obtidas no capítulo 3 foram avaliadas com relação as isotermas de sorção, temperatura de transição vítrea e pegajosidade. Além disso, foi avaliada a estabilidade do suco de uva com antocianinas encapsuladas na presença de luz.

Capítulo 5: Storage stability of phenolic compounds in powdered BRS Violeta grape juice microencapsulated with protein and maltodextrin blends: neste capítulo também foram avaliadas as quatro melhores formulações do suco de uva, obtidas no capítulo 3, quanto à estabilidade dos compostos fenólicos (antocianinas, flavonóis, ácidos hidroxicinâmicos e flavan-3-óis), atividade antioxidante e parâmetros de cor do suco de uva microencapsulado e armazenado nas temperaturas de 5, 25 e 35 ºC durante 150 dias.

Capítulo 6: neste capítulo são apresentadas as conclusões gerais deste trabalho e algumas sugestões para trabalhos futuros.

-CAPÍTULO 1-

REVISÃO BIBLIOGRÁFICA

1 REVISÃO BIBLIOGRÁFICA

1.1 SUCO DE UVA

De acordo com a legislação brasileira, o suco de uva é uma bebida não fermentada, não alcoólica, obtida da parte comestível da uva sã, fresca e madura que resulta em produto com coloração, aroma e sabor característicos da fruta de origem. O suco de uva pode ser classificado como integral, desidratado ou reconstituído. O suco integral é comercializado na sua concentração natural, sem adição de açúcar. O suco desidratado, produto em estado sólido, é obtido pela desidratação do suco integral. Por fim, o suco reconstituído é o produto obtido pela diluição do suco concentrado ou desidratado, até a concentração original do suco integral ou até o teor de sólidos solúveis totais mínimo estabelecido nos padrões de identidade e qualidade (BRASIL, 2009).

A instrução normativa nº 1/2000 (BRASIL, 2000) fixa padrões de identidade e qualidade para o suco de uva. O suco deve apresentar coloração vinho, rosada ou translúcida, teor mínimo de sólidos solúveis de 14 ºBrix, acidez total mínima, em ácido tartárico, de 0,41 g/100g e açúcares totais naturais da uva de, no máximo, 20 g/100g.

A vitivinicultura brasileira surgiu e se desenvolveu com base em uvas americanas, as chamadas uvas comuns, variedades das espécies *Vitis labrusca* e *Vitis bourquina*, usadas para a elaboração de vinhos de mesa. Entretanto, a partir de meados do século XX vinhos finos começaram a ser elaborados com uvas de variedades de *Vitis vinifera* (GUERRA et al., 2009). As cultivares brasileiras de uva vêm sendo criadas para atender às demandas do setor vitivinícola, tanto para o abastecimento do mercado interno como para a exportação da uva e de seus derivados. Sucos de *Vitis labrusca* são apreciados especialmente na Ásia e América, enquanto que na Europa, onde este sabor não é apreciado, o suco de uva é elaborado com *Vitis vinifera*.

No Brasil, a vitivinicultura vem passando por mudanças econômicas e de mercado, com produção de 1,47 milhões de toneladas de uvas no ano de 2015 (IBGE, 2015). O Rio Grande do Sul é o estado brasileiro com maior produção de uvas e derivados. No período de janeiro a novembro de 2010, a produção de suco de uva no estado foi de 57,48 milhões de litros, enquanto que, no mesmo período de 2015, a produção foi de 136,09 milhões de litros, o que representa um aumento de 136 % (UVIBRA, 2015).

Com o objetivo de aumentar a qualidade e a competitividade do vinho e do suco de uva no Brasil, a Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA Uva e Vinho) desenvolveu a uva BRS Violeta. Ela é uma cultivar hibrida, obtida a partir do cruzamento de "BRS Rúbea x IAC 1398-21", que foi lançada no ano de 2006. O vinho e o suco elaborados a partir da uva BRS Violeta apresentam intensa coloração violácea, sabor que remete framboesa e acidez relativamente baixa (CAMARGO; MAIA; NACHTIGAL, 2005).

A BRS Violeta é uma importante fonte de compostos fenólicos, com elevado teor de antocianinas, sendo considerada uma alternativa para produzir suco de uva com coloração intensa e rico em antioxidantes (CAMARGO; MAIA; NACHTIGAL, 2005; LAGO-VANZELA et al., 2014). Em um estudo composicional a uva BRS Violeta apresentou 3930 mg/kg de antocianinas, 670 mg/kg de proantocianidinas, 150 mg/kg de flavonóis e 120 mg/kg de derivados de ácidos hidroxicinâmicos (REBELLO et al., 2013)

1.1.1 Compostos fenólicos

Os compostos fenólicos são definidos como substâncias que possuem um anel aromático com um ou mais substituintes hidroxílicos, incluindo seus grupos funcionais (MALACRIDA; MOTTA, 2005). A uva, juntamente com seus derivados, constitui uma importante fonte de compostos fenólicos, que podem ser classificados entre flavonoides e não flavonoides. Do primeiro grupo fazem parte os flavan-3-óis, flavonóis e antocianinas, e ao segundo grupo pertencem os ácidos fenólicos e os estilbenos (ABE et al., 2007).

A estrutura química dos flavonoides é baseada no esqueleto de quinze carbonos, ou seja, dois anéis de benzeno (anéis A e B) ligados por um anel pirano central (anel C), cuja representação da fórmula é C_6-C_3-C_6 (Figura 1.1) (HERRMANN, 1976). As variações em substituição do anel C resultam em diferentes classes de flavonoides, enquanto as substituições dos anéis A e B originam diferentes compostos dentro de cada classe de flavonoides. Estas substituições podem incluir oxigenação, alquilação, glicosilação, acilação e sulfação (ANGELO e JORGE 2007).

A estrutura dos flavonoides favorece a sua ação antioxidante. Os hidrogênios dos grupos hidroxilas adjacentes (orto-difenóis), localizados em várias posições dos anéis A, B e C, as duplas ligações dos anéis benzênicos e a dupla ligação da função oxo (-C=O) de algumas

moléculas de flavonoides, fornecem a esses compostos uma alta atividade antioxidante (MAMEDE; PASTORE, 2004).

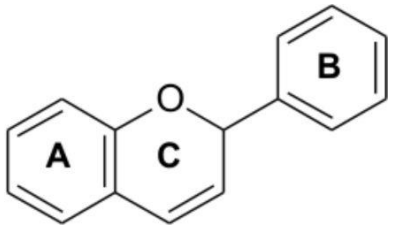
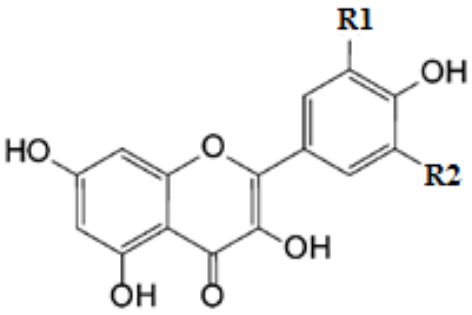


Figura 1.1 Estrutura química dos flavonoides.
Fonte: ANGELO; JORGE (2007).

Os flavonóis são encontrados na forma de glicosídeos e estão localizados na casca das uvas. Os flavonóis mais comuns nas uvas incluem kaempferol, quercetina, isoramnetina, miricetina, laricitrina e siringetina (Figura 1.2) (CASTILLO-MUÑOZ et al. 2007).



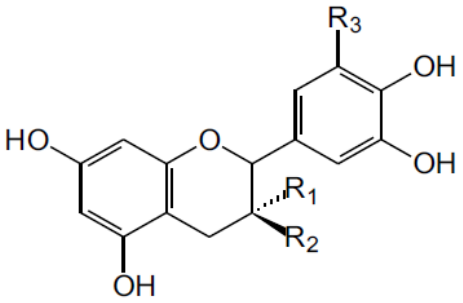
Flavonóis	**R1**	**R2**
Kaempferol	H	H
Quercetina	OH	H
Miricetina	OH	OH
Isoramnetina	OCH_3	H
Laricitrina	OCH_3	OH
Siringetina	OCH_3	OCH_3

Figura 1.2 Estrutura química dos flavonóis
Fonte: CASTILLO-MUÑOZ et al. (2007).

Os flavan-3-óis são compostos encontrados em maior quantidade nas sementes e nos engaços das uvas e são responsáveis, em grande parte, pelo sabor e adstringência dos sucos e vinhos. Eles apresentam como unidades fundamentais as estruturas monoméricas de 2-fenilbenzopiranos (C_6-C_3-C_6). A catequina e seu isômero epicatequina são os principais flavan-3-óis monoméricos presentes nas uvas (Figura 1.3) (HASLAM, 1980).

As procianidinas dímeras podem ser subdivididas em dois tipos (A e B). As procianidinas do tipo B ($C_{30}H_{26}O_{12}$) são dímeros resultantes da condensação de duas unidades de flavan-3-óis unidas por ligações C_4-C_8 (B1 a B4) ou C_4-C_6 (B5 a B8). As procianidinas do tipo A ($C_{30}H_{24}O_{12}$)

são dímeros que possuem uniões interflavanos C_4–C_8 ou C_4–C_6 e uma ligação éter entre os carbonos C_5 ou C_7 da unidade terminal com o carbono C_2 da unidade superior (RIBÉREAU-GAYON et al., 2006).

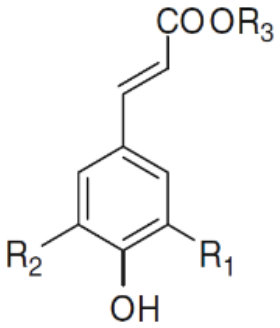


Flavan-3-óis	R1	R2	R3
Catequina	OH	H	H
Epicatequina	H	OH	H

Figura 1.3 Estrutura química dos flavan-3-óis.
Fonte: RIBÉREAU-GAYON et al., 2006.

Os compostos não flavonoides encontram-se em baixas concentrações nas uvas, com exceção dos ácidos fenólicos, que representam a terceira classe de compostos fenólicos mais abundantes nessa fruta. Os ácidos fenólicos são constituídos por um anel benzênico na molécula, são incolores em soluções hidroalcoólicas, mas podem tornar-se amarelos após sofrer oxidação (RIBÉREAU-GAYON et al., 2006).

Os ácidos fenólicos principais presentes nas uvas são os ácidos hidroxibenzóicos (C_6–C_1) e os ácidos hidroxicinâmicos (C_6–C_3). Os ácidos hidroxicinâmico são antioxidantes mais eficientes do que os ácidos hidroxibenzóico e são predominantemente encontrados na forma de ésteres tartáricos (BEER et al., 2002). A Figura 1.4 mostra a estrutura química dos ácidos hidroxicinâmicos.



Ácidos hidroxicinâmicos	R1	R2	R3
Caféico	OH	H	H
Caftárico	OH	H	Ácido tartárico
p-Coumárico	H	H	H
Ferúlico	OCH_3	H	H

Figura 1.4 Estrutura química dos ácidos hidroxicinâmicos.
Fonte: RIBÉREAU-GAYON et al., 2006.

Estudos realizados com compostos fenólicos demonstram sua capacidade antioxidante, assim como o seu possível efeito na prevenção de doenças crônicas, como câncer, problemas cardiovasculares e neurodegenerativos (DEL RIO et al., 2013). De maneira geral, a ação benéfica dos compostos fenólicos na saúde humana vem sendo relacionada com a sua atividade anti-inflamatória, antibacteriana, antiviral e com a atividade que impede, não só a aglomeração das plaquetas sanguíneas, mas também a ação de radicais livres no organismo (FANG; BHANDARI, 2010).

Os conteúdos de fenólicos nas uvas variam de acordo com a espécie, maturidade e condições climáticas. Durante a produção do suco, o tipo de extração, tempo de contato entre o suco e as partes sólidas da uva, prensagem, tratamentos térmicos, tratamentos enzimáticos e adição de dióxido de enxofre e ácido tartárico podem interferir na quantidade de fenólicos do suco pronto (MALACRIDA; MOTTA, 2005).

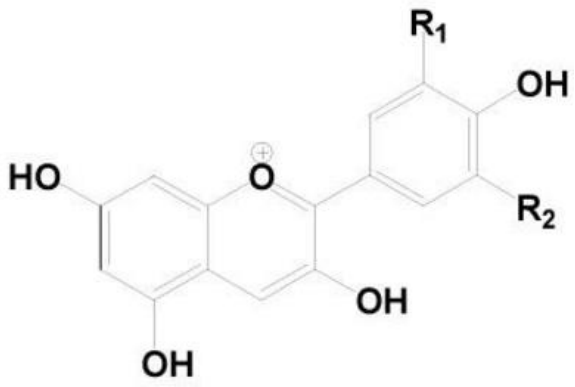
1.1.1.1. Antocianinas

Pertencentes à classe dos flavonoides, as antocianinas representam o mais importante grupo de pigmentos naturais e são responsáveis pelas cores e tons de azul, vermelho e amarelo de flores, frutos e folhas (BOBBIO; BOBBIO, 1995). As antocianinas fazem parte dos compostos fenólicos caracterizados pelo núcleo básico flavílio (cátion 2-fenilbenzopirílio) que consiste de dois anéis aromáticos unidos por uma unidade de três carbonos e condensada por um oxigênio. A molécula de antocianina é constituída por duas ou três porções: uma aglicona (denominada antocianidina), um grupo de açúcares e, frequentemente um grupo de ácidos orgânicos (BROUILLARD, 1983).

Na literatura já foram mencionadas 23 antocianidinas que diferem entre si pelo número e posição dos grupos hidroxilas e/ou metoxilas (Figura 1.5). Entretanto, apenas seis antocianidinas são frequentemente encontradas nos alimentos, sendo elas cianidina, delfinidina, pelargonidina, malvidina, peonidina e petunidina (CASTAÑEDA-OVANDO et al., 2009; TSAO, 2010).

Uma característica marcante das antocianinas está no fato de que em soluções aquosas apresentam diferentes estruturas em função do pH. De modo geral, em meio extremamente ácido (pH entre 1 e 2) as antocianinas apresentam coloração intensamente avermelhada devido ao predomínio da forma cátion flavílico (AH^+). Para um meio com pH maior que 2 é observado um equilíbrio entre o cátion flavílico e uma estrutura conhecida como pseudobasecarbinol (B). Com

o aumento do pH, as antocianinas perdem a cor até se tornarem praticamente incolores em pH aproximadamente 6, devido à predominância da espécie pseudobasecarbinol (MARÇO; POPPI; SCARMINIO, 2008).



Antocianidina	R1	R2
Cianidina	OH	H
Delfinidina	OH	OH
Pelargonidina	H	H
Malvidina	OCH_3	OCH_3
Peonidina	OCH_3	H
Petunidina	OH	OCH_3

Figura 1.5 Antocianinas majoritárias presentes nas uvas.
Adaptado de Tsao (2010).

Existe um grande interesse no estudo das antocianinas em diversas áreas, como na indústria de alimentos e de fármacos, devido a seus efeitos benéficos a saúde e por ser uma alternativa para os corantes sintéticos (MARÇO; POPPI; SCARMINIO, 2008; CASTAÑEDA-OVANDO et al., 2009). Pesquisas têm sido realizadas com o intuito de reduzir o uso de corantes alimentícios sintéticos, dando lugar ao uso de corantes de fontes naturais. As antocianinas têm um grande potencial como substituto de muitos corantes, porém a sua aplicação em escala industrial é limitada devido à sua baixa estabilidade (ESTUPINAN; SCHWARTZ; GARZON, 2011).

As antocianinas são altamente instáveis e podem ser facilmente degradadas durante o processamento e armazenamento dos alimentos. Fatores como pH, luz, temperatura, oxigênio, ação enzimática, ácido ascórbico, açúcares, dióxido de enxofre e íons metálicos aceleram a degradação das antocianinas (SHENOY, 1993; CAVALCANTI; SANTOS; MEIRELES, 2011). Ersus e Yurdagel (2007) investigaram a influência da temperatura de secagem em *spray dryer* das antocianinas de cenoura preta (*Daucus carota* L). Eles verificaram que temperaturas na faixa de 160 a 180 °C provocaram maior degradação destes compostos.

1.2 SECAGEM POR ATOMIZAÇÃO

A secagem por atomização é uma operação unitária através da qual um líquido ou pasta é atomizado em uma corrente de ar quente originando um pó. A velocidade de desidratação é muito rápida porque a área superficial das partículas é grande, e a temperatura do ar é alta (150 a 300 ºC). O produto, com alta umidade inicial, entra em contato com o ar na temperatura mais alta, desta forma, enquanto a água está sendo removida do produto, este permanece na sua temperatura de bulbo úmido, a qual geralmente não ultrapassa 50 ºC. Quando o produto está seco, o ar já se resfriou, o que diminui o risco de degradação pela temperatura (RÉ, 1998). Dessa forma, o *spray dryer* tem sido amplamente utilizado nas indústrias de alimentos e farmacêuticas na secagem de produtos termo-sensíveis.

Um dos aspectos críticos do processo é a atomização do produto. Para que a desidratação seja rápida, é importante obter a aspersão de gotas com tamanho pequeno e homogêneo. Além disso, o tamanho das gotas determinará o tamanho das partículas obtidas. Os produtos obtidos em *spray dryer* são constituídos de partículas na faixa de 10-100 μm (FUCHS et al., 2006).

De acordo com Goula, Adamopoulos e Kazakis (2004), no início da secagem, a gota tem uma superfície líquida e a evaporação de água desta superfície é rápida. A remoção da água faz com que a superfície fique mais concentrada em solutos, o que depende da velocidade de evaporação e da taxa na qual o líquido pode migrar do interior para a superfície da gota. Com o aumento da concentração, os sólidos acabam formando uma crosta, que envolve uma partícula oca. A espessura dessa crosta depende da taxa de secagem, sendo que taxas iniciais elevadas levam à formação de partículas maiores com casca fina, enquanto taxas iniciais mais baixas resultam em partículas menores, com a casca mais espessa e alta densidade.

Uma vez que o pó seco atinge o fundo do secador, ele é separado do ar e o produto é removido para processamento adicional ou para o empacotamento. A separação primária é feita através da força gravitacional e a separação entre o pó fino e o ar é realizada através de um ciclone. O ar contendo finas partículas de pó entra tangencialmente ao ciclone onde a força centrífuga causa a separação das partículas do ar, direcionando as mesmas para o fundo do ciclone. O ar flui novamente para o topo do ciclone. Em casos que o ciclone não remove todas as partículas, um filtro de tela adicional é utilizado (HELDMAN; HARTEL, 1998).

As propriedades físico-químicas do pó produzido por meio de atomização dependem de algumas variáveis do processo como as características da alimentação (viscosidade, tensão superficial e vazão), do ar de secagem (velocidade, temperatura e pressão) e das configurações do atomizador e da câmara de secagem. Problemas operacionais como a dificuldade na atomização da alimentação e pegajosidade resultam em produtos com baixa qualidade e diminuem o rendimento do processo (TONON; BRABET; HUBINGER, 2008).

O processo de atomização é normalmente utilizado na produção de sucos de fruta em pó. Porém, a atomização do produto é dificultada devido à presença de açúcares e ácidos orgânicos de baixa massa molar (BHANDARI; DATTA; HOWES, 1997). A pegajosidade e alta higroscopicidade dos sucos de frutas podem resultar em problemas tecnológicos, como a adesão do pó às paredes do secador, dificuldade de manipulação, empastamento e compactação, tornando o armazenamento e a utilização mais difícil, além de diminuir o rendimento do processo e afetar as características do produto final.

É de grande importância para a indústria melhorar o desempenho do processo de secagem por atomização. Para reduzir a pegajosidade e garantir o manuseio e o armazenamento seguro de alimentos em pó uma alternativa importante é a adição de agentes carreadores com alta massa molar. O uso de agentes carreadores melhora a estabilidade do material durante a secagem e o armazenamento e, além disso, desempenham a função de encapsulantes (ADHIKARI et al., 2009).

1.3 MICROENCAPSULAÇÃO

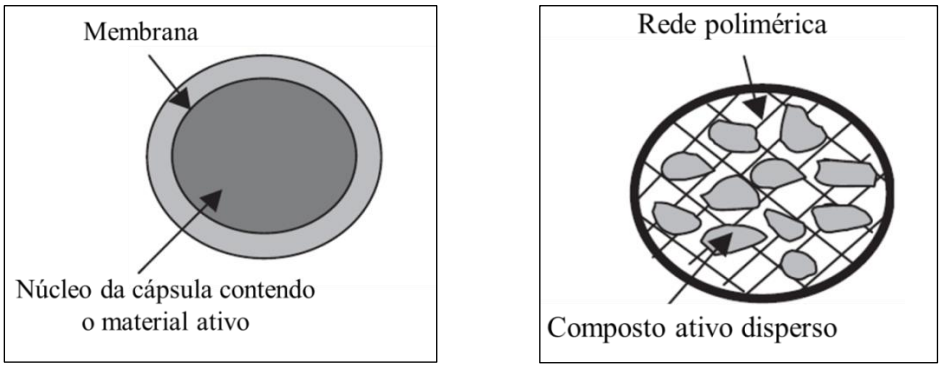
O conceito primário e o modelo mais bem-sucedido de microencapsulação são baseados nas células vivas, nas quais uma membrana natural protege os componentes celulares, e exerce função importante no metabolismo, controlando a entrada e saída de substâncias das células (FANGER, 1974). Da mesma maneira, nas microcápsulas ocorre a incorporação da substância de interesse (núcleo) em um sistema de revestimento (material de parede ou agente carreador). O material de parede age como um filme protetor, isolando a substância ativa a fim de estabilizar, proteger e permitir o controle de sua liberação (CAVALCANTI; SANTOS; MEIRELES, 2011; MADENE et al., 2006; RÉ, 1998).

Existem diversas técnicas utilizadas para a microencapsulação de ingredientes alimentícios, que se dividem em métodos físicos, químicos e físico-químicos. Destacam-se entre

os métodos físicos atomização, *spray chilling*, *spray cooling*, *spray coating*, leito fluidizado, extrusão, extrusão centrífuga, co-cristalização e liofilização. Os métodos químicos são constituídos pela coacervação, envolvimento por lipossomas e separação em fase orgânica. Enquanto os métodos físico-químicos são representados pela inclusão molecular e polimerização interfacial (JACKSON; LEE, 1991).

A escolha do método de microencapsulação depende de fatores econômicos, das propriedades físico-químicas do núcleo e do material de parede, da funcionalidade que a substância de interesse deve fornecer ao produto final, do mecanismo de liberação, do tamanho de partícula desejada, entre outros (JACKSON; LEE, 1991). A técnica de microencapsulação mais utilizada na indústria de alimentos tem sido o processo de atomização.

As micropartículas podem ser classificadas de duas formas: como reservatório, onde o núcleo fica concentrado próximo ao centro e é rodeado por um filme contínuo; ou do tipo matricial, onde o núcleo é disperso uniformemente pela matriz (RÉ, 1998). O sistema do tipo reservatório caracteriza as "verdadeiras" microcápsulas, enquanto o sistema matricial resulta nas chamadas microesferas (Figura 1.6).



(a) (b)

Figura 1.6 Ilustração das micropartículas (a) microcápsulas e (b) microesferas. Adaptado de Madene et al. (2006).

A principal diferença entre as microcápsulas e as microesferas está no fato de que, nas microesferas, uma fração do material encapsulado fica na superfície, o que é evitado pela verdadeira encapsulação. No entanto, o termo encapsulação tem sido usado tanto para microcápsulas quanto para microesferas (AZEREDO, 2005). A microencapsulação pelo processo de atomização resulta na formação de microesferas.

As principais razões para o uso da microencapsulação em alimentos são: reduzir as interações do núcleo com fatores ambientais como, luz umidade e oxigênio, retardando alterações que podem resultar em perda de aroma, alteração de cor ou perda do valor nutricional; reduzir a taxa de migração do material do núcleo para o ambiente externo; promover melhor solubilidade do núcleo e melhor incorporação em sistemas secos (AZEREDO, 2005; NESTERENKO et al., 2013). Além disso, a microencapsulação permite que a liberação do material do núcleo ocorra lentamente, ou em taxas de liberação controlada. A liberação do conteúdo da micropartícula pode ocorrer por condições pré-estabelecidas, como rompimento, solubilização, calor, pH ou por ação enzimática (NESTERENKO et al., 2013). A microencapsulação dos corantes geralmente visa protegê-los da oxidação, o que propicia um aumento na vida de prateleira, facilitando a sua incorporação e solubilização nos alimentos (FAVARO-TRINDADE; PINHO; ROCHA, 2008).

Robert et al. (2010) avaliaram a estabilidade das antocianinas do suco de romã (*Punica granatum* L.) "*in natura*" e do suco microencapsulado durante 56 dias de armazenamento na temperatura de 60 ºC. Os autores verificaram que a degradação de antocianinas no suco "*in natura*" foi mais rápida do que a degradação no suco microencapsulado, o que demonstra a importância da microencapsulação na proteção de compostos bioativos.

Mahdavee Khazaei et al. (2014) avaliaram a estabilidade das antocianinas de pétalas de açafrão (*Crocus sativus* L.) microencapsuladas com maltodextrina e goma arábica durante 10 semanas na temperatura de 35 ºC. Os autores observaram que não houve perdas significativas de antocianinas após o período de armazenamento, o que demonstra o efeito protetor do material de parede.

Idham, Muhamad e Sarmadi (2012) avaliaram a estabilidade das antocianinas de hibisco (*Hibiscus sabdariffa* L.) microencapsuladas com amido solúvel, maltodextrina, goma arábica e mistura de maltodextrina com goma arábica durante 105 dias nas temperaturas de 4, 25 e 37 ºC. Após o período de armazenamento, os autores verificaram que a mistura de maltodextrina e goma arábica apresentou menor taxa de degradação das antocianinas, quando comparado ao uso de somente um tipo de material de parede. Esse resultado indica a vantagem do uso de misturas de biopolímeros na microencapsulação.

1.4 AGENTES CARREADORES

Com o objetivo de prevenir as mudanças estruturais nos sucos de frutas obtidos pela secagem por atomização e garantir o manuseio e o armazenamento seguro de alimentos em pó, os fatores fundamentais a serem controlados são a umidade e a temperatura durante o armazenamento. Outra estratégia importante é a adição de biopolímeros aos pós alimentícios higroscópicos. Esses biopolímeros, também chamados de agentes de secagem ou agentes carreadores, também são considerados materiais encapsulantes (TELIS; MARTÍNEZ-NAVARRETE, 2012).

Os mecanismos pelos quais os agentes carreadores podem contribuir para a estabilidade dos pós higroscópicos durante o armazenamento são baseados em diferentes fatores, que podem ocorrer simultaneamente, como: competição pela umidade com os componentes do produto; atuação como uma barreira física, mesmo sem recobrir completamente a superfície dos compostos; aumento da temperatura de transição vítrea (Tg) da fase amorfa; formação de uma barreira protetora contra a umidade na superfície de partículas higroscópicas (AGUILERA; DEL VALLE; KAREL, 1995).

A escolha do carreador depende das propriedades físicas e químicas da substância de interesse, do processo de secagem e das propriedades finais desejadas. O encapsulante ideal deve apresentar baixa viscosidade em concentrações elevadas e ser de fácil manipulação durante o processo; possuir baixa higroscopicidade; não ser reativo com o material a ser encapsulado; ter habilidade de selar e reter a substância de interesse dentro da estrutura da cápsula; liberar completamente o solvente ou outro material usado durante o processo de encapsulação; ser solúvel em solventes utilizados na indústria de alimentos; apresentar boa disponibilidade e baixo custo (GHARSALLAOUI et al., 2007).

Os principais agentes carreadores empregados na indústria alimentícia são: carboidratos (amido, maltodextrina, xarope de milho, dextrose, sacarose e ciclodextrinas), celuloses (carboximetilcelulose e metilcelulose), gomas (acácia, ágar, arábica, alginato de sódio, carragena), lipídeos (ceras, parafina, diglicerídeos), proteínas (glúten, caseína, gelatina, albumina), entre outros (SHAHIDI; HAN, 1993).

Nos sucos de frutas, o agente carreador mais utilizado tem sido a maltodextrina (ABADIO et al., 2004; CANO-CHAUCA et al., 2005; GABAS et al., 2007; MARTINELLI;

GABAS; TELIS-ROMERO, 2007; TONON; BRABET; HUBINGER, 2009; KHA; NGUYEN; ROACH, 2010; ROBERT et al., 2010; TELIS; MARTÍNEZ-NAVARRETE, 2010), porém o uso de proteínas isoladas de soja e de soro de leite tem se mostrado promissor (BASTOS et al., 2012; FANG; BHANDARI, 2012). O uso de proteínas como material de parede na microencapsulação reflete uma tendência atual, especialmente nas áreas da nutrição, farmacêutica e de cosméticos (NESTERENKO et al., 2012).

A eficácia da maltodextrina como agente carreador é devido à rápida formação de filme e baixa difusividade da umidade nesses filmes. Por outro lado, as proteínas formam películas lisas e não pegajosas, de forma mais rápida que a maltodextrina. Além disso, o rendimento do processo é maior quando uma pequena quantidade de proteína é adicionada na solução a ser seca por atomização (ADHIKARI et al., 2009). Fang e Bhandari (2012) compararam a eficiência da proteína isolada de soro de leite e da maltodextrina na secagem por atomização do suco de *bayberry*. Os autores verificaram que uma pequena quantidade de proteína isolada de soro de leite (1%) é necessária para secar sucos de frutas, enquanto uma grande quantidade de maltodextrina (> 30%) é necessária para a mesma finalidade.

Dessa maneira, a mistura de maltodextrina e proteínas é uma alternativa para favorecer o processo de secagem e a proteção do material encapsulado (NESTERENKO et al., 2013; RUSLI; SANGUANSRI; AUGUSTIN, 2006), combinando propriedades específicas de cada polímero.

1.1.2 Maltodextrina

A maltodextrina é um polissacarídeo obtido pela hidrólise ácida de amidos. Consiste em unidades de α-D-glicose unidas principalmente por ligações glicosídicas (α 1→4) e apresenta uma fórmula geral igual a $(C_6H_{10}O_5)nH_2O$ (KENNEDY; KNILL; TAYLOR, 1995).

As maltodextrinas são descritas em relação à sua dextrose equivalente (DE), que é a medida do inverso do número de unidades de α-D-glicose anidro, e está ligada ao seu grau de polimerização (DP), de forma que DE = 100/DP. Assim, a especificidade das propriedades das maltodextrinas está ligada a DE e ao DP, que variam com o grau de hidrólise e o tratamento enzimático. Para ser chamado de maltodextrina, o produto deve ter DE menor do que 20 (SHAHIDI; HAN, 1993; KENNEDY; KNILL; TAYLOR, 1995).

O uso da maltodextrina se mostra eficaz quando utilizada na secagem por atomização e na microencapsulação de alimentos. A maltodextrina apresenta uma boa relação entre custo e

eficácia, tem amplo uso em alimentos, possui sabor e odor neutros, baixa viscosidade mesmo em altas concentrações e boa solubilidade (MADENE et al., 2006). A capacidade da maltodextrina em proteger o material encapsulado é atribuída à sua capacidade de formação de filme e propriedades plásticas.

Tonon, Brabet e Hubinger (2009) avaliaram o efeito da maltodextrina na secagem por atomização de suco de açaí e obtiveram pós menos higroscópicos com o uso de maiores concentrações do carreador (30%). Gabas et al. (2007) e Martinelli, Gabas e Telis-Romero (2007) analisaram os efeitos da adição de maltodextrina na secagem de sucos de abacaxi e de limão, respectivamente. Os autores verificaram que a adição de maltodextrina diminuiu a umidade de equilíbrio em uma determinada atividade de água. Telis e Martínez-Navarrete (2010) estudaram os efeitos da adição de maltodextrina sobre a temperatura de transição vítrea, isotermas de sorção e alterações de cor em pós de toranja produzidos por liofilização. Os resultados confirmaram o efeito protetor do biopolímero durante o armazenamento do produto.

1.1.3 Proteínas

As proteínas são ingredientes naturais, amplamente disponíveis e com alto valor nutritivo, sendo muito utilizadas na indústria de alimentos. Por causa dos seus diferentes grupos químicos, propriedades anfifílicas, capacidade de interação com diferentes substâncias, alta massa molar e flexibilidade na cadeia molecular, as proteínas possuem excelentes propriedades funcionais. Elas possuem propriedades úteis para encapsulação, tais como emulsificação, solubilidade, formação de filme, e capacidade de ligação com água (BASTOS et al., 2012; MADENE et al., 2006; MOLINA ORTIZ et al., 2009).

Quanto às propriedades físico-químicas, as proteínas são solúveis em uma ampla faixa de pH, exceto na faixa de pH correspondente ao ponto isoelétrico. Os tratamentos térmicos aplicados às proteínas podem afetar as propriedades funcionais do pó, possivelmente pela desnaturação proteica. A desnaturação proteica ocorre com a combinação de alta temperatura e alta atividade de água do produto (GHARSALLAOUI et al., 2007). Por isso, é difícil prever a estabilidade das proteínas no processo de secagem por atomização (KANDANSAMY; SOMASUNDARAM, 2012).

A proteína de soja é uma das mais populares proteínas de fontes vegetais utilizadas como ingredientes na formulação de alimentos. A proteína isolada de soja (SPI) é produzida a partir de

farinha desengordurada de soja e contém no mínimo 90% de proteína. As globulinas glicinina e β-conglicinina são os componentes principais da SPI, sendo que estas duas globulinas têm diferentes estruturas e propriedades funcionais (ROBERT et al., 2010). Ela é um material atóxico, relativamente barato, abundante, renovável, biocompatível e biodegradável (ORTIZ et al., 2009; NESTERENKO et al., 2012). Para a maioria das proteínas vegetais, em solução aquosa, o ponto isoelétrico está localizado num intervalo de pH entre 3 e 5. Por esta razão esses biopolímeros são normalmente utilizados em condições alcalinas, a fim de obter uma boa solubilidade de proteínas e para encapsular eficazmente substâncias ativas (NESTERENKO et al., 2013).

Tang e Li (2013) sugeriram que SPI é um bom material de parede para encapsular óleo de soja, podendo substituir algumas proteínas que possuem elevado custo, como o caseinato de sódio, nas aplicações práticas da indústria de alimentos. Rascon et al. (2011) ao utilizarem proteína isolada de soja na retenção de carotenoides da oleoresina de páprica, verificaram que este carreador resultou em boa proteção contra a oxidação. Nesterenko et al. (2012) observaram a alta retenção de α-tocoferol na secagem com SPI. Os autores associaram a alta retenção às propriedades emulsionantes da proteína de soja (como a diminuição do tamanho das gotículas das emulsões), embora modificações enzimáticas e químicas tenham sido realizadas na proteína.

A proteína de soro de leite é um subproduto de laticínios e seus constituintes mais abundantes são α-lactoglobulina e β-lactoalbumina (BASTOS et al., 2012). Essas proteínas são globulares, quase independentes do pH, mas tornam-se insolúveis no seu ponto isoelétrico, pH próximo de 5 (KANDANSAMY; SOMASUNDARAM, 2012). A proteína de soro de leite possui baixo custo, possui alto valor nutricional e boas propriedades estruturais e funcionais (BASTOS et al., 2012). Ela é de fácil manuseio, possui alta estabilidade, protege o núcleo encapsulado dos fatores deteriorantes e fornece grande versatilidade para os produtos microencapsulados (ROSENBERG, 1997).

De acordo com Kandansamy e Somasundaram (2012), a proteína de soro de leite é uma das proteínas mais utilizadas na encapsulação de corantes pelo processo de atomização. Bastos et al. (2012) utilizaram misturas de quitosana e proteína isolada de soro de leite na encapsulação do suco de caju e observaram alta estabilidade da vitamina C após 5 meses de armazenamento. Adhikari et al. (2009) reportaram que 0,5% de proteína isolada do soro de leite resultou em um rendimento do pó de soluções de sacarose superior a 80% e que o aumento da concentração de

proteínas para 1% não melhorou o rendimento. Sheu e Rosenberg (1995) avaliaram a interação da proteína de soro de leite e carboidratos na encapsulação de voláteis (éster). De acordo com os autores, as proteínas de soro de leite serviram como emulsificantes e agentes formadores de película, enquanto os carboidratos (maltodextrina e xarope de milho) atuaram como materiais formadores de matriz.

1.5 ESTABILIDADE DE ALIMENTOS EM PÓ

A estabilidade é uma característica desejável em alimentos. Do ponto de vista termodinâmico, um material é considerado estável quando se encontra em equilíbrio com as condições de temperatura e pressão do ambiente, de forma que ele não apresente alterações em seu estado físico ao longo do tempo.

A estabilidade física, química e microbiológica dos alimentos depende do conteúdo de água e de sua interação com os outros componentes do produto. Nos alimentos a água encontra-se de duas formas: água fracamente ligada, que serve como solvente e permite o crescimento dos micro-organismos e reações químicas; e água fortemente ligada ao substrato, que não serve como solvente. A atividade de água (a_w) quantifica o grau de ligação da água contida no produto e, consequentemente, sua disponibilidade para agir como solvente e participar das transformações químicas, bioquímicas e microbiológicas (FENNEMA, 1996). O estudo da atividade de água pode ser feito pela avaliação de isotermas de sorção, que descrevem a relação entre o conteúdo de água dos alimentos e a_w, em temperatura e pressão constantes.

Na indústria de alimentos as isotermas de sorção de água são consideradas ferramentas termodinâmicas úteis para predizer as interações entre a água e os componentes dos alimentos. O conhecimento de isotermas de sorção é de grande importância para vários processos alimentícios, como a desidratação e o armazenamento, sendo utilizados para calcular o tempo de secagem, predizer o comportamento de ingredientes após a mistura, selecionar embalagens, modelar as mudanças de umidade que ocorrem durante a estocagem e estimar a estabilidade durante a vida de prateleira dos alimentos, o que é muito importante, principalmente para produtos em pó (LOMAURO; BAKSHI; LABUZA, 1985).

As isotermas de sorção são úteis principalmente para caracterizar as propriedades higroscópicas do alimento desidratado. Segundo Bhandari, Datta e Howes (1997), nas condições em que ocorre o processo de desidratação, uma quantidade significativa de material permanece

no estado amorfo, provocado pela rápida remoção de água e um tempo insuficiente para a cristalização. O estado amorfo é definido como a falta de organização das moléculas, sendo oposto ao estado cristalino, o qual se caracteriza pelo melhor arranjo da estrutura (ROOS, 1995; SLADE; LEVINE, 1991).

Nos alimentos em pós que contêm açúcar, a capacidade de adsorção de água está relacionada ao seu estado físico. Em baixas atividades de água, os sólidos amorfos absorvem mais água que sólidos cristalinos. Isso ocorre porque a área superficial interna do sólido, disponível para o contato com as moléculas de água, é maior em sólidos amorfos. Também é importante considerar a habilidade da água em romper a estrutura ligada por pontes de hidrogênio nos cristais de açúcares, as quais são menos regulares em sólidos amorfos, conferindo-lhes uma maior interação com a água absorvida (KAREL; HEIDELBAUGH, 1973).

A maioria dos sólidos solúveis presentes nos sucos de frutas são açúcares de baixa massa molar, como sacarose, glicose e frutose, bem como ácidos orgânicos como os ácidos cítrico, málico e tartárico. Quando esses compostos são submetidos ao processo de atomização, a matriz sólida resultante pode estar em um estado amorfo, o qual é muito sensível a mudanças de temperatura e umidade. Essa matriz amorfa é suscetível a plasticização pela água, seguida por alterações relacionadas à temperatura de transição vítrea (TELIS; MARTÍNEZ-NAVARRETE, 2012).

A água é um plasticizante que afeta a temperatura de transição vítrea (Tg) de alimentos amorfos, sendo que o seu conteúdo, define a localização da Tg. A plasticização resulta de efeitos combinados da água e da temperatura. Dessa forma, a predição da estabilidade dos alimentos com base apenas nas isotermas de sorção não é suficiente, uma vez que algumas alterações físico-químicas e estruturais, tais como pegajosidade, colapso e cristalização não estão relacionados a um valor de monocamada, e são melhor correlacionados pela temperatura de transição vítrea (ROOS, 1995).

A transição vítrea é uma transição de fases que governa a passagem do estado vítreo para o estado gomoso. Ela ocorre quando o sólido amorfo é submetido a uma temperatura superior a Tg. Em uma faixa de temperaturas de cerca de 20 ºC ao redor da Tg inicia-se a transição de fase, cuja viscosidade diminui rapidamente à medida que a temperatura é elevada. Nesse estado, o volume livre do sistema e a mobilidade molecular aumentam rapidamente, afetando suas

propriedades físicas. O estado vítreo e o estado gomoso são estados de equilíbrio metaestável e suas propriedades podem variar com o tempo (SLADE; LEVINE, 1991).

As características viscoelásticas do material nas proximidades da Tg afetam não apenas as propriedades mecânicas do material, mas também o grau de encolhimento que tem um importante papel na taxa de secagem e na qualidade do produto desidratado. O encolhimento ou "colapso" ocorre porque a matriz polimérica não é capaz de suportar seu próprio peso. Quanto maior a viscosidade do sistema, menor será a taxa de encolhimento, de forma que este só ocorre se a temperatura de secagem for superior à Tg do material, em uma determinada umidade (ACHANTA; OKOS, 1996).

Com o objetivo de reduzir a pegajosidade e garantir o armazenamento seguro dos sucos de frutas obtidos pela secagem por atomização, é fundamental realizar o controle da umidade e armazenar o produto a baixas temperaturas. Outra estratégia importante é a utilização de aditivos de alto peso molecular, que são responsáveis pelo aumento da temperatura de transição vítrea do produto, e dessa forma são responsáveis por melhorar não somente o processo de secagem, mas também a estabilidade dos pós (TELIS; MARTÍNEZ-NAVARRETE, 2012).

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-CAPÍTULO 2-

TESTES PRELIMINARES

2 TESTES PRELIMINARES

Antes de iniciar o estudo da influência dos agentes carreadores sobre a secagem por atomização e microencapsulação das antocianinas, avaliou-se os parâmetros de secagem em *spray dryer*. As propriedades físico-químicas dos sucos em pó dependem de algumas variáveis de processo, tais como vazão de alimentação e temperatura do ar de secagem. Dessa forma, é importante avaliar os efeitos desses parâmetros sobre o processo de secagem, a fim de se obter produtos com melhores características sensoriais e nutricionais e um melhor rendimento do processo.

Como a cultivar de uva BRS Violeta ainda é pouco comercializada e somando-se o fato de que é necessário esperar pela sua safra, os testes preliminares foram realizados utilizando o suco de uva integral comercial. Como o uso de maltodextrina como agente carreador já foi bastante estudado, utilizou-se este carreador puro para a avaliação inicial da influência das variáveis do processo durante a secagem.

Após determinar os parâmetros de secagem, foi realizado um breve estudo para avaliar a concentração dos agentes carreadores (misturas de maltodextrina e proteínas), assim como a proporção de proteínas em relação à quantidade total de agente carreador, necessários para realizar a secagem do suco de uva por atomização.

2.1 MATERIAIS E MÉTODOS

2.1.1 Materiais

Para determinar as melhores condições de secagem em *spray dryer* (Item 2.2), foi utilizado como matéria prima suco de uva natural, com 16,7 ºBrix de sólidos solúveis, obtido no comércio local. Como agente carreador foi utilizado maltodextrina (DE-10 MOR-REX 1910, Corn Products, Brasil).

Para avaliar a concentração e proporção de agentes carreadores (Item 2.3), assim como, para realizar todas as fases seguintes deste trabalho, foi utilizado suco extraído da uva da cultivar BRS Violeta, com 14 ºBrix de sólidos solúveis. Como agentes carreadores foram utilizados maltodextrina (DE-10 MOR-REX 1910, Corn Products, Brasil), proteína isolada de soja (Supro 783, TovaniBenzaquen, Brasil) e proteína concentrada de soro de leite (WPC80, Alibra, Brasil).

2.1.2 Secagem em *spray dryer*

As amostras foram submetidas à secagem por atomização em um mini *spray dryer* (modelo B-290, marca Büchi, Suiça), com bico atomizador de 0,7 mm de diâmetro. A pressão do compressor foi ajustada em 6 bar.

Para determinar as melhores condições de secagem no *spray dryer* foram avaliados os seguintes parâmetros: temperatura do ar de secagem, de 140 à 170 ºC; vazão de alimentação, de 2 à 6 mL/min; e o fluxo de ar de secagem, de 300 à 500 L/h. Após a secagem, o suco em pó foi acondicionado em embalagem plástica, revestida externamente com papel alumínio, permanecendo em dessecador com sílica gel para não absorver umidade do ar ambiente.

2.1.3 Rendimento do processo

O rendimento do processo de secagem foi calculado pela razão entre a quantidade de sólidos presentes após a secagem e a quantidade de sólidos presentes na mistura antes de entrar no secador. Os resultados foram expressos em porcentagem.

2.1.4 Umidade

A umidade foi determinada por gravimetria, em estufa a vácuo (Marconi modelo MA 030) a 70 °C, sob pressão reduzida, ≤ 100 mm Hg (13,3 kPa), até peso constante (AOAC, 1990).

2.1.5 Solubilidade

A solubilidade das microesferas foi determinada de acordo com o método descrito por Eastman e Moore (1984), modificado por Cano-Chauca et al. (2005), onde 1 g de amostra (base seca) foi adicionada a 100 ml de água destilada e agitada durante 5 minutos. A solução foi transferida para uma centrífuga a 3000×g por 5 minutos. Uma alíquota de 25 mL do sobrenadante foi transferida para placa de Petri previamente tarada, então foi aquecida em estufa a 105ºC por 5 horas. A porcentagem de solubilidade foi calculada pela diferença de peso.

2.1.6 Mudanças na cor

Os parâmetros de cor foram determinados usando colorímetro Hunter Lab (Color Flex EZ, USA), com iluminante D65 e ângulo de observação de 10º. Nesse sistema, L* representa a

claridade (0 para preto até 100 para branco) e as coordenadas cromáticas a* (+ a* vermelho, – a* verde) e b* (+ b* amarelo e – b* azul). Os parâmetros a* e b* foram usados pra calcular a intensidade cromática (C*) e o ângulo de tonalidade cromática (h), conforme as equações a seguir:

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

$$h = \text{arctg}\left(\frac{b}{a}\right) \quad (2)$$

2.1.7 Planejamento experimental

Para encontrar as melhores condições de secagem, um planejamento fatorial completo foi realizado considerando-se quatro fatores (variáveis independentes) com dois níveis de cada uma das variáveis. As variáveis independentes avaliadas foram a temperatura do ar de secagem (140 - 170 ºC), vazão de alimentação (2 - 6 mL/min), fluxo de ar (300 - 500 L/h), e a proporção de maltodextrina em relação aos sólidos solúveis do suco, maltodextrina (1 - 1,5 g agente carreador/g sólidos solúveis do suco), incluindo dois ensaios no ponto central. Os resultados foram avaliados estatisticamente por análise de variância (ANOVA) a um nível de significância de 5% ($p \leq 0,05$).

2.2 AVALIAÇÃO DOS PARÂMETROS DE SECAGEM EM *SPRAY DRYER*

A influência da temperatura do ar de secagem, vazão de alimentação, fluxo de ar e concentração de maltodextrina (CAC, g de carreador/g sólidos solúveis do suco) sobre o rendimento e umidade do pó estão apresentados na Tabela 2.1.

Tabela 2.1 Rendimento do processo para diferentes condições de secagem em *spray dryer* e umidade do suco de uva em pó.

Tratamento	CAC*	Temperatura do ar (ºC)	Fluxo de ar (L/h)	Vazão de alimentação (mL/min)	Rendimento (%)	Umidade (b.u., %)
1	1,00	140	300	2	17.38	1,09 ± 0,10
2	1,50	140	300	2	6.28	1,00 ± 0,09
3	1,00	170	300	2	8.36	0,89 ± 0.03
4	1,50	170	300	2	7.25	2,04 ± 0,09
5	1,00	140	500	2	45.57	1,54 ± 0,01
6	1,50	140	500	2	42.17	1,02 ± 0,03
7	1,00	170	500	2	39.98	1,63 ± 0,39
8	1,50	170	500	2	40.82	1,33 ± 0,15
9	1,00	140	300	6	8.78	2,66 ± 0,01
10	1,50	140	300	6	2.08	2,31 ± 0,08
11	1,00	170	300	6	9.57	2,39 ± 0,75
12	1,50	170	300	6	4.59	1,60 ± 0,18
13	1,00	140	500	6	36.42	2,08 ± 0,18
14	1,50	140	500	6	32.04	2,10 ± 0,24
15	1,00	170	500	6	34.56	1,93 ± 0,06
16	1,50	170	500	6	35.70	2,28 ± 0,12
17	1,25	155	400	4	32.04	1,90 ± 0,35
18	1,25	155	400	4	34.66	0,57 ± 0,13

2.2.1 Umidade

Todos os pós apresentaram baixo conteúdo de umidade, que variou de 0,57 a 2,66%, em base úmida. Como é possível observar na Figura 2.1, o aumento na vazão de alimentação influenciou negativamente o conteúdo de umidade do suco de uva em pó ($p \leq 0,05$).

Isso ocorre porque altas taxas de alimentação resultam em um curto tempo de contato entre o material e o ar de secagem, tornando o processo de transferência de calor menos eficiente, resultando em baixas taxas de secagem (TONON; BRABET; HUBINGER, 2008).

Além disso, o aumento na vazão de alimentação também resulta em um aumento no número de gotas na câmara de secagem, dessa forma o ar de secagem não é suficiente para secar eficientemente toda a água presente nas gotas.

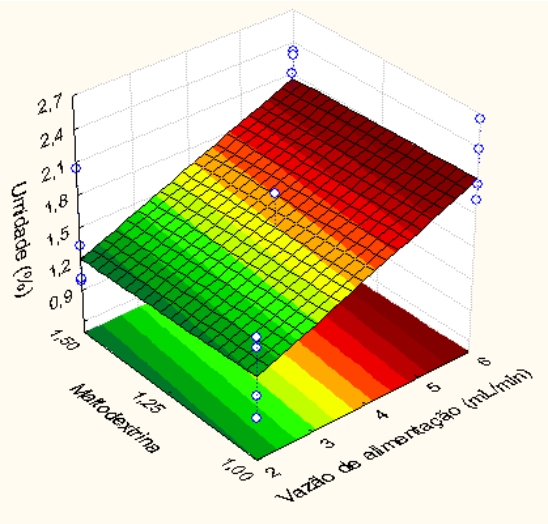

Figura 2.1 Influência da vazão de alimentação e da concentração de maltodextrina sobre a umidade dos pós.

O conteúdo de umidade é o fator que mais afeta a estabilidade dos pós. A água atua como plastificante e diminuem a temperatura de transição vítrea, causando mudanças importantes no produto, como pegajosidade, aglomeração e colapso (FERRARI; GERMER; AGUIRRE, 2012).

2.2.2 Rendimento de processo

O aumento do fluxo de ar apresentou efeito positivo sobre o rendimento da secagem (Tabela 2.1, Figura 2.2). Esse resultado está de acordo com Fazaeli et al. (2012), que realizaram a secagem por atomização do suco de amora. Elevadas vazões de alimentação demonstraram efeito negativo sobre o rendimento. Como já era esperado, elevada taxa de fluxo de ar combinado com baixa vazão de alimentação favorecem a secagem do produto.

O baixo fluxo de ar e a alta vazão de alimentação resultaram no gotejamento das amostras, sendo assim, parte do material que passou pelo *spray dryer* não foi atomizado. Na Figura 2.3 está apresentada a tentativa de secagem correspondente ao tratamento 10, a qual foi realizada com fluxo de ar de 300 L/h e vazão de alimentação de 6 mL/min. Nessas condições foi impossível realizar a secagem do suco de uva.

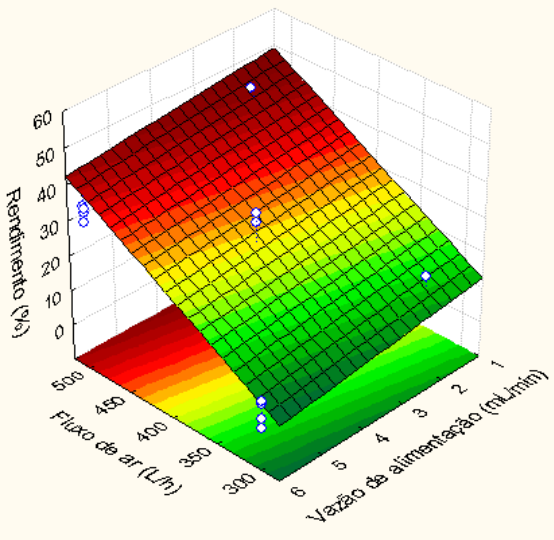

Figura 2.2 Influência do fluxo de ar e vazão de alimentação no rendimento do processo. (Temperatura do ar = 140 °C; Concentração de maltodextrina = 1)

Figura 2.3 Condições de secagem insatisfatórias resultaram no gotejamento da amostra.

2.2.3 Solubilidade

Nenhuma das variáveis estudadas apresentou efeito significativo sobre a solubilidade do suco de uva em pó, que variou de 95 a 100%. Cano-Chauca et al. (2005) verificaram que a adição de maltodextrina na secagem do suco de manga produz um pó com alto grau de solubilidade, superior a 90%. De acordo com esses autores, a boa solubilidade em água das

micropartículas deve-se à alta polaridade da maltodextrina. Abadio et al. (2004) avaliaram o efeito da maltodextrina sobre as propriedades físicas de suco de abacaxi em pó e observaram valor médio de solubilidade de 81%.

2.2.4 Mudanças na cor

O fluxo de ar e a concentração de maltodextrina aumentaram significativamente a claridade (L*) dos pós (Figura 2.4A). A interação entre fluxo de ar e vazão de alimentação também foi significativa, e altos valores dessas variáveis resultaram em maior L*.

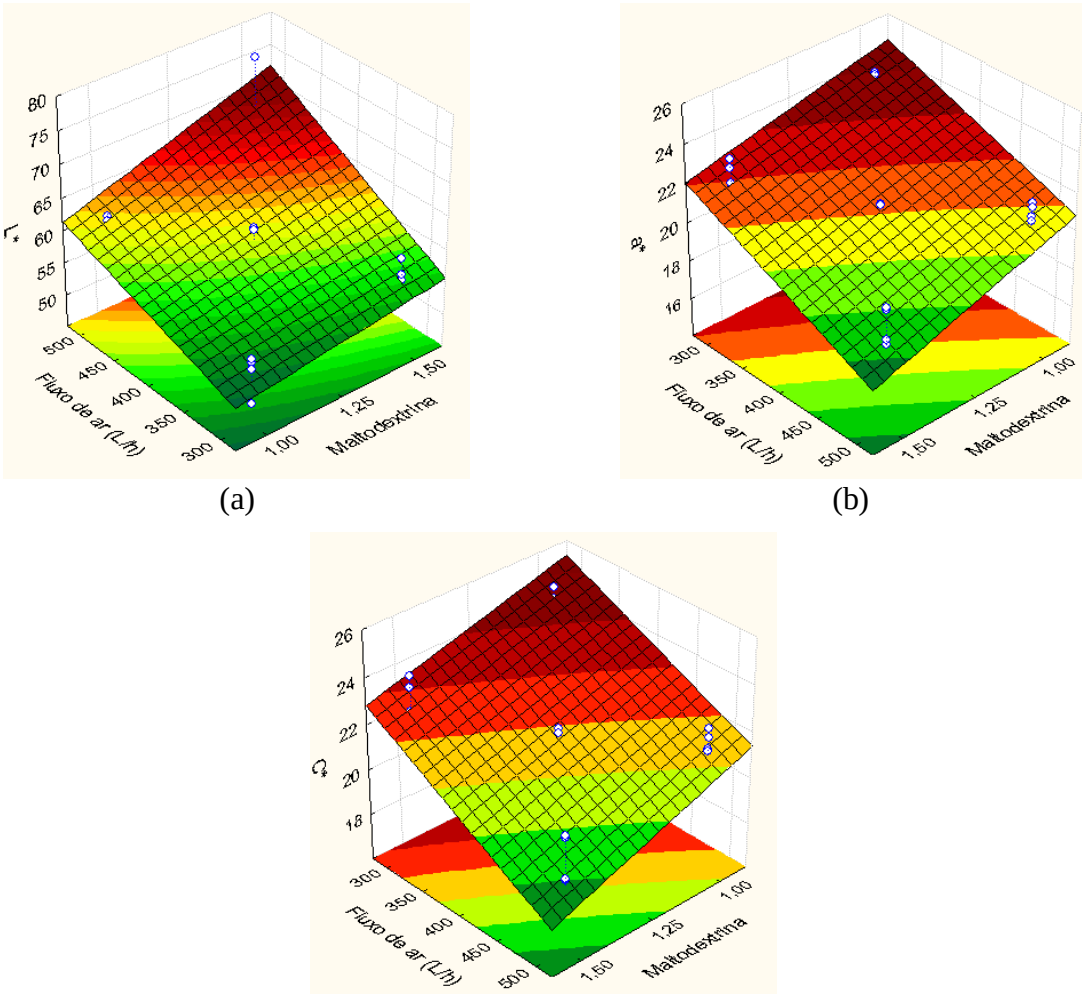

Figura 2.4 Influência do fluxo de ar e maltodextrina no parâmetro de cor do suco de uva em pó. (a) L*; (b) a*; e (c) C*. (Temperatura do ar = 140 °C; Vazão de alimentação = 2 mL/min).

A claridade dos pós aumenta com o aumento da concentração de maltodextrina, o que ocorre porque o carreador causa uma diluição no suco, resultando na perda de cor (TONON; BRABET; HUBINGER, 2009). Resultados semelhantes foram observados para suco de amora produzido por atomização (FERRARI; GERMER; AGUIRRE, 2012).

O fluxo de ar e o conteúdo de maltodextrina influenciaram negativamente o valor de a* (Figura 2.4B), indicando uma redução na coloração vermelha dos pós. A interação entre fluxo de ar e temperatura de secagem também foi significativa, assim baixos valores dessas variáveis resultaram em maiores valores de a*. O valor de b* não foi influenciado por nenhuma variável estudada.

A intensidade cromática (C*) foi influenciada ($p \leq 0,05$) pela concentração de maltodextrina e fluxo de ar (Figura 2.4C). A interação entre fluxo de ar e vazão de alimentação também foi significativa. O baixo fluxo de ar, baixa concentração de maltodextrina e baixa vazão de alimentação resultaram em maiores valores de C*, o que demonstra maior intensidade na coloração do produto. Kha et al. (2010) também observaram a mesma influência da maltodextrina na secagem por atomização da fruta Gac (*Momordica cochinchinensis*). O ângulo de tonalidade cromática (h) não foi influenciado por nenhuma das variáveis estudadas.

2.2.5 Escolha dos parâmetros de secagem

De maneira geral, a baixa vazão de alimentação resultou em pós com baixa umidade e elevado rendimento. A secagem utilizando maior fluxo de ar resultou em bom rendimento, por outro lado, afetou a coloração do produto. Ao observar a Tabela 2.1, verifica-se que o baixo fluxo de ar (300 L/h) não apresentou rendimentos aceitáveis (2,1 a 17,4%). Menor concentração de maltodextrina e menor temperatura de secagem resultaram nos melhores parâmetros de cor.

Dessa forma, concluiu-se que os melhores parâmetros para realizar a secagem do suco de uva são: baixa concentração de agente carreador; menor temperatura do ar de secagem, 140 ºC; maior fluxo de ar, 500 L/h; e menor vazão de alimentação, 2 mL/min. As melhores condições de secagem (temperatura, fluxo do ar e vazão de alimentação) obtidas nessa etapa foram adotadas para as fases seguintes deste trabalho (Capítulos 3, 4 e 5).

2.3 AVALIAÇÃO DA CONCENTRAÇÃO E PROPORÇÃO DE CARREADORES

A partir dos resultados apresentados no item 2.2 concluiu-se que a menor concentração de maltodextrina (CAC = 1) apresentou os melhores resultados globais na secagem do suco de uva. Por outro lado, o comportamento de misturas de proteína de soja e maltodextrina (SM) e misturas de proteína de soro de leite e maltodextrina (WM) durante a secagem por atomização eram desconhecidos. Após realizar uma pesquisa na literatura verificou-se que alta concentração de maltodextrina (10 a 40%) seria necessária para secar suco de frutas em *spray dryer* (TONON; BRABET; HUBINGER, 2009; ROBERT et al., 2010; FANG; BHANDARI, 2012) enquanto uma pequena quantidade de proteína de soja (1%) seria suficiente para secar suco de fruta (FANG; BHANDARI, 2012).

Para as misturas de proteína de soja e maltodextrina (SM), foram avaliada as concentrações de agente carreador (CAC, g agente carreador/g sólidos solúveis do suco) na faixa de 0,25 a 1 sobre o rendimento do processo de secagem (Tabela 2.2). A proporção de proteína de soja na mistura de carreadores (g proteína/100 g de agente carreador total) foi mantida em 10%. Ao secar suco de uva utilizando SM nas concentrações de 0,25 e 0,5, o rendimento não foi aceitável, enquanto que, o uso das concentrações de 0,75 e 1 g agente carreador/g sólidos solúveis do suco apresentou bom rendimento.

Tabela 2.2 Avaliação da concentração de agente carreador (CAC) e proporção de proteína de soja sobre o rendimento da secagem do suco de uva.

Tratamento	CAC*	Proporção de proteína (%)**	Rendimento (%)
1	0,25	10	0,00
2	0,50	10	13,03
3	0,75	10	47,00
4	1,00	10	43,79

*CAC: g agente carreador/g sólidos solúveis do suco. ** Proporção de proteína (%): g proteína/100 g de agente carreador total.

Nas misturas de proteína de soro de leite e maltodextrina (WM), foram avaliadas as concentrações de agente carreador de 0,25 à 0,75 sobre o rendimento (Tabela 2.3). A menor CAC apresentou bom rendimento de secagem. Dessa forma, foram realizadas secagens utilizando CAC = 0,25 e diferentes proporções da proteína de soro (g proteína/100 g de agente

carreador total). Então, concluiu-se que o aumento da quantidade de proteína na mistura (10%, 20% e 30%) resultou em maior rendimento do processo de secagem.

Tabela 2.3 Avaliação da concentração de agente carreador (CAC) e proporção de proteína de soro sobre o rendimento da secagem do suco de uva.

Ensaio	CAC*	Proporção de proteína (%)**	Rendimento (%)
1	0,25	10	17,79
2	0,25	20	28,98
3	0,25	30	49,71
4	0,50	10	41,37
5	0,75	10	51,88

*CAC: g agente carreador/g sólidos solúveis do suco. ** Proporção de proteína (%): g proteína/100 g de agente carreador total.

Dessa forma, as concentrações de agentes carreadores que foram escolhidas para serem avaliadas no Capítulo 3 foram na faixa de 0,75 a 1,25 g de carreador/g sólidos solúveis do suco para SM e 0,25 a 0,75 g de carreador/g sólidos solúveis do suco para WM. Nos sistemas SM e WM, a proporção de proteína utilizadas em relação à quantidade total de agentes carreadores foi de 10 a 30%.

A seguir está apresentado um exemplo de como foram realizados os cálculos da concentração de agente carreador (misturas de maltodextrina e proteínas) e a proporção de proteína (em relação à quantidade total de carreador) utilizada na secagem do suco de uva.

A concentração de agente carreador (CAC) foi expressa como g agente carreador/g sólidos solúveis do suco. Dessa maneira, quando a CAC = 0,75 foi adicionada no suco de uva que continha 14 ºBrix, significa que foi adicionado 0,75 gramas de carreador para cada 1 grama de sólidos solúveis do suco. Assim, em 100 gramas de suco, que possuía 14 gramas de sólidos solúveis, foi adicionado 10,5 gramas de agente carreador.

A proporção de proteínas na mistura de carreadores foi expressa como g proteína/100 g de agente carreador total. Ao utilizar a CAC = 0.75 e a proporção de proteína = 10%, a quantidade de carreadores adicionado em 100 gramas de suco foi de 10,5 gramas. Essa quantidade de carreador (10,5 gramas) era composta por 10% de proteína (1,05 gramas) e 90% de maltodextrina (9,45 gramas).

2.4 CODIFICAÇÃO DOS TRATAMENTOS PARA OS CAPÍTULOS 3, 4 E 5

O Capítulo 3 apresenta o estudo da influência da concentração de diferentes agentes carreadores (misturas de maltodextrina e proteínas), assim como diferentes proporções de proteínas nas misturas, na secagem do suco de uva e microencapsulação das antocianinas. Para isso, foi realizado um planejamento experimental que resultou em 20 tratamentos. Os quatro tratamentos que apresentaram os melhores resultados globais foram escolhidos para realizar os estudos nos Capítulos 4 e 5.

Como as codificações dos melhores tratamentos apresentam números provenientes do planejamento experimental (3SM, 7SM, 4WM e 6WM), essas codificações foram modificadas nos Capítulos 4 e 5, conforme Tabela 2.4.

Tabela 2.4 Composição dos quatro melhores tratamentos escolhidos no Capítulo 3 e correspondente codificação utilizada nos Capítulos 4 e 5.

Composição	**Capítulo 3**	**Capítulo 4**	**Capítulo 5**
CAC= 1,25, 10% de proteína	3SM	1SM	1SM
CAC = 1, 5,86% de proteína	7SM	2SM	2SM
CAC = 0,75, 30% de proteína	4WM	1WM	1WM
CAC = 0,85, 20% de proteína	6WM	2WM	2WM

CAC: g agente carreador/g sólidos solúveis do suco.
Proporção de proteína (%): g proteína/100 g de agente carreador total.
SM: Mistura de proteína de soja e maltodextrina; WM: mistura de proteína de soro de leite e maltodextrina.

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-CAPÍTULO 3-

SPRAY DRYING OF GRAPE JUICE FROM HYBRID CV. BRS VIOLETA: MICROENCAPSULATION OF ANTHOCYANINS USING PROTEIN/MALTODEXTRIN BLENDS AS DRYING AIDS

Trabalho aceito para publicação no "Journal of Food Processing and Preservation"

3 SPRAY DRYING OF GRAPE JUICE FROM HYBRID CV. BRS VIOLETA: MICROENCAPSULATION OF ANTHOCYANINS USING PROTEIN/MALTODEXTRIN BLENDS AS DRYING AIDS

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ABSTRACT

Grape juice contains high amounts of anthocyanins, with great potential for substituting synthetic food dyes. Carrier agents used in spray drying entraps anthocyanins, allowing their preservation. This work appraised whey protein/maltodextrin (WM) and soy protein/maltodextrin (SM) blends as alternative carriers for spray drying of grape juice and encapsulation of anthocyanins. The effects of carrier agent concentration (*CAC*) and ratio protein/carrier agent (*R*) on grape juice powder properties were evaluated. The grape juice powders presented good solubility, low water content, and high anthocyanin retention. WM blends resulted in higher yields and higher anthocyanin retention (from 77.9 to 94%) than SM blends, whereas SM blends leaded to higher encapsulation efficiency (> 97%). Increasing *CAC* and *R* resulted in brighter powders, but reconstituted juices presented color parameters similar to those of fresh juice. WM and SM were suitable for encapsulating anthocyanins of grape juice, resulting in powders with potential applications in food industry.

Keywords: natural pigments, encapsulation, preservation, anthocyanins

PRACTICAL APPLICATIONS

The grape cultivar BRS Violeta contains high levels of anthocyanins and is an alternative to produce antioxidant-rich and highly colored grape juice. Spray drying is applied for producing powdered grape juice with high anthocyanin content. In this technique, the addition of whey and soy proteins blended with maltodextrin as carrier agents avoid problems such as stickiness, which is negative to process yield and product quality. Moreover, the use of carrier agents in spray drying promotes the microencapsulation of bioactive compounds, allowing their protection and preservation during processing and storage. The grape juice powder from cv. BRS Violeta can be applied in the food industry as a potential substitute for synthetic food dyes, in addition to being a promising additive for incorporating anthocyanins into functional foods.

3.1 INTRODUCTION

Grapes and their derived products are important natural sources of phenolic compounds. EMBRAPA Grape & Wine (Brazilian Corporation of Agricultural Research, Unit Grape & Wine) developed the hybrid cultivar BRS Violeta ("BRS Rubea" × "IAC 1398-21") in 2006. This cultivar contains high levels of anthocyanins and is an alternative to produce highly colored and antioxidant-rich grape juice, as well as dehydrated products with potential use as dietary supplements (CAMARGO; MAIA.; NACHTIGAL, 2005). Rebello et al. (2013) found 3950 mg of anthocyanins/kg of grape BRS Violeta (expressed as malvidin 3.5-diglucoside equivalents).

Powdered fruit juices are good alternatives of convenient and healthy ingredients to formulated foods. Powdered juices rich in natural pigments are potential substitutes for synthetic food dyes, allowing their use with double functionality: promoting food coloring and providing health benefits through their bioactive properties (WROLSTAD; CULVER, 2012).

Spray drying is an alternative for producing powdered grape juice with high anthocyanin content, although anthocyanins are unstable and losses can occur during drying, reducing nutritional and functional attributes of the juice (TONON; BRABET; HUBINGER, 2010). In addition, spray drying of fruit juices is difficult due to their hygroscopic and thermoplastic behavior at high temperature and humidity, a consequence of their high content of sugars and organic acids with low molar mass (ADHIKARI et al., 2004). Spray dried powders with low molar mass compounds are usually in an amorphous, non-equilibrium state that is very sensitive to changes in temperature and water content, being susceptible to glass transition related changes, including stickiness, caking, and color changes (TELIS; MARTÍNEZ-NAVARRETE, 2010).

The use of drying aids to entrap an active agent in a biopolymer matrix, protecting it against unfavorable environmental conditions, is an important strategy to allow spray drying of foods susceptible to degradation or water plasticization. The encapsulating matrix protects sensitive ingredients during storage, preserves flavors, protects food against nutritional losses, and may add nutritive materials to food after processing, providing additional attractiveness for the products (PHISUT, 2012).

In general, maltodextrins and gum Arabic are drying adjuvants in spray drying of fruit juices (TELIS; MARTÍNEZ-NAVARRETE, 2012). Maltodextrins are inexpensive, have

widespread use in foods, possess low viscosity at high solid content, and good solubility. Gum Arabic, despite the suitable properties that have facilitated its extensive use as carrier agent, is an expensive ingredient with availability and costs subjected to fluctuation, which have motivated research for alternative encapsulation matrices (MADENE et al., 2006). Whey and soy proteins are widely available, natural ingredients with high nutritional value. They have good properties for encapsulation, such as emulsification, solubility, film forming, and water binding capacity (MOLINA ORTIZ et al., 2009; BASTOS et al., 2012).

The efficacy of maltodextrin as drying adjuvant is due to its rapid film forming property and low water diffusivity in these films. On the other hand, proteins form smooth and non-sticky films much earlier than maltodextrin, and powder recovery is higher when a small amount of protein is added to the spray dried solution (ADHIKARI et al., 2009). Maltodextrin/protein blends favor protection of bioactive compounds and present good drying properties (NESTERENKO, et al., 2013). Fang and Bhandari (2012) observed that a small amount of whey protein (1%) is required to dry fruit juices, while large amounts of maltodextrin (> 30%) are needed for the same purpose, pointing out that addition of large carrier amounts increases cost and may alter product flavor and taste.

Microencapsulation of anthocyanins using maltodextrins (FERRARI; GERMER; AGUIRRE, 2012; TONON.; BRABET; HUBINGER, 2010), whey proteins (FANG; BHANDARI, 2012) and soy proteins (ROBERT et al., 2010) has been investigated, however application of maltodextrin mixtures with whey or soy proteins as drying aids for anthocyanins has been barely reported. Based on these considerations, this study aimed to investigate the potential of maltodextrin blends with whey or soy proteins as carrier agents for encapsulation of grape juice anthocyanins by spray drying.

3.2 MATERIAL AND METHODS

3.2.1 Materials

Fresh grapes (cv. BRS Violeta) were provided by EMBRAPA Grape and Wine (Jales, Brazil). Maltodextrin DE-10 (Mor-Rex 1910, Corn Products, Brazil), whey protein concentrated (WPC) (WPC80, Alibra, Brazil) and soy protein isolate (SPI) (Supro 783, TovaniBenzaquen, Brazil) were the carrier agents.

3.2.2 Grape juice preparation

A batch of juice was processed from fresh grapes by steam extraction (60 min, 75-85 °C) according to the procedure described by Rizzon et al. (1998), sieved (270 mesh) to remove potassium bitartrate crystals, and stored at –18 ºC until use (Appendix A). The juice had total solid content of 15.18 ± 0.02 g/100 g (w/w), 14.0 ± 0.1 ºBrix, pH 3.84 ± 0.006, reducing sugar 123.25 ± 17.77 g/L, acidity (% tartaric acid) of 0.60 ± 0.004, ash 0.43 ± 0.004 g/100 g (w/w), and 1405.50 ± 3.74 mg/L total anthocyanins (FRANCIS, 1982).

3.2.3 Sample preparation

Blends of WPC/maltodextrin (WM) and SPI/maltodextrin (SM) used as carriers were added to juice at different ratios of carrier agent concentration to soluble solids of juice (*CAC*), and at different ratios of protein to total carrier agent (*R*). Experiments followed a 2^2 rotatable central composite design (Table 3.1).

Carriers were added to juice under agitation, until complete dissolution. The effects of *CAC* and *R* on yield, water content, solubility, anthocyanin retention, color, and encapsulation efficiency of powdered juice were evaluated by response surface methodology, applying analysis of variance (ANOVA) at 5% probability.

3.2.4 Spray drying

Spray drying was performed in a concurrent spray dryer (B-290, Büchi, Switzerland), with spray nozzle (orifice diameter = 0.7 mm), operating at: inlet air temperature = 140 ºC; feed flow rate = 2 mL/min; airflow rate = 500 L/h. The grape juice powders obtained were packed

into metallized plastic bags. The bags were then stored in a desiccator containing silica gel until evaluation. Drying yield was calculated as the ratio between total solids recovered and solids present in juice before drying.

3.2.5 Characterization of powdered juice

3.2.5.1 Water content

Powder water content was determined gravimetrically, drying samples in vacuum oven (70 ºC) until constant weight (AOAC, 1990).

3.2.5.2 Solubility

Powder samples (1 g) were added to 100 mL of distilled water, agitated for 5 min, and centrifuged (3000×g, 5 min). Aliquots (25 mL) of the supernatant were transferred to weighed Petri dishes and oven dried (105 ºC, 5 h). Solubility (%) was calculated as the weight difference (CANO-CHAUCA et al., 2005).

3.2.5.3 Anthocyanin retention

Powder samples (0.05 g) were extracted with 95% ethanol/1.5 N HCl (85:15, v:v), vortexed for 5 min and brought to 50 mL with the extracting solution. Samples were protected from light and refrigerated (4 ºC, 12 h) (FRANCIS, 1982). Absorbance was measured in UV-vis spectrophotometer (SP-220, Biospectro) at 520 nm, and total anthocyanin content was calculated using molar absorbance and molar mass corresponding to malvidin-3,5-glucoside. Anthocyanin retention was calculated as the ratio between total anthocyanin content (mg/100 g of dry matter) in powder and in juice before drying.

3.2.5.4 Encapsulation efficiency of anthocyanins (EE)

Powder samples (0.2 g) were washed with 10 mL of ethanol 99.5% to extract unencapsulated anthocyanins without capsule disruption (NORI et al., 2011). The mixture was stirred for one minute, filtered, and the supernatant was analyzed for anthocyanins as described in anthocyanin retention. Encapsulation efficiency was calculated as:

$$EE\ (\%) = \frac{W2 - W1}{W2} \quad (1)$$

in which W1 = anthocyanins in the supernatant and W2 = total anthocyanins in the powder.

3.2.5.5 Color

CIELab coordinates (L*, a*, b*) were measured using a Hunter Lab colorimeter (Color Flex EZ, USA), with D65 illuminant and 10º observation angle. Hue angle (h*), chroma (C*), and total color differences (ΔE*) between fresh grape juice and powdered juice after reconstitution in water were calculated by Equations (2) – (4) (TELIS.; MARTÍNEZ-NAVARRETE, 2010).

$$h^* = arctg\left(\frac{b^*}{a^*}\right) \quad (2)$$

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (3)$$

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (4)$$

For reconstitution, juice powder was mixed with distilled water keeping always the same proportion between solids coming from fresh juice and water. As fresh juice presented 14 °Brix, the mass of powder was calculated to give a ratio of 14 g of solids from juice to 86 g of water.

3.2.5.6 Scanning electron microscopy (SEM)

Powder samples were attached to double-sided adhesive tape mounted on SEM stubs, covered with gold layer (9 nm) under vacuum, and observed in scanning electronic microscope (FEI - Inspect S50) working at 5 kV, with 1000× and 5000× of magnification.

3.2.5.7 Particle size distribution

Particle size distribution was analyzed by laser light scattering (LA-900, Horiba Instruments, Inc., Japan). Sample amount was adjusted to obtain refractive reading unit.

3.3 RESULTS AND DISCUSSION

The drying yield, solubility, anthocyanin retention, and encapsulation efficiency of juice powders produced with different *CAC* and *R* (Table 3.1) presented different behaviors for blends WM and SM, and the mathematical models obtained for each response were tested for adequacy and fitness using ANOVA only considering the significant terms ($p < 0.05$).

Table 3.1 Treatments corresponding to the experimental design and respective results of process yield, solubility, anthocyanin retention, and encapsulation efficiency.

Treatments	***CAC****	***R* (%)****	**Yield (%)**	**Solubility (%)**	**Anthocyanin retention (%)**	***EE* (%)**
1WM	0.25 (-1)	10 (-1)	41.47	92.91 ± 4.53	80.01 ± 0.15	62.81 ± 1.54
2WM	0.25 (-1)	30 (+1)	57.22	93.10 ± 1.41	87.80 ± 0.71	69.48 ± 0.80
3WM	0.75 (+1)	10 (-1)	75.48	96.96 ± 0.34	83.61 ± 1.74	78.77 ± 2.86
4WM	0.75 (+1)	30 (+1)	74.59	91.91 ± 0.93	91.31 ± 3.16	80.20 ± 1.15
5WM	0.15(-1.41)	20 (0)	3.15	96.13 ± 3.82	77.89 ± 0.94	84.10 ± 0.66
6WM	0.85(+1.41)	20 (0)	74.75	93.96 ± 0.88	94.04 ± 0.19	81.14 ± 0.97
7WM	0.5 (0)	5.86 (-1.41)	75.58	96.51 ± 0.11	86.67 ± 0.94	68.19 ± 1.14
8WM	0.5 (0)	34.14(+1.41)	74.25	92.72 ± 1.10	83.66 ± 0.82	72.25 ± 0.82
9WM	0.5 (0)	20 (0)	74.27	93.33 ± 1.09	88.86 ± 3.54	72.27 ± 1.25
10WM	0.5 (0)	20 (0)	72.58	94.68 ± 2.04	85.54 ± 0.21	78.47 ± 0.71
1SM	0.75 (-1)	10 (-1)	49.31	94.25 ± 0.58	60.87 ± 0.52	97.12 ± 1.32
2SM	0.75 (-1)	30 (+1)	48.93	91.26 ± 1.99	58.49 ± 1.65	97.10 ± 0.36
3SM	1.25 (+1)	10 (-1)	51.53	95.03 ± 0.82	63.96 ± 3.71	98.86 ± 0.20
4SM	1.25 (+1)	30 (+1)	51.74	88.69 ± 1.74	63.82 ± 7.25	99.11 ± 0.17
5SM	0.65 (-1.41)	20 (0)	49.41	91.21 ± 0.77	62.42 ± 3.83	97.41 ± 0.33
6SM	1.35 (+1.41)	20 (0)	47.48	88.92 ± 1.28	67.46 ± 4.15	99.17 ± 0.11
7SM	1 (0)	5.86 (-1.41)	55.40	94.93 ± 1.62	71.61 ± 0.94	98.48 ± 0.12
8SM	1 (0)	34.14(+1.41)	56.12	87.43 ± 2.02	63.33 ± 3.30	97.76 ± 0.03
9SM	1 (0)	20 (0)	54.25	91.47 ± 0.45	64.12 ± 3.37	97.43 ± 0.12
10SM	1 (0)	20 (0)	53.05	91.56 ± 2.86	60.76 ± 1.53	98.19 ± 0.01

**CAC*: g of carrier agent/g of soluble solids of the juice;
***R* (%): g dry protein/100 g dry carrier agent.

The results for models describing yield (for WM and SM treatments) and encapsulation efficiency (for SM treatments) indicate that these were adequate, showing significant regression, low residual values, no lack of fit, and satisfactory coefficients of determination (Table 3.2).

Table 3.2 Analysis of variance (ANOVA) of the mathematical models for yield (for WM and SM treatments) and encapsulation efficiency (for SM treatments).

Source of Variation	**Sum of squares**	**Degrees of freedom**	**Mean square**	**F calculated**
		Yield WM		
Regression	4494.09	1	4494.09	70.17
Residue	512.35	8	64.04	
Total SS	5006.44	9		
		Yield SM		
Regression	61.14	1	61.14	28.82
Residue	16.97	8	2.12	
Total SS	78.11	9		
		***EE* SM**		
Regression	4.87	1	4.87	38.21
Residue	1.02	8	0.13	
Total SS	5.89	9		

Value F critical = 5.32

Despite all the responses evaluated had shown higher F-calculated values compared to critical values, the coefficients of determination for anthocyanin retention (WM treatments) and solubility (WM and SM treatments) were not satisfactory, hindering generation of predictive models and response surfaces.

3.3.1 Yield

Independently of the protein used, the carrier agent concentration (CAC) presented significant effect ($p < 0.05$) on drying yield (Tables 3.1 and 3.2), whereas the ratio of protein/total carrier agent (R) did not affect yield significantly.

In WM treatments, values of CAC in the range of 0.5 - 0.85 g of carrier agent/g of soluble solids of juice resulted in the highest powder recovery, around 75% (Figure 3.1). The proposed model to represent yield for blends WM (Equation 5) showed R^2 of 0.89.

According to Fang and Bhandari (2012), 30% of maltodextrin is a minimum amount to efficient spray drying (criteria of 50% powder recovery) of fruit juices. In the present study, using CAC = 0.25 (25% of carrier in relation to juice soluble solids) resulted in yield of 57% (Table 3.1), demonstrating that addition of a small amount of WPC to maltodextrin improved spray drying of grape juice. On the other hand, the lowest level of carrier agent concentration (CAC = 0.15) resulted in very low yield (3.15%), which is not acceptable. Bustos-Garza, Yáñez-Fernández and Barragán-Huerta (2013) used whey protein to microencapsulate taxanthin oleoresin and obtained yield of 63%, which was lower than that found in this study for WPC/maltodextrin blends.

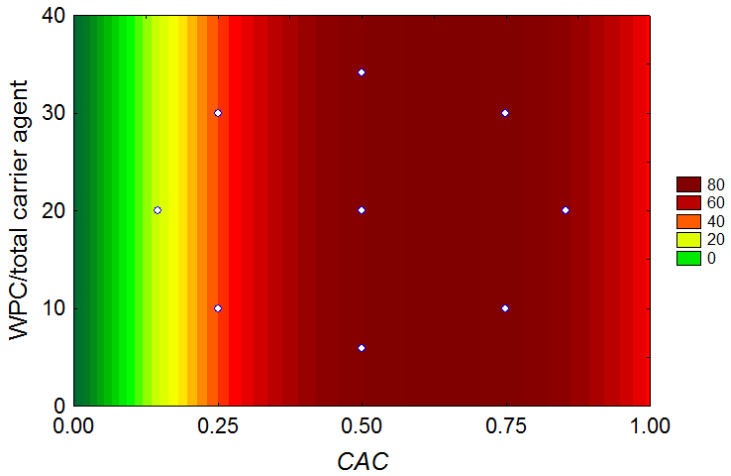

Figure 3.1 Contour plot for spray drying yield of grape juice powder using different values of carrier agent concentration (CAC) and WPC/total carrier agent ratio.

$$\text{Yield } (\%)_{\text{WM}} = 75.78 + 19.08CAC - 16.80CAC^2 \quad (5)$$

Powder losses during drying may have resulted from a combination of powder deposition on dryer chamber, which commonly occurs in sugar-rich foods due to stickiness, as well as from pumping out of fine particles through the dryer filter (FANG; WANG; BHANDARI, 2013).

Using blends SM resulted in yields varying from 47.5 to 56.1%. Using CAC = 1, independently of the SPI/total carrier agent ratio, resulted in the highest yield. When using higher or lower values of CAC, powder recovery decreased. These results indicate that, for the

drying conditions applied in this study, the optimal CAC for blends SM was 1 g of carrier agent/g of soluble solids of grape juice. Nesterenko et al. (2012), using soy protein in the microencapsulation of α-tocopherol obtained yield of 83%, which was greater than the maximum yield found in this study. The obtained model (Equation 6) was also suitable for prediction of yield using blends SM (R^2 = 0.78).

$$\text{Yield } (\%)_{\text{SM}} = 54.37 - 3.30CAC^2 \quad (6)$$

3.3.2 Water content

Powders obtained with blends WM showed water contents from 0.79 to 5.26% (dry basis), whereas for blends SM moisture varied from 0.60 to 2.86% (dry basis). For both carrier blends, water content of juice powder was not affected by CAC or R, and most of formulations resulted in water contents lower than 5%, a characteristic limit to water content proposed for spray dried products (FANG; WANG; BHANDARI, 2013).

3.3.3 Solubility

Grape juice dried with protein/maltodextrin blends presented high solubility. In WM treatments the powder solubility varied from 92 to 97%, while for blend SM it was slightly lower, ranging from 87 to 95% (Table 3.1). Using maltodextrin as carrier, Cano-Chauca et al. (2005) also noted high solubility of powdered mango juice (> 90%).

Powder solubility was not influenced by CAC, which is in agreement with Kha, Nguyen and Roach (2010) that studied production of gac powder using maltodextrin. According to Phisut (2012), increasing maltodextrin concentration did not reduced powder solubility. This effect may be explained by the high water solubility of maltodextrin. Yousefi, Emam-Djomeh and Mousavi (2011) reported that solubility is strongly affected by carrier type and, in some cases, by carrier concentration.

Powder solubility was negatively influenced by increasing R, although the obtained models were not predictive. Nevertheless, reduction in solubility was small and all systems showed good characteristics of rehydration. Frequently, the functional properties of proteins are limited by their relatively poor solubility, particularly close to the isoelectric point (pI). Whey

protein has pI at pH near 5.0 (DUONGTHINGOC et al., 2013) and soy protein near 4.5 (WANG; ZHANG; MUJUMDAR, 2010).

3.3.4 Anthocyanin retention

Anthocyanin retention was greatly affected by the protein used with maltodextrin (Table 3.1). In WM treatments, the retention was higher, varying from 77.9 to 94%, whereas powders produced with blends SM retained between 58.5 and 71.6% of anthocyanins present in the juice before drying. Nayak and Rastogi (2010) reported similar values to anthocyanin retention in *Garcinia indica* Choisy fruits using maltodextrin as drying aid (65.1 - 79.8%).

Despite mathematical models obtained for anthocyanin retention could not be considered predictive due to low coefficient of determination, in WM treatments there was a trend that larger values of CAC resulted in greater anthocyanin retention. On the other hand, in blends SM, CAC did not affect the compound retention. The ratio R did not influence anthocyanin retention either for WPC or for SPI.

In SM treatments, even using higher values of CAC in relation to blends WM, there was lower anthocyanin retention. The solid level is an important parameter influencing core retention in encapsulated systems (NESTERENKO, et al., 2013). This influence could be explained by reduction of core molecule mobility when entrapped in the wall material, in addition to lower time needed to form the protective shell, both induced by higher solid content. However, over a critical solid concentration an abrupt increase in viscosity may occur, leading to significant fall in encapsulation efficiency.

3.3.5 Encapsulation efficiency

In general, both carrier blends resulted in adequate encapsulation of anthocyanins, although SM blends resulted in higher EE than WM blends, indicating better encapsulating ability of soy protein (Table 3.1). In WM treatments, CAC and R did not significantly influence EE. Powders presented $62.8\% \leq EE \leq 84.1\%$, demonstrating the fair ability of these carrier formulations to encapsulate anthocyanins of grape juice. Lim et al. (2012) reported EE of 90.12% for pitaya seed oil encapsulated with whey protein/maltodextrin. Millqvist-Fureby, Elofsson and Bergenståhl (2001), using only WPC to stabilize emulsions of canola oil, found

that EE decreased with increasing protein denaturation and observed that poor encapsulation may be due to less flexible protein structure after denaturation.

In SM treatments, EE was higher, varying from 97.10 to 99.17%, increasing significantly ($p < 0.05$) with increasing CAC (Figure 3.2). The mathematical model (Equation 7) obtained for EE showed $R^2 = 0.83$, being predictive (Table 3.2). The ratio R in systems WM and SM did not affect EE of anthocyanins in powdered juice.

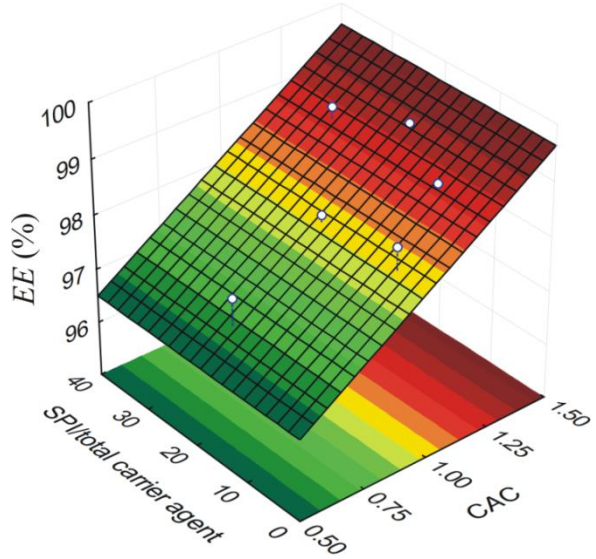

Figure 3.2 Influence of carrier agent concentration (CAC) and SPI/total carrier agent ratio in encapsulation efficiency (EE) of anthocyanins in grape juice powder.

$$\text{EE } (\%)_{\text{SM}} = 98.06 + 0.78\text{CAC} \quad (7)$$

Higher amounts of carrier result in faster polymer precipitation on the dispersed phase surface, preventing core diffusion across phase boundary. Furthermore, increasing viscosity of the solution delays core diffusion within polymer droplets (JYOTHI et al., 2010). The excellent encapsulation of anthocyanins with higher concentrations of SM blend is consistent with the results of SEM, which indicated that increasing CAC resulted in formation of more spherical particles.

Using soy protein, Robert et al. (2010) observed that EE reached higher values for anthocyanins than polyphenol, showing the ability of this carrier to bind anthocyanins, which could be related to the flavylium cation of anthocyanins. Deng et al. (2014), using only soy

protein or soy protein/starch blend, also found that EE varied considerably with composition of encapsulating materials. Robert et al.(2010) reported greater EE of anthocyanins using soy protein (35.8–100%) and maltodextrin (89.4–100%) as wall materials.

3.3.6 Color

Color characteristics of powdered juice were affected by CAC and by R. The mathematical models obtained to L*, C*, and h* for WM and SM treatments were predictive according to ANOVA (Table 3.3). Surface plots based on generated models (Equations 8 – 13) are shown in Figure 3.3.

Table 3.3 Analysis of variance (ANOVA) of the mathematical models for *L**, *C** and *h** for WM and SM treatments.

Source of Variation	**Sum of squares**		**Degrees of freedom**		**Mean square**		**F calculated**	
	WM	**SM**	**WM**	**SM**	**WM**	**SM**	**WM**	**SM**
				L*				
Regression	643.16	303.53	1		643.16	303.53	356.53	71.38
Residue	14.43	34.02	8		1.80	4.25		
Total SS	657.59	337.55	9					
				C*				
Regression	46.87	98.39	1		46.87	98.39	34.08	111.37
Residue	11.00	7.07	8		1.37	0.88		
Total SS	57.87	105.46	9					
				h*				
Regression	472.77	334.14	1		472.77	334.14	287.91	259.09
Residue	13.14	10.32	8		1.64	1.29		
Total SS	485.91	344.46	9					

Value F critical = 5.32

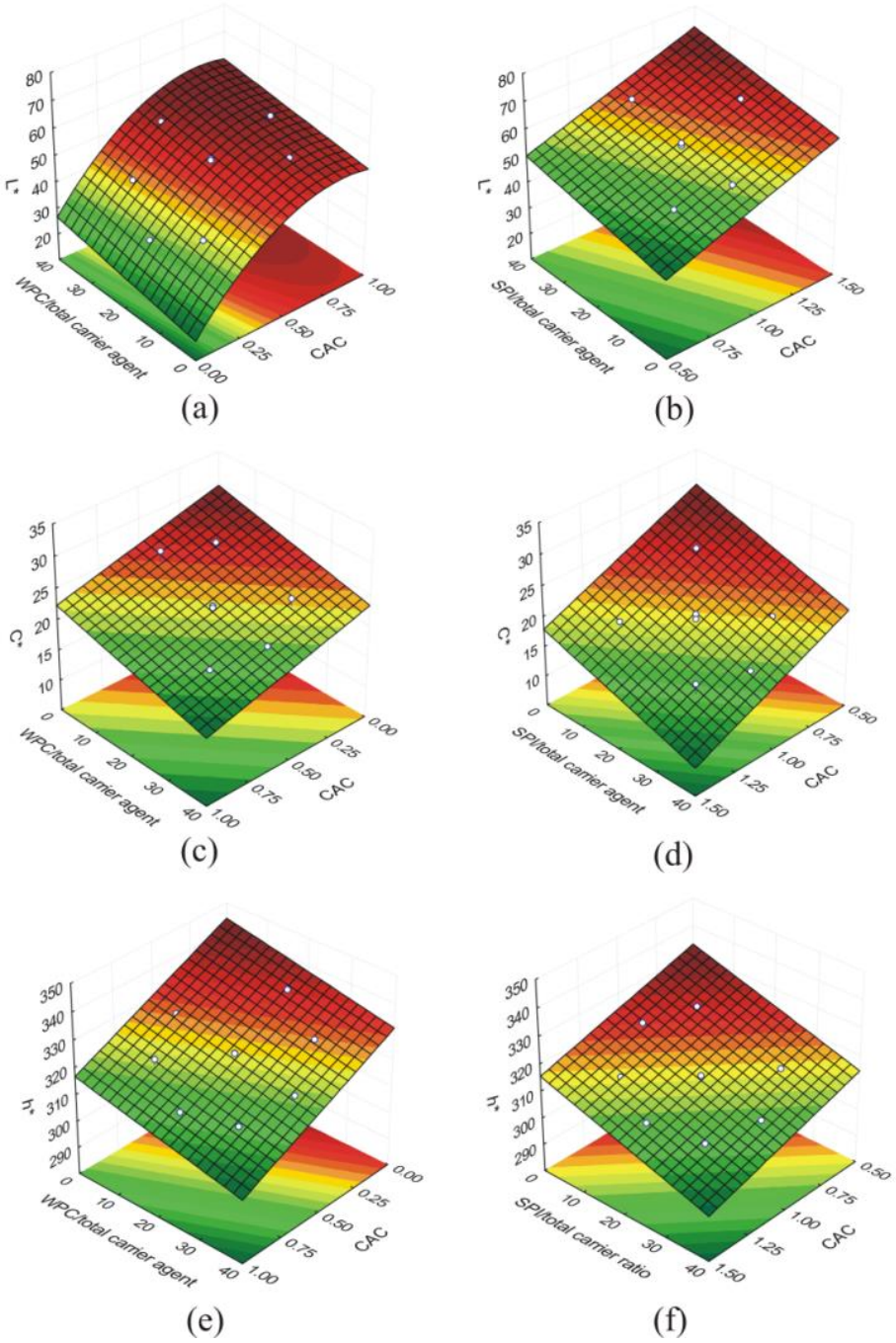

Figure 3.3 Influence of carrier agent concentration (CAC) and protein/total carrier agent ratio in the color parameters of grape juice powder: lightness for (a) WM and (b) SM; chroma for (c) WM and (d) SM; hue for (e) WM and (f) SM.

Lightness (L*) was significantly affected ($p < 0.05$) by both CAC and R. The response models Equation (8) with $R^2 = 0.98$, and Equation (9), with $R^2 = 0.90$, to first and second model presented below, indicate that increasing CAC and R resulted in brighter powders (Figure 3.3 A

and B). The increase in lightness is due to the dilution effect caused by carrier addition to juice, resulting in loss of color.

Similar results were found to powdered juice of açai (TONON; BRABET; HUBINGER, 2009), gac fruit (KHA; NGUYEN; ROACH, 2010) and blackberry (FERRARI; GERMER; AGUIRRE, 2012), microencapsulated with maltodextrin. The juice dried with SM presented higher values of L* (45.38 to 64.34) than WM (31.57 to 57.77), probably because of the largest amount of carrier.

$$L^*_{\mathrm{WM}} = 50.36 + 8.30CAC - 2.96CAC^2 + 2.29R \quad (8)$$

$$L^*_{\mathrm{SM}} = 55.94 + 5.61CAC + 2.55R \quad (9)$$

$$C^*_{\mathrm{WM}} = 22.76 - 1.83CAC - 1.58R \quad (10)$$

$$C^*_{\mathrm{SM}} = 19.46 - 2.86CAC - 2.03R \quad (11)$$

$$h^*_{\mathrm{WM}} = 323.96 - 6.95CAC - 3.28R \quad (12)$$

$$h^*_{\mathrm{SM}} = 314.90 - 4.43CAC - 4.70R \quad (13)$$

Values of chroma (in the range of 19.23 – 26.73 for WM, and 13.72 – 24.77 for SM) and hue (varying from 313.1 – 335.8 for WM, and 305.7 – 324.3 for SM) of juice powder were located in the fourth quadrant of CIELab color chart, corresponding to purple color. Increasing values of CAC and R decreased C* (Figure 3.3 C and D), indicating grayish chromaticity, whereas higher chroma values at low CAC and R demonstrate more saturated or vivid color. Similar findings were reported by Kha, Nguyen and Roach (2010). Increasing CAC and R also leaded to decrease in h* (Figure 3.3 E and F), which means that color became more blue-purple than red-purple. Comparison of h* between powdered and fresh grape juice (h* = 355.8) shows that carrier addition resulted in lower hue angles and that this effect was more accentuated for blends SM than WM.

3.3.6.1 Comparison between color of powdered and reconstituted grape juice

Considering that most applications of juice powder involve solubilization in water, it is relevant to evaluate the effects of CAC and R on color of reconstituted grape juice. Four treatments of juice powders, selected with basis on best global results for yield, anthocyanin retention and EE (Table 3.1), were reconstituted in water and had their color compared with fresh grape juice (Table 3.4).

The $\Delta E^* < 1.5$ is considered small, indicating that the powdered grape juice is almost identical to the original by visual observation. For $1.5 \leq \Delta E^* \leq 5$, the color difference can already be distinguished, and this difference becomes evident for $\Delta E^* > 5$ (OBÓN et al., 2009). The reconstituted juice of 4WM and 6WM treatments resulted in $\Delta E^* > 5$ (Table 3.4), indicating visual differences in color compared to fresh juice. Nevertheless, 3SM and 7SM treatments resulted in $\Delta E^* < 5$, demonstrating that SM blends had lower influence on juice coloration, which may be attributed either to lower interference of SPI than WPC on reconstituted juice color or to better protection of anthocyanins by SPI than WPC during drying. Yousefi, Emam-Djomeh and Mousavi (2011) stated that the coloring agent of pomegranate juice might have being absorbed into the carrier agent and being protected from severe damage during spray drying.

Table 3.4 Color comparison between fresh and reconstituted grape juice.

Treatments	***L****	***a****	***b****	**Δ*E****
	Fresh grape juice			
	1.66 ± 0.10	3.34 ± 0.04	-0.25 ± 0.06	
	Reconstituted grape juice			
4WM	13.32 ± 0.03	8.56 ± 0.10	-8.36 ± 0.04	15.14 ± 0.07
6WM	7.61 ± 0.03	8.33 ± 0.02	-6.16 ± 0.03	9.76 ± 0.03
3SM	1.97 ± 0.02	2.52 ± 0.03	-2.13 ± 0.04	2.08 ± 0.03
7SM	1.44 ± 0.09	2.24 ± 0.06	-1.35 ± 0.03	1.57 ± 0.02

3.3.7 Microstructure of powder particles

Powders produced using WM blend showed much agglomeration, probably caused by stickiness. Particles were not spherical and some of them presented wrinkled surface, although

there was no evidence of indentation or apparent pores. 6WM treatment (Figure 3.4 A), corresponding to the greatest CAC (0.85) and intermediate R (20%), resulted in the most suitable morphology when compared to other WM blends.

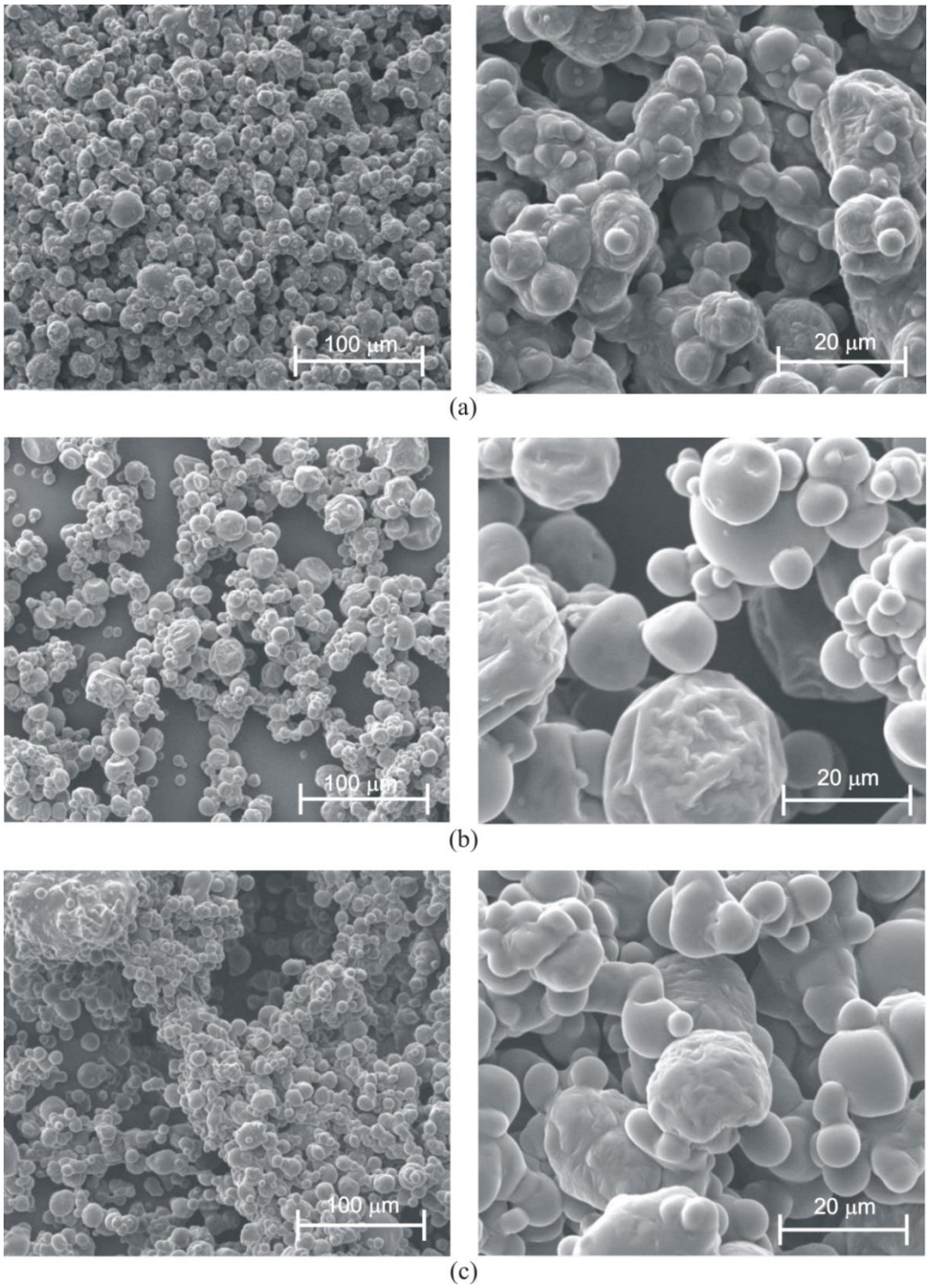

Figure 3.4 Scanning electron microscopic images with magnification 1000× (left) and 5000× (right) of powdered grape juice microencapsulated with: (a) 6WM; (b) 3SM; and (c) 8SM.

Lim et al. (2012) used whey protein/maltodextrin blends as wall material and obtained much agglomeration. Using only whey protein, other authors found no well-defined, agglomerated microcapsules (BUSTOS-GARZA; YÁÑEZ-FERNÁNDEZ; BARRAGÁN-HUERTA, 2013), or particles with deep dents and surface wrinkles (BARANAUSKIENĖ et al., 2006). Regarding SM blends, SEM micrographs showed particles not completely spherical (Figure 3.4 B), but with higher sphericity than WM blends. There was some agglomeration (Figure 3.4 C) but it was possible to observe individual particles, in addition to wrinkled surfaces.

Carrier concentration was decisive when using blends SM. 1SM, 2SM, and 5SM treatments, which had lower CAC, showed poor ability of particle formation, whereas 3SM treatment (Figure 3.4 B), 4SM, and 6SM, with higher CAC, resulted in the most suitable particles. The presence of protein contributed considerably to particle agglomeration: 8SM treatment, with the highest R (34.14%), resulted in higher agglomeration (Figure 3.4 C). Using soy protein, Deng et al. (2014) obtained particles with indented surface and wrinkled surface morphology. To soy protein with lactose, Tang and Li (2013) found high agglomeration and presence of dents.

Ferrari, Germer and Aguirre (2012) and Lim et al. (2012) found that particles produced using only maltodextrin as drying aid showed smooth surface. Thus, it is possible to hypothesize that the irregularities observed in particle surface are due to presence of proteins in carrier formulations. Gharsallaoui et al. (2010) noted that in protein/carbohydrate blends, proteins serve as emulsifying and film-forming agents, while polysaccharides act as matrix forming material.

3.3.8 Particle size

The powder formulations selected for reconstitution and color evaluation (4WM, 6WM, 3SM, and 7SM), were also analyzed regarding particle size distributions. 4WM treatment (CAC = 0.75; R = 30%) presented unimodal size distribution (Figure 3.5 A), with diameters between 2 and 30 µm, and average diameter of 11.57 µm. On the other hand, 6WM treatment (CAC = 0.85; R = 20%) presented bimodal size distribution: the first peak included larger volume of particles (> 8%), with diameters between 2 and 30 µm, similar to 4WM treatment; the second peak enclosed lower volume of particles (< 2%) with size between 50 and 400 µm. The presence of

larger particles can be attributed to agglomeration, which was previously observed by SEM. Particles 6WM showed average diameter of 34 µm.

Using maltodextrin, Tonon, Brabet and Hubinger (2010) obtained average diameter of 10.08 µm to açai powder, whereas diameter of blackberry powder varied between 12.52 and 34.18 µm (FERRARI; GERMER; AGUIRRE, 2012). Bastos *et al*. (2012), using whey protein isolated obtained smaller particles (6.17 µm). Carneiro et al. (2013), studying maltodextrin/WPC to spray drying flaxseed oil, observed wider size distribution (0.02 to 160.0 µm).

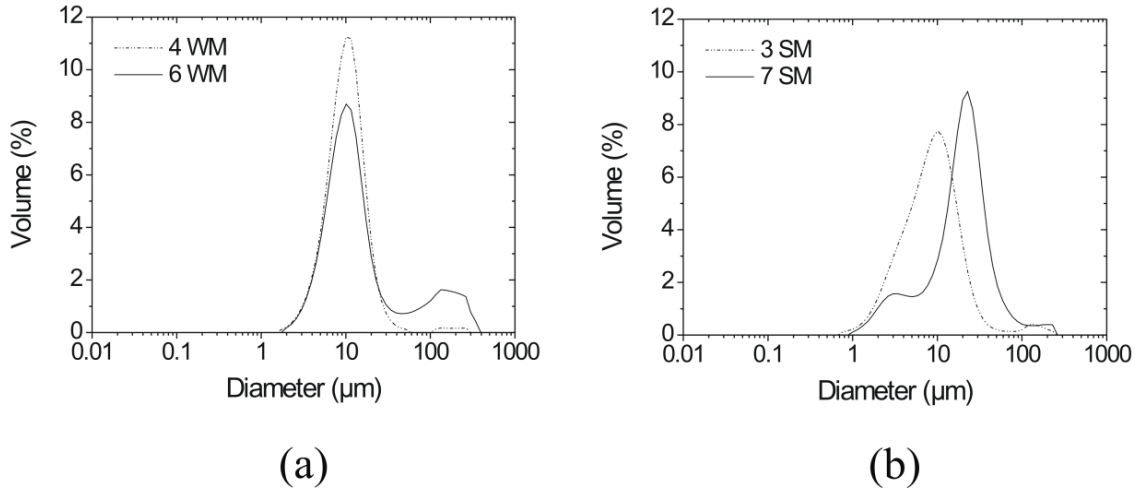

Figure 3.5 Particle size distributions of powdered grape juice: (a) WPC/maltodextrin blends; (b) SPI/maltodextrin blends.

The 3SM particles (CAC = 1.25; R = 10%) presented unimodal size distribution, with diameters between 1 - 40 µm and average diameter of 12.49 µm (Figure 3.5 B). Particles 7SM (CAC = 1; R = 5.86%) presented bimodal size distribution: the first peak, partially superimposed to the second one, included small particle volume (< 2%) and small diameters, between 0.9 and 5 µm; the second peak presented greater volume (> 9%) and large size range, from 5 to 250 µm.

Molina Ortiz et al. (2009), encapsulating casein hydrolysate by spray drying with SPI also found bimodal size distribution, but smaller particle size (11.32 µm).

3.4 CONCLUSIONS

The soy or whey protein used with maltodextrin to formulate carrier blends had different effects on properties of spray dried grape juice. Higher carrier concentrations resulted in higher yield and in higher particle sphericity, independently of the protein type. In addition, increasing carrier concentration, in general, leaded to better powder properties, such as increased anthocyanin retention with WM blends, and higher encapsulation efficiency with SM blends. Particles produced with WM presented some agglomeration, whereas SM resulted in particles that were more spherical, which could explain the higher encapsulation efficiency observed in these powders. Increasing carrier agent concentration and increasing ratio of protein/carrier agent resulted in brighter powders. Nevertheless, after reconstitution in water, the rehydrated juice presented color parameters similar to those of fresh juice, with better results for powders prepared with SM than WM. In conclusion, whey and soy proteins blended with maltodextrin helped spray drying of grape juice, resulting in good retention and good encapsulating efficiency of anthocyanins. Spray dried grape juice powders are a promising food additive for incorporating anthocyanins of BRS Violeta grape into functional foods as well as developing innovative and healthy food products.

ACKNOWLEDGMENTS

To São Paulo Research Foundation (FAPESP), grants 2012/09074-4 and 2010/09614-3 for the financial support, and Brazilian Agricultural Research Company - Grape and Wine (Jales, Brazil).

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-CAPÍTULO 4-

WATER SORPTION, GLASS TRANSITION TEMPERATURE AND STABILITY OF ANTHOCYANINS IN GRAPE JUICE MICROENCAPSULATED IN BLENDS OF PROTEIN AND MALTODEXTRIN

4 WATER SORPTION, GLASS TRANSITION TEMPERATURE AND STABILITY OF ANTHOCYANINS IN GRAPE JUICE MICROENCAPSULATED IN BLENDS OF PROTEIN AND MALTODEXTRIN

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ABSTRACT

Powdered grape juice from grape cultivar BRS Violeta was produced by spray drying and the resulting powders were analyzed aiming to correlate the encapsulation matrix formulation with the physical-chemical stability of the material during storage. Sorption isotherms by the gravimetric method, glass transition temperature (Tg) through differential scanning calorimetry, stickiness by compression tests, and light stability of anthocyanins throughout 90 days were evaluated. Blends of soy protein with maltodextrin (SM) and whey protein with maltodextrin (WM) were used as carrier agents. The carrier agent concentration (CAC, g of carrier agent/g of soluble solids of the juice) varied between 0.75 and 1.25, whereas the ratio of protein in the carrier agent (g dry protein/100 g total carrier agent) varied from 5.86% to 30%. The GAB model was well fitted to data of water vapor adsorption and the curves were classified as type II. The glass transition temperature decreased with the increase of water content, as well as the compression force showed a strong dependence on the water activity. The encapsulation matrix formulated with soy protein and maltodextrin, containing 10% protein and applied at CAC of 1.25 g of carrier agent/g of soluble solids of the juice (1SM) resulted in the less hygroscopic powder, with higher compression force, indicating less stickiness, and showed the best anthocyanin protection, consequently presenting the lower total color differences along storage.

Keywords: spray drying, carrier agents, water content, stickiness, light stability.

4.1 INTRODUCTION

Brazilian viticulture has been going through great market and economic changes, with production of 1.47 million tons of grapes (IBGE, 2015) and commercialization of 136.09 million liters of grape juice in 2015 (UVIBRA, 2015). As an alternative to increase quality and competitiveness of wine and grape juice produced in Brazil, the Brazilian Agricultural Research Corporation launched, in 2006, the hybrid cultivar BRS Violeta from the crossing between "BRS Rubea × IAC 1398-21". This cultivar has high sugar content and color intensity and is a good alternative for blending of wines or juices, in order to obtain final products with improved quality (CAMARGO; MAIA; RITSCHEL, 2005). Compositional studies demonstrated that juices and wines produced with BRS Violeta grape are antioxidant-rich and an important source of phenolic compounds (LAGO-VANZELA et al., 2013; REBELLO et al., 2013).

Grape juice is a major source of phenolic compounds, which have many health benefits, such as high antioxidant activity, as well as anti-inflammatory, antibacterial, and antiviral functions (FANG; BHANDARI, 2010). Furthermore, they can provide favorable effects in protection against the development of chronic diseases, including cancer, cardiovascular, and neurodegenerative pathologies (DEL RIO et al., 2013). Among the main phenolic compounds in grape juice are the anthocyanins. The use of these pigments as dyes is very attractive to the food industry because they are not only natural alternatives to synthetic dyes, but also beneficial to health. However, anthocyanins are very unstable during processing and storage of products, and factors such as light, temperature, pH, porosity, presence of organic acids and exposure to oxygen cause their degradation (SKREDE; WROLSTAD; DURST, 2000; SAGAR; SURESH KUMAR, 2010). Microencapsulation is an alternative to improve the conservation and to produce powdered grape juice with high anthocyanin content.

Spray drying and storage of powdered fruit juices present technical difficulties due to their high content of sugars and organic acids with low molar mass. The presence of these compounds makes the product very sensitive to changes in temperature and moisture (TELIS; MARTÍNEZ-NAVARRETE, 2010). Water sorption isotherms are useful for predicting the interactions of water and product components. They provide information for assessing drying, packaging, and storage of food. The sorption properties can be affected by structural transformations and phase transitions, both of which are time-dependent phenomena. Typical structural alterations occurring in amorphous foods stored at temperatures above glass transition

temperature (Tg) include agglomeration, stickiness, collapse and crystallization (RIGHETTO; NETTO, 2005). Knowledge of the sorption properties is highly important for predicting the physical state of a food.

The use of carrier agents is an important strategy to allow spray drying of food materials which are susceptible to degradation or water plasticization. These carrier agents may act as encapsulant, protecting the active material entrapped in the dried matrix (RÉ, 1998; PHISUT, 2012). The choice of polymers used as carrier agents is very important to microparticles' stability and the degree of protection of the active core. Maltodextrins are inexpensive and possess properties such as low viscosity at high solid content and good solubility; in addition, they have low hygroscopicity (MADENE et al., 2006). The efficacy of maltodextrin as drying adjuvant is due to its rapid film or shell forming property and the relatively low water diffusivity in these films (ADHIKARI et al., 2009). Whey and soy proteins are widely available, with high nutritional value and good properties for encapsulation, such as emulsification, solubility, film-forming and water binding capacity (FENNEMA, 2000). The mixture of maltodextrin and protein favors the protection of bioactive compounds, presents good drying properties (NESTERENKO et al., 2013), and combines specific properties of each polymer in microencapsulation.

Therefore, the aim of this work was to investigate the effect of maltodextrin blended with proteins as carrier agents on water sorption, stickiness and glass transition temperature of spray dried grape juice, as well as evaluating the storage stability of microencapsulated anthocyanins in the presence of light.

4.2 MATERIAL AND METHODS

4.2.1 Materials

Fresh grapes (*Vitis labrusca*, cv. BRS Violeta) were provided by EMBRAPA Grape and Wine (Jales, Brazil). Whey protein concentrated (WPC) with 80% protein content (WPC80, Alibra, Brazil), soy protein isolate (SPI) with 92.8% protein content (Supro 783, Tovani Benzaquen, Brazil) and maltodextrin DE-10 MOR-REX 1910 (Corn Products, Brazil) were used as carrier agents.

4.2.2 Grape juice preparation

A batch of juice was processed from fresh grapes by steam extraction (60 min, 75-85 °C) according to the procedure described by Rizzon et al. (1998) (Appendix A), sieved (270 mesh) to remove potassium bitartrate crystals, and stored at –18 ºC until use. The juice had total solid content of 15.18 ± 0.02 g/100 g (w/w), 14.0 ± 0.1 ºBrix, pH 3.84 ± 0.006, reducing sugar 123.25 ± 17.77 g/L, acidity (% tartaric acid) of 0.60 ± 0.004, ash 0.43 ± 0.004 g/100 g (w/w), and 1405.50 ± 3.74 mg/L total anthocyanins.

4.2.3 Sample preparation

Blends of soy protein isolate/maltodextrin (SM) and whey protein concentrated/maltodextrin (WM) were added to the grape juice under magnetic agitation, until complete dissolution. The juices to be dried were formulated with different carrier agent concentration (CAC, g of carrier agent/g of soluble solids of the juice) and ratio of protein in the carrier agent (R, g dry protein/100 g total carrier agent), which are presented in Table 4.1.

These formulations were selected on a previous study (MOSER; SOUZA; TELIS, 2016) in which carrier agent concentrations and proportions of protein in the microencapsulated grape juice were evaluated. In that study, it was observed that higher carrier concentration resulted in higher drying yield and better powder properties, as well as in greater anthocyanin retention. Moreover, higher carrier concentration improved encapsulation efficiency and resulted in the most suitable morphology.

Table 4.1 Carrier agent concentration (CAC) and ratio of protein in the total carrier agent (R) used in the spray drying of the grape juices.

Treatments	**CAC** (g of carrier agent/g of soluble solids of the juice)	**R (%)**
1SM	1.25	10.00
2SM	1.00	5.86
1WM	0.75	30.00
2WM	0.85	20.00

4.2.4 Spray drying

The microencapsulation of anthocyanins from grape juice was performed in a mini spray dryer (model B-290, Büchi, Switzerland), which operates concurrently and has a spray nozzle with an orifice of 0.7 mm diameter. Based on preliminary trials, the processing conditions used were inlet air temperature of 140 ºC, feed flow rate of 2 mL/min, and air flow rate of 500 L/h.

4.2.5 Sorption isotherms

Equilibrium moisture contents were determined by the static gravimetric method (JOWITT, 1987) at temperatures 25 ºC. Saturated salt solutions of LiCl, CH_3COOK, $MgCl_2$, K_2CO_3, $Mg(NO_3)_2$, $NaNO_2$ and NaCl were prepared to provide relative humidity in the range of 11–76% (ROCKLAND, 1960; GREENSPAN, 1976). Each salt solution was transferred to a jar in a sufficient quantity to occupy a space of about 1.5 cm depth. About 1 g of grape juice powder was placed into small plastic containers, which in turn were placed on a support inside each jar in order to avoid contact with the salt solution. The jars were hermetically closed and placed in a temperature-controlled chamber. The required time for equilibration was 3–4 weeks, based on the change in samples weight, which did not exceed 0.1%. The equilibrium moisture content was determined in a vacuum oven, at 70 ºC until constant weight (AOAC, 1990).

The theoretical Brunauer–Emmett–Teller (BET) and Guggenheim–Anderson–de Boer (GAB) models were fitted to experimental water sorption data and are shown in equations 1 and 2, respectively.

$$w_e = \frac{w_m C_{BET} a_w}{\left[(1-a_w)(1-a_w+C_{BET}a_w)\right]} \quad (1)$$

$$w_e = \frac{w_m C_{GAB} K_{GAB} a_w}{\left[(1-K_{GAB}a_w)(1-K_{GAB}a_w+C_{GAB}K_{GAB}a_w)\right]} \quad (2)$$

in which w_e is the equilibrium water content (g water/g dry matter), w_m is the monolayer moisture content, a_w is the water activity and C_{BET}, C_{GAB}, K_{GAB} are constants of the models.

The goodness of fit of BET and GAB models was evaluated by the determination coefficient (R^2) and mean relative error (E), calculated as

$$E = \frac{1}{N}\sum_{i=1}^{N}\frac{\left|V_e - V_p\right|}{V_e} \quad (3)$$

in which N is the population of experimental data, V_e is the experimental value and V_p is the predict value.

4.2.6 Glass transition temperature

The glass transition temperature (Tg) was measured in a differential scanning calorimeter (DSC8000, Perkin Elmer, Shelton, CT) equipped with a intracooler (Intracooler 3, Perkin Elmer, Netherlands). About 5 mg of grape juice powders were placed directly in aluminum pans and conditioned in different water activity (aw at 0.11, 0.23, 0.33, 0.43, 0.52, 0.64 and 0.75) in desiccators at 25 ºC. After equilibrium was reached, samples were rapidly sealed and analyzed. The samples were heated at 35 ºC/min, with temperature ranging from -80 at 120 ºC, using an empty pan as reference. Nitrogen at 20 mL/min was used as the purge gas. The midpoint of the glass transition was considered as the characteristic Tg. All analyzes were performed in triplicates.

4.2.7 Stickiness

The stickiness analyses of grape juice powder was determined according to Telis and Martínez-Navarrete (2010) in a texture analyzer TA-XT Plus (Stable Micro Systems, Ltd., UK),

with the use of a 10 mm diameter cylindrical probe. The samples, conditioned in different water activity (aw from 0.11 to 0.75) at 25 ºC, were placed in an aluminum sample holder and, then, compressed for a fixed distance of 3 mm at a constant rate of 0.1 mm/s.

4.2.8 Light stability

Grape juice powders were packed into low-density polyethylene bags, sealed and stored in a controlled temperature chamber at 25 ºC, under incident light of 3500 lux using fluorescent lamps of 15 W placed at a distance of 16 cm. The samples were distributed uniformly across the chamber, ensuring that illumination reached all the material. Samples were analyzed at 0, 2, 4, 7, 15, 30, 45, 60 and 90 days of storage, regarding water content, total anthocyanins and powder color. The analyses were performed in triplicate.

4.2.8.1 Water content

The water content of the grape juice powder was determined by the gravimetric method. Samples were weighted and dried in a vacuum oven at 70 ºC until constant weight (AOAC, 1990).

4.2.8.2 Total anthocyanins

Anthocyanins were extracted from grape juice powder according to the procedure described by Francis (1982). A powder sample of 0.05 g was extracted with 95% ethanol/1.5 N HCl (85:15, v:v), vortexed for 5 min and brought to 50 mL with the extracting solution. The sample was protected from light and refrigerated (4 ºC, 12 h). Absorbance was measured in UV-vis spectrophotometer (SP-220, Biospectro) at 520 nm, and total anthocyanin content was calculated using molar absorbance and molar mass corresponding to malvidin-3,5-glucoside.

4.2.8.3 Color changes

Color attributes (CIE L*, a* and b* values) were determined using a Hunter Lab colorimeter (Color Flex EZ, USA) with D65 illuminant and 10º observation angle. Total color differences (ΔE^*), chroma (C^*) and hue angle (h^*) of grape juice powders were calculated along time of storage regarding the color in the zero time, according to Equations 4, 5 and 6.

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (4)$$

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (5)$$

$$h^* = arctg\left(\frac{b^*}{a^*}\right) \quad (6)$$

4.2.8.4 Statistical analysis

Analyses were performed in triplicate and the results were subjected to analysis of variance and mean comparison of Tukey's test at 5% probability.

4.3 RESULTS AND DISCUSSION

4.3.1 Sorption isotherms

The BET and GAB models were fitted to experimental water sorption data for powdered grape juice produced with blends of whey protein concentrated/maltodextrin (WM) and soy protein isolate/maltodextrin (SM) and stored at 25 ºC. Both models presented satisfactory determination coefficients (R^2). The mean relative errors were slightly lower for the GAB model, thus, it was chosen to represent the sorption data of powdered grape juice (Figure 4.1).

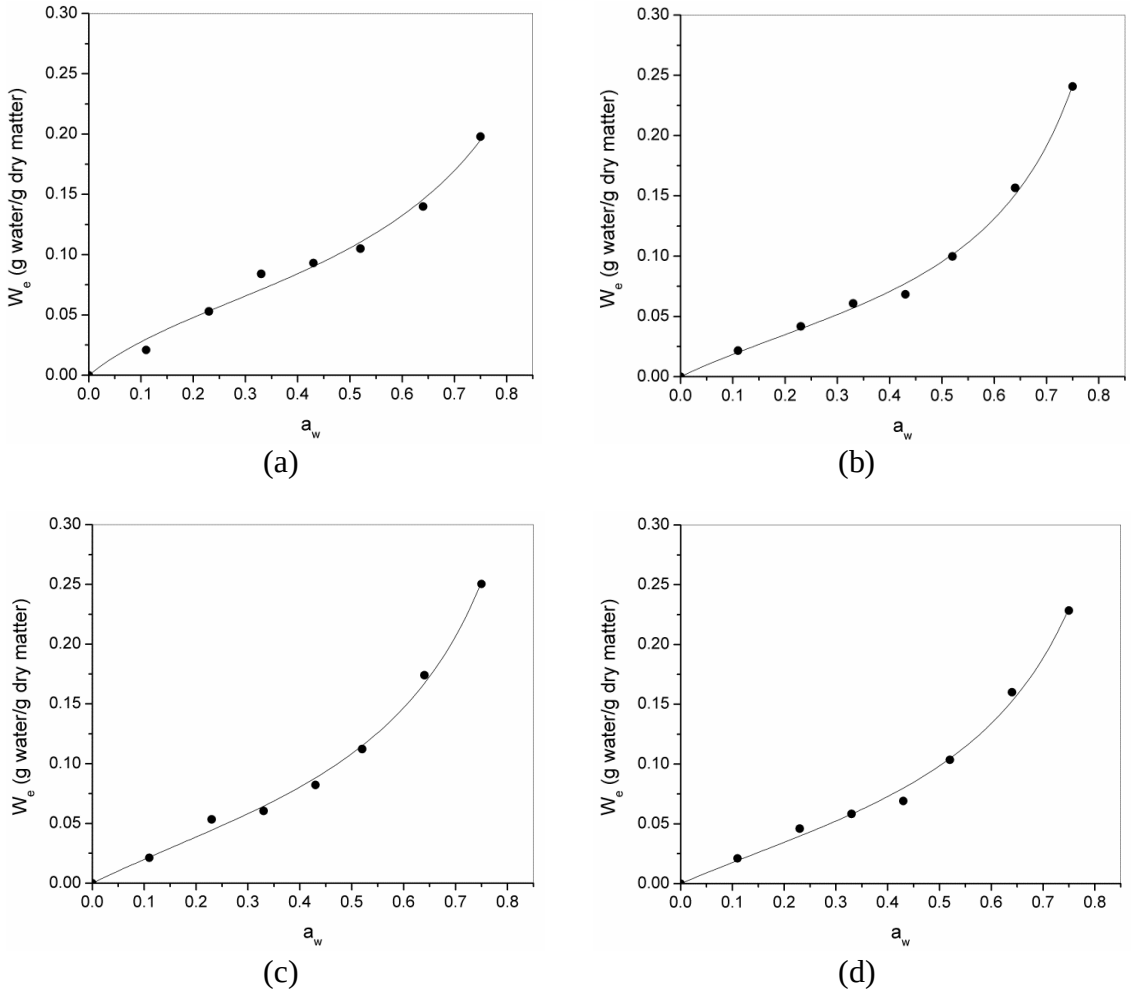

Figure 4.1 Sorption isotherms of powdered grape juice (a) 1SM; (b) 2SM; (c) 1WM; (d) 2WM at 25 ºC. The solid lines represent the fitted GAB model.

The curves presented sigmoidal shape and, according to Brunauer et al. (1938), they can be classified as type II. This type of curve was also observed for spray dried blackberry pulp

with maltodextrins of different dextrose equivalent (GIRALDO-GÓMEZ et al., 2011), spray dried sugarcane added of maltodextrin (LARGO-AVILA et al., 2014), and spray dried mango with maltodextrin and skimmed milk (CANO-HIGUITA et al., 2015).

The equilibrium water content increased slowly at a_w from 0.1 to 0.5, while at a_w above these values there was a steep rise for all treatments. In this region ($a_w > 0.5$), water has a predominant influence on the powder stability because, since it is in the form of free molecules, it can dissolve constituents, which results in acceleration of undesirable reactions (RIGHETTO; NETTO, 2005).

The estimated parameters of GAB model for powdered grape juice produced with WM and SM blends are presented in Table 4.2. The monolayer moisture content (w_m) is an important parameter that indicates the amount of water that is strongly adsorbed to specific sites at the food surface and is considered an important value to assure food stability (TONON et al., 2009). The w_m values for powdered grape juices varied from 5.8 % to 7.5 % (dry basis). Tonon et al (2009) and by Righetto and Netto (2005) obtained w_m values of 5 % for spray dried açai juice and immature acerola juice, respectively, using maltodextrin as drying aid.

Table 4.2 Parameters of GAB model fitted to experimental data for powdered grape juice.

	1SM	**2SM**	**1WM**	**2WM**
w_m	0.073 ± 0.000	0.058 ± 0.000	0.075 ± 0.000	0.070 ± 0.000
C_{GAB}	5.54 ±0.01	3.46 ± 0.03	2.91 ±0.01	2.74 ± 0.02
K_{GAB}	0.88 ± 0.00	1.04 ± 0.00	0.98 ± 0.00	0.98 ± 0.00
R^2	0.976	0.994	0.992	0.99
E (%)	10.10	5.13	5.73	6.32

Where: w_m = g water/ g dry matter; C_{GAB} and K_{GAB} are constants of the GAB model.

Energy constants *C* and *K* influence the sigmoidal shape of the isotherms. The *C* determines the more or less pronounced form of the "knee" in the lower a_w range, whereas *K* determines the isotherm profile in the higher a_w range (RIGHETTO; NETTO, 2005).

Changes on the physical characteristics of powdered grape juice at different relative humidity at 25 ºC were visually observed (Figure 4.2). In water activity from 0.43 to 0.75, the juices microencapsulated with SM showed very similar behavior to each other. The juices

microencapsulated with WM also showed similar behavior to each other, but different behavior if compared to the SM.

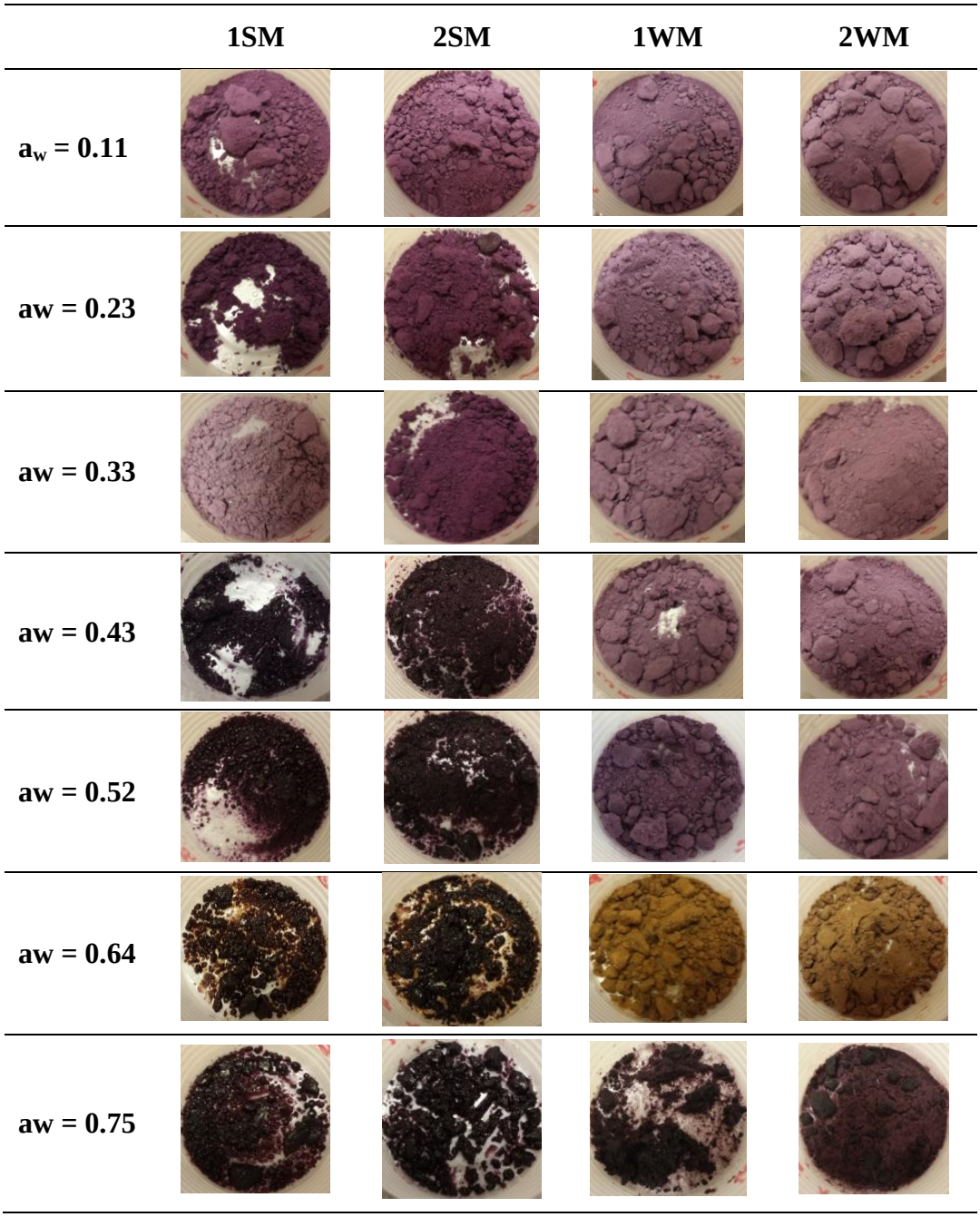

Figure 4.2 Powdered grape juice formulated with SM and WM equilibrated at different water activity at 25 ºC after 28 days.

The powdered grape juices produced with SM, when stored at a_w of 0.33 or lower, remained as free-flowing powders, whereas when stored at a_w above 0.43 showed the formation

of dark and hard blocks that resulted from collapse, an advanced stage in caking associated with a pronounced loss of system integrity. The chemical composition, humidity and temperature are decisive factors for caking of amorphous particles. According to Hartmann and Palzer (2011), several stages of caking can be distinguished. In the initial phase, the powder starts to get sticky and particles adhere to each other, which results in stickiness. There is the formation of brittle lumps and progressing agglomeration. In a later phase, particles missing a stabilizing inner structure, which are built of non-dissolving substances, lose their structure and shape. The powder structure collapses, and a highly viscous, foam-like, amorphous melt is obtained.

The powdered grape juices produced with WM were more stable than those produced with SM. When the WM powders were stored at a_w of 0.52 or lower, they maintained themselves as free-flowing powders. At a_w of 0.64, powders showed agglomeration and could not flow easily. Only at a_w of 0.75 it was possible to perceive the beginning of collapse. Frascareli et al. (2012) observed a high physical stability of coffee oil microcapsules produced with pure whey protein or whey protein/maltodextrin blends.

4.3.2 Glass transition temperature

The glass transition temperature and water content of powdered grape juices, previously conditioned to different water activities at 25 ºC, are presented in Table 4.3. In all treatments, the glass transition temperature decreased with the increase of water content, confirming the plasticizing effect of water on this property.

At a_w in the range of 0.11 to 0.52, 1WM treatment, which had a lower carrier agent concentration (CAC) and lower amount of maltodextrin, showed Tg slightly lower than the other treatments. On the other hand, at a_w of 0.43 and 0.52 the treatment 1SM showed Tg slightly higher than the other treatments, which demonstrates that it was less plasticized by water. This is probably related to the fact that 1SM had the higher CAC and high amount of maltodextrin in the blend. Righetto and Netto (2005) found very low glass transition temperature to immature acerola juice (Tg = 0.5 ºC, at a_w = 0.43) and observed that the use of maltodextrin, increased Tg values and improved the product stability.

Table 4.3 Glass transition temperature (Tg) and water content (*w*, g water/g solids) of powdered grape juice stored at different water activities (a_w).

a_w	1SM		2SM		1WM		2WM	
	w	**Tg**	***w***	**Tg**	***w***	**Tg**	***w***	**Tg**
0.11	0.021	90.3 ± 1.9	0.021	91.8 ± 1.0	0.021	87.0 ± 4.1	0.021	90.1 ± 2.6
0.23	0.050	83.8 ± 10.2	0.040	89.6 ± 1.1	0.051	nd	0.044	nd
0.33	0.078	nd	0.057	nd	0.057	59.6 ± 4.7	0.055	70.5 ±1.9
0.43	0.085	65.3 ± 4.2	0.064	57.8 ± 2.9	0.076	53.3 ± 4.0	0.065	61.6 ± 2.3
0.52	0.095	59.6 ± 2.7	0.091	55.1 ± 1.3	0.101	48.6 ± 1.2	0.094	55.6 ± 1.6
0.64	0.123	nd	0.135	52.9 ± 0.4	0.148	46.3 ± 2.7	0.138	44.1 ± 1.2
0.75	0.165	nd	0.194	50.7 ± 1.9	0.200	nd	0.186	36.0 ± 3.3

nd – not determined

The Tg values obtained for powdered grape juice were higher to SM and WM than those obtained by Tonon et al. (2009) for spray dried açai juice added of maltodextrin. At a_w in the range of 0.1 to 0.7, those authors observed Tg values between 70 ºC and -14 ºC, respectively.

Roos (1991) reported that the Tg values of maltodextrin with 10DE varied from 103 ºC to 30 ºC for a_w in the range of 0.1 to 0.7, whereas Frascareli et al. (2012) observed that Tg of whey protein isolate were between 55 ºC and 33 ºC for a_w in the range of 0.11 to 0.7. In addition, Chen and Zhang (2005) observed Tg of 67 ºC for dry soy protein isolate. Based on these reported results for glass transition temperatures of the pure carriers, it is clear that, at lower a_w, maltodextrin has higher Tg values than proteins but, on the other hand, with increasing a_w, maltodextrin is more plasticized by water.

When amorphous food materials reach their glass transition temperature, a drastic decrease in the viscosity and increase in molecular mobility may occur (TELIS; MARTÍNEZ-NAVARRETE, 2010). In this way, is very important to keep the powdered grape juice below its respective Tg, since structural changes may deplete the protective ability of the encapsulating matrices.

4.3.3 Stickiness

Mechanical compression tests were carried out to quantify the plasticizing effect of water on the mechanical properties and evaluate the stickiness of powdered grape juices (Figure 4.3).

The force attained during the compression test in the powdered grape juice showed a strong dependence on the water activity, in all treatments.

The 1SM treatment presented higher compression force, mainly at a_w of 0.1 and 0.2, indicating that powdered grape juice particles were more resistant to deformation and consequently less sticky. The higher compression force of 1SM was followed, in magnitude, by 1WM, 2WM and 2SM, except at a_w of 0.2, in which 2WM was higher than 1WM.

In the 1WM and 2SM treatments the force decreased at a_w of 0.22, while in the 1SM and 2WM treatments, the force sudden decreased at a_w of 0.33. The abrupt decrease in the compression force can be taken as an indication of powder susceptibility to imminent collapse and, in such a way, samples 1SM and 2WM would stand for higher a_w before collapsing than 1WM and 2SM treatments.

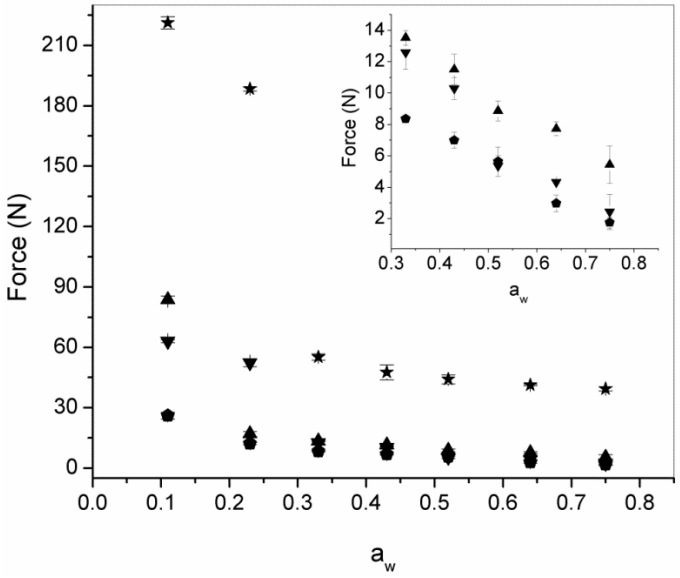

Figure 4.3 Stickiness of powdered grape juice formulated with: ★ 1SM; ⬟ 2SM; ▲ 1WM; ▼ 2WM.

4.3.4 Light stability

The light stability of powdered grape juice stored at 25 ºC, during 90 days, was evaluated regarding to moisture, anthocyanin retention and color. In all treatments, the moisture content increased during storage period (Figure 4.4).

During the first 30 days of storage, the powdered grape juice absorbed a higher amount of water; on the other hand, after this period the moisture increased slowly. The 1SM treatment

presented the lowest moisture values, showing that it was less hygroscopic than the other treatments.

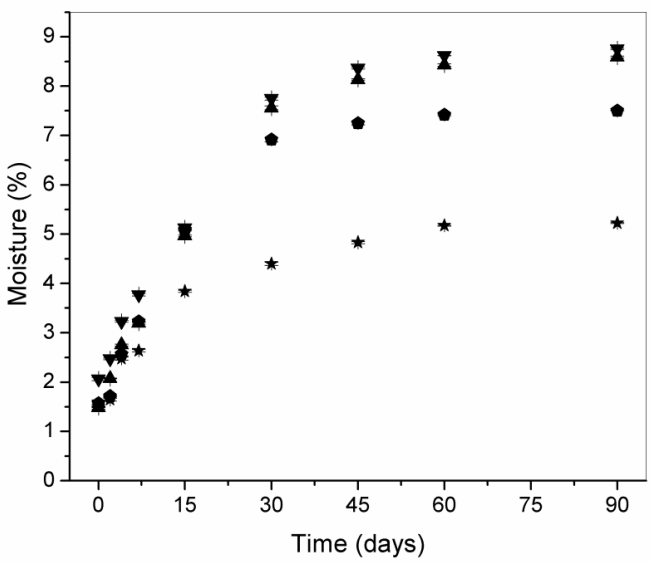

Figure 4.4 Moisture of powdered grape juice formulated with: ★ 1SM; ⬟ 2SM; ▲ 1WM; ▼ 2WM, stored at 25 ºC under exposure to light, during 90 days.

4.3.4.1 Anthocyanin retention

The grape juice was microencapsulated with carrier blends of varied composition, presenting consequently different retention and encapsulation efficiency of anthocyanins. The total anthocyanins in grape juice powders, obtained at the initial storage time (0 day) and expressed as mg/100 g powdered juice (excluding the mass of carrier agents), were: 1SM = 717; 2SM = 677; 1WM = 815; and 2WM = 776. In order to study separately the effect of light, excluding the difference in anthocyanin concentrations among the four treatments, the content of anthocyanins was expressed as C/C_0 (%), with C_0 representing the anthocyanin content at the initial storage time.

The stability of microencapsulated anthocyanins is shown in Figure 4.5. The grape juice formulated with 1SM showed the lowest loss of anthocyanins along time, followed by 1WM, 2WM and 2SM treatments. This result revealed that 1SM was an effective protector wall against degradation by light.

At 30 days of storage, a fast decrease in the amount of anthocyanins was observed in all treatments. The reduction in anthocyanin content was of 27% for 1SM, 32% for 1WM, 39% for 2WM, and 44% for 2SM treatments. During the following 60 days of storage, the losses in anthocyanins were of 1%, at the maximum, showing the stabilization of the phenolic compound and the effectiveness of microencapsulation. Zuanon, Malacrida and Telis (2012) obtained curcumin microcapsules by coacervation complex using gelatin and gum Arabic and, after 35 days of storage under light incidence, those authors observed high degradation rate of curcumin (60 - 72%).

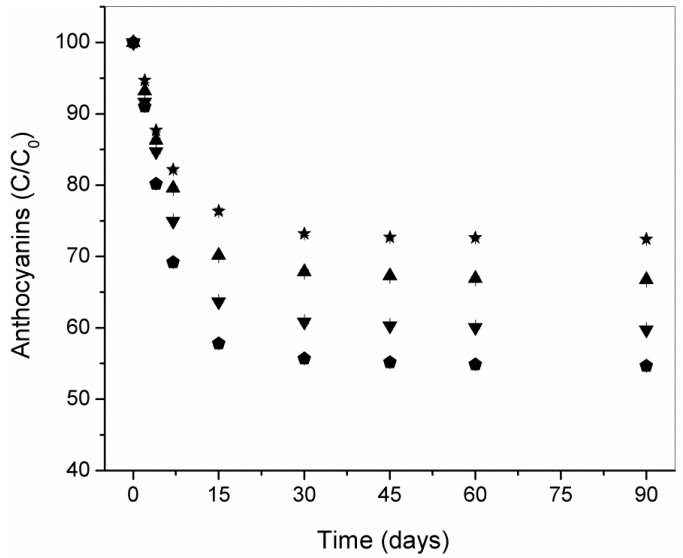

Figure 4.5 Stability of microencapsulated anthocyanins formulated with: ★ 1SM; ⬟ 2SM; ▲ 1WM; ▼ 2WM, stored at 25 ºC under exposure to light, during 90 days.

The fast decrease in the amount of anthocyanins at 30 days of storage may be attributed to degradation of the non-encapsulated compounds, which remained at the surface of the microspheres (CANO-HIGUITA; MALACRIDA; TELIS, 2015). The anthocyanin outside the microspheres shows greater contact with oxygen, or even to the material in contact with the oxygen present in the interior of pores, resulting in their degradation (TONON; BRABET; HUBINGER, 2010).

The higher water adsorption, which occurred at 30 days of storage, also can be responsible for the faster initial degradation of anthocyanins. In addition, 1SM treatment, which showed the lowest water absorption during storage, also showed the lowest degradation rate of

anthocyanin. According to Tonon, Brabet and Hubinger (2010), the increase in water content implies in higher molecular mobility, which can result in degradation of the compound. In this way, it is important to protect the powdered juice from water, because moisture also can be responsible for anthocyanins degradation.

4.3.4.2 Color changes

The color changes of powdered grape juice stored under exposure to light during 90 days are shown in Table 4.4. The total color difference was observed in the powdered juice in each time, with reference to the beginning of the storage.

Table 4.4 Total color differences (ΔE^*), chroma (C^*) and hue angle (h^*) of powdered grape juice stored at 25 ºC under exposure to light, during 90 days.

Days	ΔE^*				C^*				h^*			
	1SM	2SM	1WM	2WM	1SM	2SM	1WM	2WM	1SM	2SM	1WM	2WM
0	-	-	-	-	19.62^{a}	23.74^{b}	24.01^{c}	19.67^{a}	324.12^{a}	340.05^{b}	329.36^{ab}	328.96^{ab}
2	0.94^{a}	1.22^{a}	6.64^{ab}	1.19^{a}	19.03^{a}	23.42^{b}	26.73^{b}	19.96^{a}	325.90^{a}	340.31^{b}	336.73^{a}	329.61^{abc}
4	1.17^{a}	6.66^{b}	10.82^{c}	0.78^{a}	18.72^{a}	25.92^{bc}	15.28^{a}	18.90^{a}	324.85^{a}	326.36^{a}	336.14^{bc}	329.05^{a}
7	1.41^{a}	7.53^{b}	9.89^{bc}	1.14^{a}	18.53^{a}	27.49^{c}	17.11^{ab}	19.28^{a}	325.04^{a}	325.52^{a}	325.59^{a}	331.06^{abc}
15	1.74^{a}	2.37^{a}	6.39^{a}	2.32^{a}	18.32^{a}	23.18^{b}	18.55^{b}	19.88^{a}	325.36^{a}	345.44^{bc}	329.16^{ab}	332.85^{abc}
30	2.89^{a}	12.07^{c}	7.86^{ab}	10.43^{b}	17.76^{a}	17.85^{a}	18.57^{b}	18.14^{a}	325.90^{a}	346.72^{c}	339.57^{c}	337.89^{abc}
45	3.00^{a}	12.16^{c}	7.93^{ab}	10.48^{b}	17.68^{a}	17.77^{a}	18.51^{b}	18.08^{a}	325.95^{a}	346.82^{c}	339.64^{c}	337.97^{bc}
60	3.06^{a}	12.24^{c}	7.98^{ab}	10.52^{b}	17.63^{a}	17.70^{a}	18.47^{b}	18.04^{a}	326.00^{a}	346.91^{c}	339.69^{c}	338.03^{c}
90	3.09^{a}	12.29^{c}	8.02^{abc}	10.55^{b}	17.61^{a}	17.66^{a}	18.44^{b}	18.01^{a}	325.99^{a}	346.94^{c}	339.76^{c}	338.05^{c}

Means with different letters in the same column indicate significant differences ($p < 0.05$) according Tukey's test.

In general, 1SM treatment did not present significant changes in ΔE^*, C^* and h^* during the storage period. Microspheres formed with 1SM maintained the same color at initial and final storage times, which demonstrates the high light stability of the product.

The 2SM, 1WM and 2WM treatments presented changes in the total color differences during the storage period. At 30 days of storage, ΔE^* higher than 5 was observed, which can be related to degradation of anthocyanins that occurred in the same period. According to Obón et al. (2009), a difference in color from 0 to 1.5 can be considered small and almost identical for visual observation, in the range from 1.5 to 5 the difference in color can be distinguished, whereas the difference in color is evident for ΔE^* higher than 5. Estupiñan, Schwartz, and Garzón (2011)

evaluated the stability of isotonic model beverages colored with freeze-dried Andes berry anthocyanins, with and without maltodextrins as carrier agent. The authors observed that after 71 days of storage under exposure to light, the addition of maltodextrin increased stability of color. Zuanon, Malacrida and Telis (2012) observed ΔE^* around 20 to curcumin microcapsules formulated with gelatin and gum Arabic, when stored at 35 days under exposure to light.

The 2WM treatment did not show changes in the value of C^* during storage. On the other hand, 2SM and 1WM showed a small decrease in the value of chroma, which means that these powders presented a less vivid color. Estupiñan, Schwartz, and Garzón (2011) also observed changes in C^* for freeze-dried anthocyanins with maltodextrin, when stored at 71 days under illumination.

Hue angle of juice powders were located in the fourth quadrant of CIELab color chart, corresponding to purple color. In the 2SM, 1WM, and 2WM treatments, the h^* decreased after 90 days of storage with reference to the beginning of the storage. Although still presenting the purple color, changes occurred in the sample's color with respect to the purple tone.

4.4 CONCLUSIONS

The sorption isotherms in conjunction with data on glass transition temperature and compression force indicated that powdered grape juice encapsulated in the matrix composed by soy protein isolate/maltodextrin in proportions corresponding to samples 1SM presented lower higrocopicity and lower susceptibility to structural collapse among the studied matrix formulations. At the same time, stability assays carried out along 90 days of storage under exposure to light showed that the formulation 1SM also resulted in the smaller degradation rates of anthocyanins, as well as in the lower total color differences referring to samples at the beginning of the storage period. The results permitted to conclude that the blend 1SM is suitable to encapsulate grape juice, producing powders that can be manipulated and stored with relative safety provided that water activity is kept under 0.3, which can be attained by using appropriated package specifications.

ACKNOWLEDGMENTS

To São Paulo Research Foundation (FAPESP), grants 2012/09074-4 and 2010/09614-3for the financial support, and Brazilian Agricultural Research Company - Grape and Wine (Jales, Brazil).

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-CAPÍTULO 5-

STORAGE STABILITY OF PHENOLIC COMPOUNDS IN POWDERED BRS VIOLETA GRAPE JUICE MICROENCAPSULATED WITH PROTEIN AND MALTODEXTRIN BLENDS

Trabalho submetido à "Food Chemistry"

5 STORAGE STABILITY OF PHENOLIC COMPOUNDS IN POWDERED BRS VIOLETA GRAPE JUICE MICROENCAPSULATED WITH PROTEIN AND MALTODEXTRIN BLENDS

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ABSTRACT

The stability of the phenolic compounds, antioxidant activity and color parameters of microencapsulated powdered grape juice - stored at 5, 25 and 35 ºC for up to 150 days - was evaluated. Powdered grape juice from the red grape cultivar BRS Violeta was produced by spray drying. Blends of soy protein (S) or whey protein (W) with maltodextrin (M) were used as the carrier agents. The carrier agent concentration (CAC = g of carrier agent/g of juice soluble solids) varied from 0.75 to 1.25, whereas the percentage of protein in the carrier agent (g dry protein/100 g total carrier agent) varied from 5.86 % to 30 %.The treatment 1SM, which had the largest amount of carrier agent and a low ratio of soy protein, allowed for conservation of the total anthocyanins (after 120 days of storage at all the temperatures, it maintained more than 90% of the anthocyanins), whereas the treatment 1WM (lowest amount of carrier agent and highest ratio of whey protein) did not provide good protection. The degradation of the anthocyanins followed first order kinetics. The 1SM treatment, when stored at 5 ºC, presented the lowest degradation rate and the longest half-life time (approximately 39 months). The WM

treatments showed correlation between the polymerized anthocyanins and storage time, but no correlation with temperature was found. The principal component analysis was applied to highlight the effects of time and temperature on individual anthocyanins and the individual anthocyanin profile changed slightly during storage. With respect to the 2SM, 1WM and 2WM treatments, the longer the storage time, the greater the proportion of *p*-coumaroylated anthocyanins present. The flavonols, hydroxycinnamic acid derivatives (HCAD) and flavan-3-ol were analyzed at zero time (0 day) and at the end of the storage time (150 days). The 1SM treatment led to a significant loss of flavonols at the end of the storage time, but this did not happen with the 1WM treatment. All the samples showed a significant loss of HCAD in the powder at the end of the storage time, independent of the storage temperature and a loss of flavan-3-ol after 150 days of storage at 35 ºC. Despite all the aforementioned losses in the phenolic compounds studied, the time and temperature did not influence the antioxidant activity of the powder, nor did it influence the color of the reconstituted grape juice after 150 days of storage.

Keywords: phenolic compounds, antioxidant activity, microencapsulation, stability, temperature.

5.1 INTRODUCTION

Grape is one of the most important natural sources of phenolic compounds. The grape BRS Violeta is a hybrid cultivar, obtained in 2006 from a cross between BRS Rubea and IAC 1398-21 by the Brazilian Agricultural Research Corporation (*Empresa Brasilera de Pesquisa Agropecuária*, EMBRAPA). This cultivar contains high levels of anthocyanins and other polyphenols, becoming an alternative to produce highly colored and antioxidant-rich grape juice (CAMARGO; MAIA; NACHTIGAL, 2005; LAGO-VANZELA et al., 2014). A previous study demonstrated that the BRS Violeta grape is a good source of phenolic compounds, mainly found in the skin, such as anthocyanins (3930 mg/kg), flavonols (150 mg/kg), hydroxycinnamic acid derivatives (120 mg/kg) and proanthocyanidins (670 mg/kg) (REBELLO et al., 2013).

Anthocyanins are red colored pigments, and their extracts are increasingly attractive to the food industry as natural alternatives to synthetic dyes (BUENO et al., 2012). In addition, anthocyanins and other polyphenols provide health benefits due to their high biological activity, including antioxidant, anti-inflammatory, antibacterial and antiviral functions (FANG; BHANDARI, 2010). Furthermore, they can provide favorable effects in the protection against chronic diseases, including cancer and cardiovascular and neurodegenerative pathologies (DEL RIO et al., 2013).

Nevertheless, the application of anthocyanins as natural colorants on a commercial scale is limited because of their relatively low stability to processing and storage conditions (ESTUPINAN; SCHWARTZ; GARZON, 2011). During the storage of grape juice powders the degradation of sensitive components may occur, the losses depending on the temperature, pH, exposure to oxygen, porosity, light and the presence of organic acids (SAGAR; SURESH KUMAR, 2010). Knowledge of the degradation kinetics of the anthocyanins is a very important factor in the prediction of food quality loss. Several studies have shown that the degradation of anthocyanins follows first order kinetics, i.e., the anthocyanin content decreases with time (ROBERT et al., 2010; IDHAM; MUHAMAD; SARMIDI, 2012; FLORES; SINGH; KONG, 2014; SOUZA et al., 2014).

In the food industry, microencapsulation techniques have been widely used to protect food ingredients against degradation. In this process, the immobilization and incorporation of biologically active compounds inside solid particles (microspheres) occurs, providing stability of the compound structure and its protection. In addition, microencapsulation facilitates light- and

heat-labile molecules, such as anthocyanins, to increase their stability and lengthen their shelf-life (CAVALCANTI; SANTOS; MEIRELES, 2011).

Spray drying is the most common technique applied in microencapsulation, and is an alternative for the production of grape juice powder with a high anthocyanin content. The choice of polymers as carrier agent, which acts as the wall material, strongly determine the stability of the microparticles and the degree of protection of their active core. The efficacy of maltodextrin is due to its rapid film or shell forming property and the relatively low water diffusivity of these films. On the other hand, proteins form smooth and non-sticky films or shells much earlier than maltodextrin, and the powder recovery is higher when a small amount of protein is used (ADHIKARI et al., 2009). The mixture of maltodextrin and protein favors the protection of bioactive compounds (NESTERENKO et al., 2013) and combines the specific properties of each polymer.

Previous research showed that, during storage at 60 ºC, the degradation of polyphenols and anthocyanins in fresh juice was faster than in microencapsulated juice, showing the importance of microencapsulation in the protection of bioactive compounds (ROBERT et al., 2010). Khazaei et al. (2014) verified the strong protective effect of encapsulation and wall materials against heat with respect to the stability of anthocyanins. Idham, Muhamad & Sarmidi (2012) noted that the combination of two carrier agents (maltodexrin and gum Arabic) resulted in the lowest anthocyanin degradation rate, when compared to the use of just one carrier agent.

As a result of all their benefits, the use of novel and inexpensive technologies has encouraged the food industry to increase the shelf life of grape juice, as well as to preserve its unstable compounds. Based on these considerations, the aim of this study was to investigate the stability of the phenolic compounds, antioxidant activity and color parameters of BRS Violeta grape juice microencapsulated by spray drying with whey protein concentrate/maltodextrin and soy protein isolate/maltodextrin blends. The study was extended over a storage period of 150 days at three different storage temperatures, namely 5, 25 and 35 ºC.

5.2 MATERIAL AND METHODS

5.2.1 Materials

BRS Violeta grape was provided by EMBRAPA Grape and Wine (Jales, Brazil). Whey protein concentrated 80% (Alibra, Brazil), soy protein isolate 92.8% (Tovani Benzaquen, Brazil) and maltodextrin DE-10 MOR-REX 1910 (Corn Products, Brazil) were used as the carrier agents.

All the solvents were of HPLC quality and all the chemicals used were of analytical grade (>99%). The water was of Milli-Q quality. For the identification of the phenolic compounds, the following commercial standards from Phytolab (Vestenbergsgreuth, Germany) were used: malvidin 3-glucoside, malvidin 3,5-diglucoside, peonidin 3,5-diglucoside, trans-piceid, trans-caftaric acid, (-)-epigallocatechin and (-)-gallocatechin. The following commercial standards from Extrasynthese (Genay, France) were also used: cyanidin 3-glucoside, cyanidin 3,5-diglucoside, procyanidins B1 and B2, kaempferol, quercetin, isorhamnetin, myricetin, syringetin and the 3-glucosides of kaempferol, quercetin, isorhamnetin and syringetin. In addition the following commercial standards from Sigma Aldrich (Tres Cantos, Madrid, Spain) were used: trans-resveratrol, caffeic acid, (+)-catechin, (-)-epicatechin, (-)-epicatechin 3-gallate and (-)-gallocatechin 3-gallate. Other non-commercial flavonol standards such as myricetin 3-glucoside, quercetin 3-glucuronide and laricitin 3-glucoside were previously isolated from Petit Verdot grape skins (CASTILLO-MUÑOZ et al., 2009) and procyanidin B4 was kindly supplied by Prof. Fernando Zamora (Department of Biochemistry and Biotechnology, Universitat Rovira i Virgili, Spain).

All the standards were used for identification and quantitation by way of calibration curves covering the expected concentration ranges. When a standard was not available, the quantitation was done using the calibration curve of the most similar compound: malvidin 3,5-diglucoside for the 3,5-diglucoside anthocyanin type and malvidin 3-glucoside for the 3-glucoside type, quercetin 3-glucoside for the flavonol 3-glycosides and their free aglycones, caffeic acid for the hydroxycinnamic acid derivatives, (+)-catechin for polymeric flavan-3-ols (total proanthocyanidins), individual flavan-3-ol monomers and dimers by their corresponding standards, considering their total sum as (+)-catechin equivalents.

5.2.2 Grape juice preparation

The grape juice was prepared by the steam extraction method and the temperature of extraction was maintained at 75-85 °C for 60 minutes. The juice was filtered through a 270 mesh on the Tyler standard screen scale to remove the potassium bitartrate crystals, and then stored in a freezing chamber at –18 ºC until used (RIZZON; MANFROI; MENEGUZZO, 1998). The grape juice had a total solids content of 15.18 ± 0.02 g/100 g (w/w), 14.0 ± 0.1 ºBrix, pH 3.84 ± 0.006, acidity (% tartaric acid) of 0.60 ± 0.004, ash of 0.43 ± 0.004 g/100 g (w/w) (AOAC, 2006), reducing sugar content of 12.32% and 1965.38 mg/L ± 3.74 of total anthocyanins.

5.2.3 Microencapsulation

The grape juice was spray dried in a mini spray dryer (model B-290, Büchi, Switzerland) using the following processing conditions: inlet air temperature 140 ºC, feed flow rate 2 mL/min and air flow 500 L/h.

Blends of the whey protein concentrate (W) or soy protein isolate (S) with maltodextrin (M) were used as the carrier agents. The carrier agent concentration (CAC, = g of carrier agent/g of juice soluble solids) and the percentage (w/w) of protein in the carrier agent (g dry protein/100 g total carrier agent) used were: 1SM, CAC 1.25, 10.00%; 2SM, CAC 1.00, 5.86%; 1WM, CAC 0.75, 30.00%; and 2WM, CAC 0.85, 20.00%. The carrier agents were added to the juice with magnetic stirring until complete dissolution (MOSER; SOUZA; TELIS, 2016).

These protein/maltodextrin systems were selected based on a previous study in which the carrier agent concentrations and proportion of protein in the microencapsulated grape juice were evaluated (MOSER; SOUZA; TELIS, 2016). In the samples formulated with the WM blend, the CAC varied between 0.15 and 0.85, whereas with the SM blend the CAC values ranged from 0.65 to 1.35. In this study, it was observed that high carrier concentrations resulted in higher drying yields and better powder properties, such as an increase in anthocyanin retention. Moreover, higher carrier concentrations improved the encapsulation efficiency and resulted in a more suitable morphology, whereas lower carrier concentrations showed poor microcapsule formation ability. Increasing the carrier concentration and protein/carrier agent ratio resulted in brighter powders, but the rehydrated juice presented a color similar to that of the fresh juice. The use of larger amounts of carrier resulted in better anthocyanin microencapsulation of properties.

5.2.4 Storage stability of grape juice powders

One gram portions of the powders were packed into metallized plastic bags, sealed under vacuum, and stored in a refrigerator or incubator at three different temperatures: 5 ºC representing the refrigeration temperature (aiming at the use of the powder as an additive in low temperature stored products), 25 ºC, representing room temperature, and 35 ºC, which is one of the temperatures recommended for accelerated shelf-life studies (TONON; BRABET; HUBINGER, 2010).

The powdered juice was reconstituted by mixing the necessary mass of juice powder with distilled water so as to always maintain the same proportion between the solids coming from the fresh grape juice and the water. Before carrying out any tests, the residual insoluble particles of the carrier agents were removed from the juice by centrifugation (14000 rpm) or filtration (0.20 µm, polyester membrane, Chromafil PET 20/25, Macherey-Nagel, Düren, Germany).

The samples were analyzed after the following storage times: 0, 7, 15, 30, 45, 60, 90, 120 and 150 days, with respect to the contents of individual, total and polymeric anthocyanins, the antioxidant capacity and the color parameters. Flavonols, hydroxycinnamic acid derivatives, flavan-3-ol monomers, B-type dimers and the total proanthocyanidin content were only analyzed at zero time (0 day) and the final (150 days) storage time. The analyses were carried out in triplicate (three bags were used for each storage time analyzed) and the results were expressed as mg/100 g of dry juice matter (excluding the mass of the carrier agents).

5.2.4.1 Anthocyanins

The polymeric anthocyanin content (expressed as a percentage with respect to the total anthocyanins) was estimated using the sulfur dioxide bleaching method with a Shimadzu UV spectrophotometer (UV-1800, Kyoto-Japan) at 520 nm (RIBÉREAU-GAYON; STONESTREET, 1965). The concentration (mg/L) was determined from a calibration curve obtained with standard malvidin-3,5- diglucoside solutions.

The degradation kinetics were calculated from the total anthocyanins as analyzed by HPLC. The reaction rate constants (k) and half-life time ($t_{1/2}$) were calculated from the first-order kinetics as described in Equations 1 and 2.

$$-\ln\left(\frac{C_t}{C_0}\right) = kt \quad (1)$$

$$t_{1/2} = \frac{\ln(2)}{k} \quad (2)$$

where C_0 is the initial anthocyanin content, and C_t is the anthocyanin content at reaction time (t).

The variation of *k* as a function of the temperature fitted the Arrhenius-type equation well (Equation 3), allowing for the calculation of the activation energy (E_a).

$$\ln k = \ln k_0 - \frac{E_a}{RT} \quad (3)$$

where E_a is the activation energy, R is the ideal gas constant and T is the absolute temperature.

5.2.4.2 HPLC–DAD–ESI-MSn analysis of phenolic compounds

The HPLC separation, identification and quantitation of the grape juice phenolic compounds were carried out using an Agilent 1100 Series system (Agilent, Germany) equipped with DAD (G1315B) and LC/MSD Trap VL (G2445C VL) electrospray ionization mass spectrometry (ESI-MSn) system, coupled to an Agilent ChemStation (version B.01.03) data-processing unit. The mass spectra data were processed using the Agilent LC/MS Trap software (version 5.3).

Anthocyanins, flavonols and hydroxycinnamic acid derivatives from the microencapsulated grape juice were separately analyzed after the adaptation of previously described methods (CASTILLO-MUÑOZ et al., 2007; CASTILLO-MUÑOZ et al., 2009) to the use of narrow bore, smaller particle size, chromatography columns (REBELLO et al., 2013).

For the analysis of anthocyanins, 20 μL of the reconstituted juice was injected directly into the HPLC system after filtration (0.20 μm, polyester membrane, Chromafil PET 20/25, Machery-Nagel, Düren, Germany) and dilution (1:4) with 0.1N HCl. For flavonols and hydroxycinnamic acid derivatives, anthocyanin-free extracts were obtained by SPE on Bond Elute Plexa PCX (Agilent; 6 cm^3, 500 mg of adsorbent) cartridges (CASTILLO-MUÑOZ et al., 2007).

The anthocyanin and non-anthocyanin compounds were analyzed according to a previously described method (LAGO-VANZELA et al., 2011). The juice samples were injected (10 µL for anthocyanin analysis and 20 µL for non-anthocyanin flavonol analysis) into a Zorbax Eclipse XDB-C18 reversed-phase column (2.1 x 150 mm; 3.5 µm particle; Agilent, Germany) maintained at 40 ºC.

An ion trap ESI-MS/MS detector was used in both positive (anthocyanins) and negative (non-anthocyanin) ion modes for identification with the following parameters: dry gas, N_2, 8 L/min; drying temperature, 325 °C; nebulizer, N_2, 50 psi; ionization and fragmentation parameters optimized by direct infusion of appropriate standard solutions (malvidin 3,5-diglucoside in positive ionization mode; quercetin 3-glucoside and caffeic acid in negative ionization mode); scan range, 50–1200 m/z. The identification was based mainly on spectroscopic data (UV–vis and MS/MS) obtained from authentic standards or using previously reported data (NIXDORF; HERMOSIN-GUTIERREZ, 2010; LAGO-VANZELA et al., 2013; REBELLO et al., 2013; BARCIA et al., 2014; LAGO-VANZELA et al., 2014). For quantification, DAD-chromatograms were obtained at 520 nm (anthocyanins), 360 nm (flavonols) and 320 nm (hydroxycinnamic acid derivatives) and calibration curves were obtained for the most representative compound of each type of phenolic compound: malvidin 3,5-diglucoside for anthocyanins, quercetin 3-glucoside for flavonols and caffeic acid for hydroxycinnamic acid derivatives. The analyses were carried out in duplicate for each of the triplicate samples.

5.2.4.3 Flavan-3-ol monomers and B-type procyanidin dimers

For the analysis of the flavan-3-ol monomers and procyanidin B-type dimers from microencapsulated grape juice, 0.30 mL of the reconstituted juice was diluted with 1.5 mL of water: formic acid (98.5:1.5) in a chromatographic vial that was sealed and used for direct injection into the HPLC system. The analysis was carried out using a HPLC Agilent 1200 series system equipped with DAD (Agilent, Germany) and coupled to an AB Sciex 3200 TRAP (Applied Biosystems) with a triple quadrupole, turbo spray ionization (electrospray assisted by thermo-nebulization) mass spectroscopy system (ESI-MS/MS). The chromatographic system was managed by an Agilent ChemStation (version B.01.03) data-processing unit. The mass spectra data was processed by the Analyst MSD software (Applied Biosystems, version 1.5). The

diluted samples were injected (20 µL) into an Ascentis C18 reversed-phase column (150 mm x 4.6 mm with 2.7 µm of particle size), maintained at 16 ºC. The solvents and gradients used for this analysis and the two MS scan types used (Enhanced MS - EMS and Multiple Reaction Monitoring –MRM) as well as all the mass transitions (m/z) for identification and quantitation were all of HPLC grade (LAGO-VANZELA et al., 2011). For quantification, an external catechin standard was injected at the beginning and end of every sample injection sequence. The mean value of the areas obtained for this external standard was used for the correction of the response factors previously obtained for every compound analyzed (flavan-3-ol monomers and procyanidin B-type dimers) with respect to catechin in a series of standard solutions containing a fixed concentration of catechin and variable concentrations of each compound analyzed.

5.2.4.4 Antioxidant activity

The antioxidant capacity was determined by reaction with a methanolic solution (6×10^{-5} mol L^{-1}) of the DPPH (2,2-diphenyl-1-picrylhydracyl, Fluka Chemie) radical after 25 minutes of reaction, and quantified using calibration curves prepared with methanolic solutions of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Fluka Chemie), under the same conditions (BRAND-WILLIAMS; CUVELIER; BERSET, 1995). The absorbance was measured in a Shimadzu UV spectrophotometer (UV-1800, Kyoto-Japan) at 515nm.

5.2.4.5 Color parameters

The color parameters of the reconstituted grape juice were measuring using a Portable Spectrophotometer CM-2500c (Konica Minolta Inc.) calibrated for the CIELab color coordinates, with a D65 illuminant and 10º observation angle. In this system, L* denotes lightness on a 0-100 scale from black to white; a*, (+) red and (-) green color components; and b*, (+) yellow and (–) blue color components. The total color differences (ΔE*) were calculated at each storage time evaluated, with respect to the color at the start of storage, and described by Equation 4:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (4)$$

5.2.5 Statistical analysis

Statistical analyses were carried out using the SAS System for Windows version 9.0. The regression analysis was used to correlate the total or polymeric anthocyanins, antioxidant activity and color with respect to the time of storage at different temperatures. The ANOVA analysis, followed by the Student t test, was applied to discriminate the differences between the concentrations of flavonols, hydroxycinnamic acid derivatives and flava-3-ols (monomers and dimers) at the start (0 days) and end of the storage period (150 days). The relationship between individual anthocyanins and the storage conditions was determined using the principal component analysis (PCA).

5.3 RESULTS AND DISCUSSION

5.3.1 Evolution of phenolic compounds during storage

5.3.1.1 Anthocyanins

The grape juice was microencapsulated with carrier agents of varied compositions, thus presenting different values for the retention and encapsulation efficiency of the anthocyanins. The total anthocyanins in the grape juice powders, obtained at the start of the storage time (0 day), were analyzed by HPLC and expressed as mg/100 g powdered juice, with: 1SM = 764; 2SM = 993; 1WM = 970; 2WM = 585. In order to study the effect of time and temperature separately, the amounts of anthocyanins at the start of the storage time were considered as 100%, excluding the differences in anthocyanin concentrations between the four treatments.

All the powdered juices stored at 5 ºC showed the smallest losses of anthocyanins (2–11%), which suggested that microencapsulation, combined with storage at low temperature, helped maintain the majority of the anthocyanins contained in the product (Figure 5.1). Only the 1WM treatment presented a correlation ($p < 0.05$) for the anthocyanins content with the storage time at 5 ºC.

For all treatments storage at 25 and 35 °C showed linear correlations between the time and anthocyanin content. After 120 days of storage, the 1SM treatment maintained more than 90% of the initial content of anthocyanins, but after 150 days a more pronounced reduction was observed (Figure 5.1A).

The grape juice powder formulated with 1SM, which had the largest amount of carrier agent (CAC = 1.25) and a relatively low protein ratio (10%) when compared with the WM treatments (30% in 1WM and 20% in 2WM), appeared to show the best conservation of the anthocyanins. On the other hand, the 1WM treatment, which had the lowest amount of carrier agent (CAC = 0.75) and the highest whey protein ratio (30%), did not protect the anthocyanins well.

The improvement in stability of the samples formulated with larger amounts of carrier agents and lower protein proportions, and consequently with a greater amount of maltodextrin, can be associated with the complexing of the flavylium cation form of the anthocyanins with dextrins, preventing their transformation to other less stable forms. Furthermore, maltodextrin

has an additional stabilizing effect due to its ability to reduce reactant mobility (ESTUPINAN; SCHWARTZ; GARZON, 2011). According to Souza (2014) the samples containing less carrier agent are more hygroscopic and therefore absorb more water, facilitating the degradation reactions. In the previous study of the present authors, a greater encapsulation efficiency of the anthocyanins was observed when using SM blends instead of WM blends. These results may help to explain why the use of SM showed greater protection of the anthocyanins than samples formulated with WM (MOSER; SOUZA; TELIS, 2016).

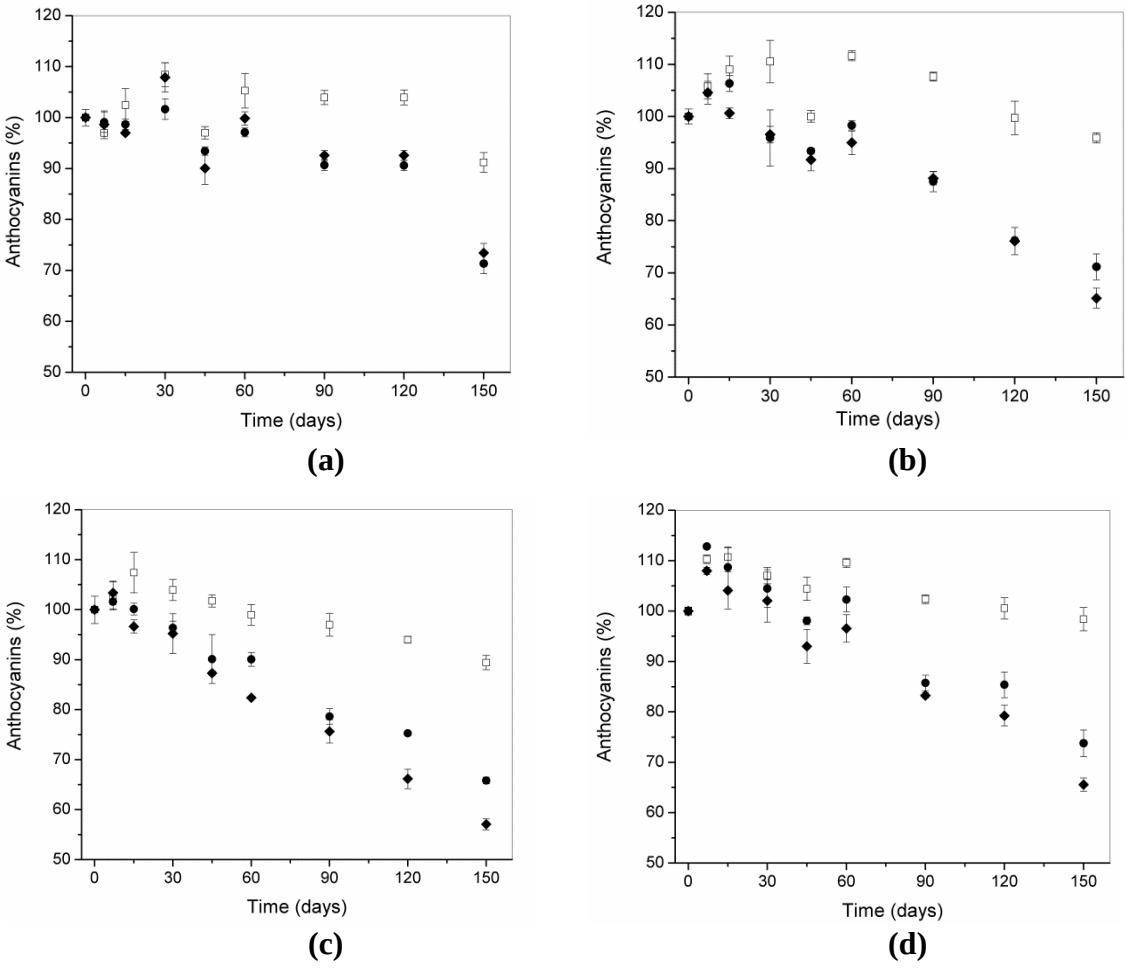


Figure 5.1 Total anthocyanins in grape juice microencapsulted with: (a) 1SM; (b) 2SM; (c) 1WM; and (d) 2WM, storage at □ 5, ● 25 and ◆ 35 ºC, for 150 days.

Understanding degradation mechanisms is important to maximize the nutritional and sensory quality of products. Thus the degradation kinetics of the anthocyanins was monitored throughout the storage period, and the reaction rate constant (*k*) and half-life time ($t_{1/2}$)

parameters are shown in Table 5.1. The anthocyanins of the juice powder followed first-order kinetics, showing linear degradation with respect to time.

Table 5.1 Degradation kinetic parameters and activation energy (Ea) of microencapsulated anthocyanins in powdered grape juice stored at 25 and 35 ºC for 150 days.

Treatment	Temperature	*k* (days^{-1})	t½ (days)	R^2	Ea (kJ/mol)
1SM	5 ºC	0.0006	1195	0.90	20.85
	25 ºC	0.0015	471	0.77	
	35 ºC	0.0013	545	0.62	
2SM	5 ºC	0.0012	573	0.93	14.83
	25 ºC	0.0019	361	0.86	
	35 ºC	0.0022	309	0.89	
1WM	5 ºC	0.0011	654	0.95	29.12
	25 ºC	0.0027	252	0.97	
	35 ºC	0.0035	198	0.98	
2WM	5 ºC	0.0007	950	0.83	24.66
	25 ºC	0.0014	495	0.60	
	35 ºC	0.0021	330	0.83	

The treatments with 2SM, 1WM and 2WM stored at 35 ºC showed greater anthocyanin degradation than samples stored at 25 ºC. The opening of the pyrylium ring and formation of a chalcone can be the first step in the degradation of anthocyanins (PATRAS et al., 2010).

The 1WM treatment, which contained the largest amount of whey protein in the blend, presented the highest degradation rate, suggesting that this carrier agent is not sufficiently effective to protect the anthocyanins. Flores et al. (2014) observed the disadvantage of using only whey protein as the wall material as compared to combining it with polysaccharide. Comparing the temperatures of 25 and 35 ºC, the 1SM treatment stored at 35 ºC presented the lowest degradation rate and the longest half-life time (approximately 18 months). These results suggest that 1SM is more suitable for microencapsulation and preserving the anthocyanins of grape juice stored at temperatures of 25 and 35 ºC. Lago-Vanzela et al. (2014), studied the accelerated aging of wines made from BRS Violeta grapes for 120 days, and found higher

reaction rate constants than found in the present study, with *k* values of 0.0078 and 0.0140 at storage temperatures of 25 and 35 ºC, respectively.

The activation energy (E_a) values for the microencapsulated grape juice are shown in Table 5.1. The highest E_a value was found for the 1WM treatment, followed by 2WM, 1SM and 2SM, respectively. The results suggest that the activation energy was influenced by the concentration of maltodextrin. The two soy protein and maltodextrin blends, which had higher concentrations of carrier agents and higher ratios of maltodextrin in the mixture, presented smaller E_a values, when compared to the whey protein and maltodextrin blends.

A similar result was found by Carrillo-Navas et al. (2011) for passion fruit juice microencapsulated using mesquite gum, gum arabic and maltodextrin blends, and stored at 25, 35 and 40 ºC. The authors observed that for concentrations of 17% and 66% of maltodextrin in the blends, the E_a values were 30.6 kJ/mol and 19.9 kJ/mol, respectively. Vrentas (1978) suggested that the activation energy for diffusion may be described as the energy required to create a "hole" large enough to accommodate a diffusing molecule. The hole is created by the separation of polymer segments as the diffusing molecule moves through them (SOOTTITANTAWAT, 2005).

5.3.1.1.1 Polymerization of anthocyanins

The contribution of polymeric anthocyanins to the total anthocyanin content was estimated from the degree of bisulfite bleaching, which is greatly affected by the protein type used as the carrier agent in conjunction with maltodextrin. In the SM treatments (from 4 to 10.8% of total anthocyanins), the low polymerization did not present a linear correlation between the polymeric anthocyanins and the storage time or temperature (Figure 5.2A).

In contrast, in the treatment with WM, there was a positive linear correlation between the polymerized anthocyanins and the storage time, but no correlation with temperature was found (Figure 5.2B). The storage time resulted in an increase in anthocyanin polymerization from 8.7 to 33.6%, which was greater in the first 60 days of storage, and then followed by an apparent stabilization. Nevertheless, at the end of the storage time, the amount of polymerized anthocyanins increased again. An increase in polymeric pigments and losses of monomeric anthocyanins may be due to several factors, including residual enzymatic activity or condensation reactions between the anthocyanins and other phenolic compounds, such as flavan-

3-ols (BROWNMILLER; HOWARD; PRIOR, 2008). The residual enzymatic activity can be discarded as a result of the probable destruction of the enzymes by the heat treatments used during the grape juice extraction and spray drying.

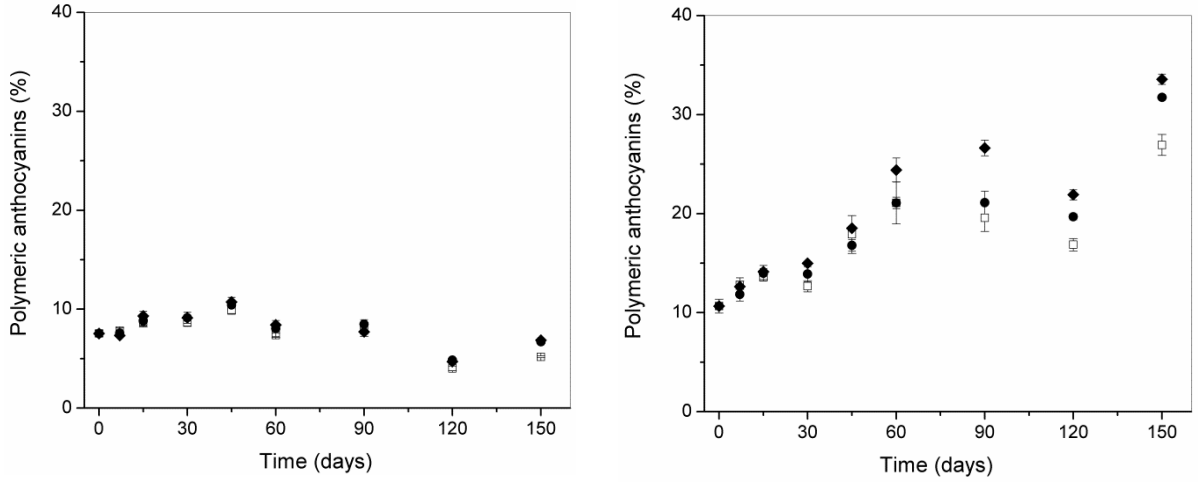


Figure 5.2 Percentage of polymeric anthocyanins with respect to the total anthocyanins microencapsulated with (a) 1SM and (b) 2WM and stored at □ 5 ºC, ● 25 ºC and ◆ 35 ºC for 150 days.

5.3.1.1.2 Individual anthocyanins

The evolution of the main individual anthocyanins in the grape juice powder during storage was evaluated by HPLC. In both the fresh grape juice and the microencapsulated grape juice, 21 monomeric anthocyanins derived from delphinidin, cyanidin, petunidin, peonidin and malvidin were found. The chromatographic anthocyanin profile was practically the same at the start and end of storage, although a decrease in the intensity of the signal was evident (Figure 5.3).

The Principal Component (PC) analysis was applied to the data with the aim of highlighting the relationships between individual anthocyanin percentages and the storage time or temperature. Figure 5.4 shows the representation of samples corresponding to the treatments 1SM, 2SM, 1WM and 2WM in the planes determined by the first Principal Component (PC1) and second Principal Component (PC2), as well as the percentage of variance explained by each PC.

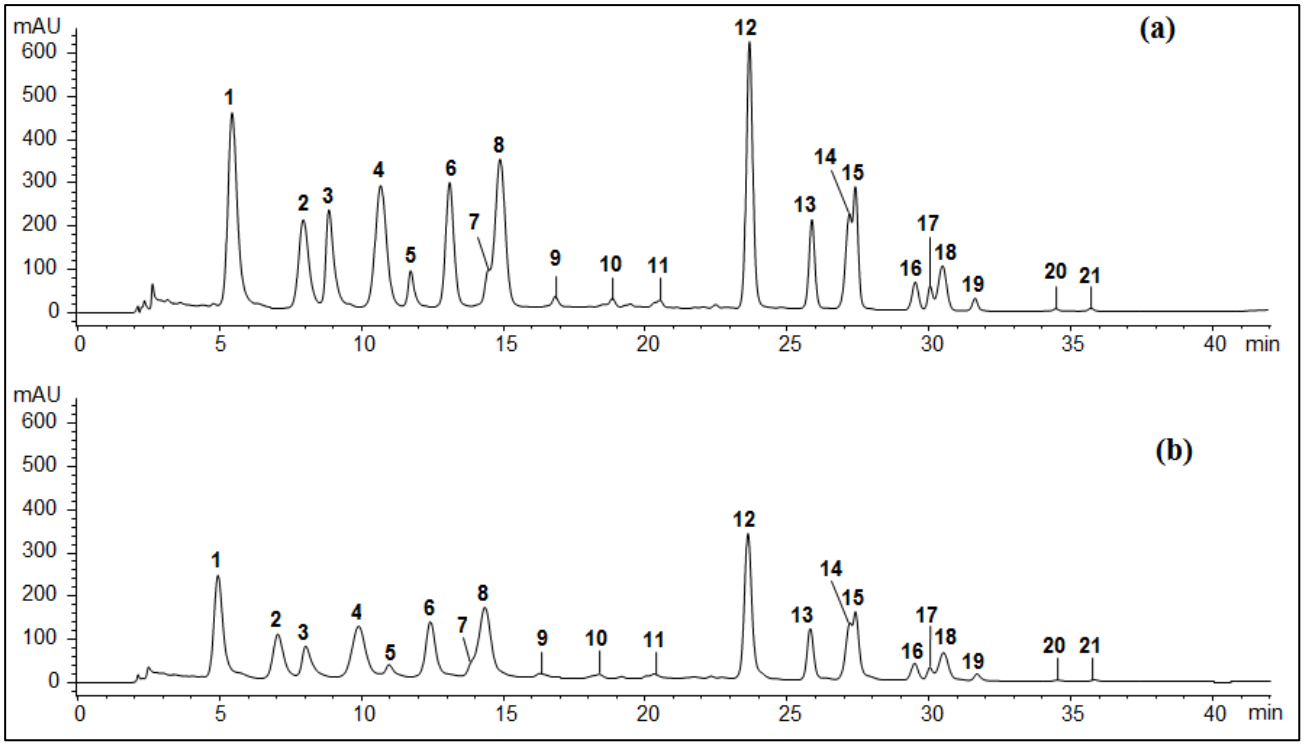
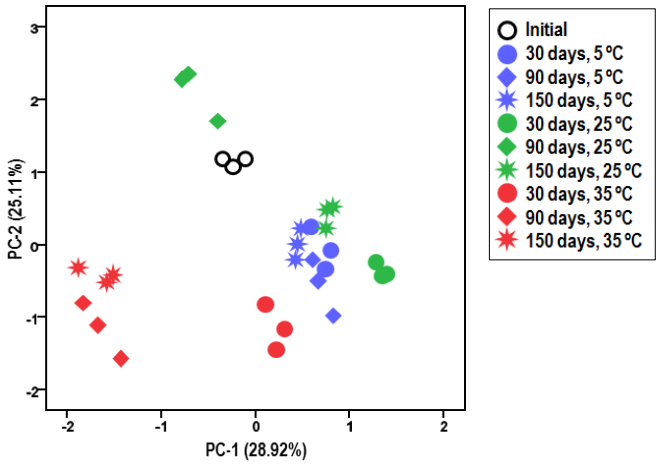


Figure 5.3 Chromatographic profile obtained at 520 nm of the 1WM treatment stored at 35 ºC: (a) zero storage time and (b) final storage time.

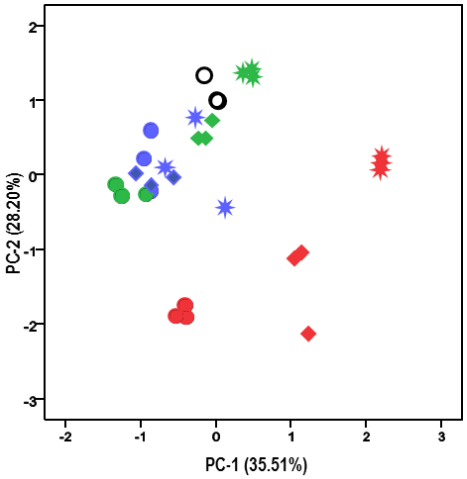
Peak assignation: 1, dp-3,5-diglc; 2, cy-3,5-diglc; 3, dp-3glc; 4, pt-3,5-diglc; 5, cy-3glc; 6, pn-3,5-diglc; 7, dp-3-acglc-5glc; 8, mv-3,5-diglc; 9, pt-3glc; 10, mv-3glc; 11, dp-3-cfglc-5glc; 12, dp-3-cmglc-5glc; 13, cy-3-cmglc-5glc; 14, pt-3-cmglc-5glc; 15, dp-3cmglc; 16, pn-3-cmglc-5glc; 17, cy-3cmglc; 18, mv-3-cmglc-5glc; 19, pt-3cmglc; pn-3cmglc; 20, mv-3cmglc.

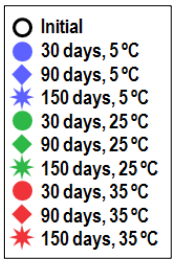
Abbreviations: dp, delphinidin; cy, cyanidin; pt, petunidin; pn, peonidin; mv, malvidin; 3,5-diglc, 3,5-diglucosides; 3-glc, 3-glucoside; 3-acglc-5-glc, 3-(6″-acetyl)-glucoside-5-glucoside; 3-cfglc-5-glc, 3-(6″-caffeoyl)-glucoside-5-glucoside; 3-cmglc-5-glc, 3-(6″-p-coumaroyl)-glucoside-5-glucoside; 3-cmglc, 3-(6″-p-coumaroyl)-glucoside.



Loadings for more correlated variables	PC-1 (28.92%)	PC-2 (25.11%)	PC-3 (17.67%)
mv-3-cmglc-5-glc	**-,962**	-,127	-,114
dp-3-glc	**,888**	,389	-,139
pt-3,5-diglc	**,851**	,369	,183
cy-3-cm glc-5glc	**-,828**	-,364	-,263
pn-3-cmglc-5glc	**-,817**	,336	-,447
pt-3-cmglc	,177	**,970**	-,036
cy-3-cmglc	-,085	**,948**	-,175
mv-3-cmglc	,329	**,932**	-,082
pn-3-cmglc	,311	**,920**	-,070
mv-3-glc	,259	,156	**,921**
dp-3-cfglc-5-glc	,233	-,317	**,818**

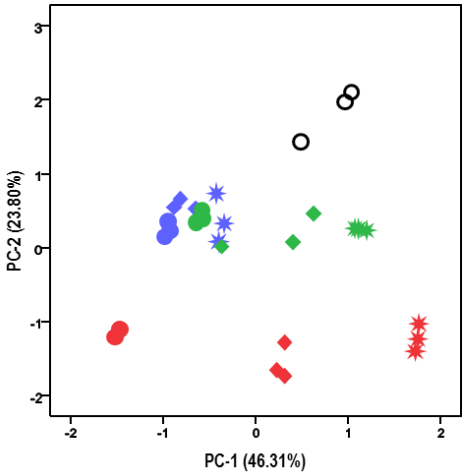
(a)

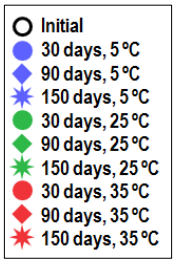




Loadings for more correlated variables	PC-1 (35.51%)	PC-2 (28.20%)	PC-3 (12.88%)
pt-3,5-diglc	**-,961**	,153	,155
mv-3-cmglc-5-glc	**,942**	-,184	,022
cy-3-cm glc-5glc	**,932**	-,054	,128
dp-3-cmglc-5 glc	**,906**	,324	-,093
dp-3-glc	**-,828**	,435	,215
pn-3-cmglc-5glc	**,818**	,473	-,226
mv-3-cmglc	-,235	**,944**	,069
pn-3-cmglc	-,245	**,929**	-,020
cy-3-cmglc	,242	**,925**	-,066
pt-3-cmglc	,396	**,881**	,080
mv-3,5-diglc	,131	**-,869**	-,121
cy-3 glc	-,107	-,036	**,885**

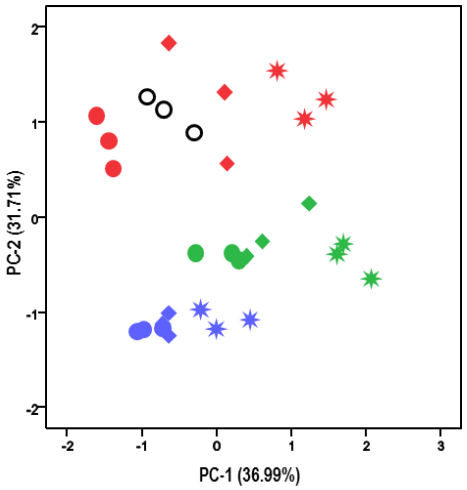
(b)

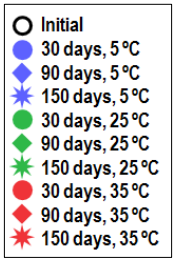




Loadings for more correlated variables	PC-1 (46.31%)	PC-2 (23.80%)	PC-3 (14.91%)
cy-3-cmglc	**,978**	,020	-,091
mv-3-cmglc	**,969**	,060	-,055
pt-3-cmglc	**,960**	-,078	,126
pn-3-cmglc	**,945**	-,160	,114
pt-3-cmglc-5 glc	**,896**	-,109	,109
pn-3-cmglc-5glc	**,876**	-,392	-,217
cy-3-cm glc-5glc	**,826**	-,492	,243
dp-3-cmglc-5 glc	**,822**	-,534	,085
pt-3-glc	-,357	**,856**	-,227
dp-3-glc	-,521	**,837**	,021
cy-3 glc	-,018	,393	**,894**
pn-3,5-diglc	-,270	-,240	**,846**

(c)





Loadings for more correlated variables	PC-1 (36.99%)	PC-2 (31.71%)	PC-3 (13.59%)
mv-3-cmglc	**,970**	,062	,088
cy-3-cmglc	**,907**	,361	,008
pn-3,5-diglc	**-,904**	-,246	-,136
cy-3 glc	**-,861**	,025	,196
pn-3-cmglc	**,848**	,303	-,243
dp-3-cfglc-5-glc	,061	**-,942**	,001
pt-3-glc	-,157	**-,864**	,435
dp-3-glc	-,354	**-,832**	,381
cy-3,5-diglc	-,181	-,127	**,966**

(d)

Figure 5.4 Principal component analysis (plot of the samples in the PC-1 vs. PC-2 graph; tables of the "loadings" of the most correlated variables and percentage of total variance explained by

each PC) as applied to individual anthocyanin percentages in relation to storage time and temperature in the treatments: (a) 1SM, (b) 2SM, (c) 1WM, (d) 2WM.

In the 1SM treatment the percentages of *p*-coumaroylated derivatives of anthocyanidin-3-glucosides (3-glc) were more correlated with PC2. The proportion of these anthocyanins decreased in relation to the initial sample for all the temperatures and storage times, except for the sample stored for 90 days at 25 ºC, which appears to be an anomaly. On the other hand, some *p*-coumaroylated derivatives of anthocyanidin-3,5-diglucosides (3,5-diglc) with negative loadings, together with non-acylated derivatives of both 3,5-diglc and 3-glc, with positive loadings, were more correlated with PC1. The mixture of anthocyanins correlated with PC1 is difficult to interpret, in addition, they are not grouped with a time criteria: at 5 ºC there are no differences between these variables; at 25 ºC the temporal logic is reversed, samples that appeared after 150 days now appearing between 30 and 90 days; and at 35 ºC there was no differentiation between the samples stored for 90 and for 150 days, and they showed a greater proportion of *p*-coumaroylated derivatives of anthocyanins 3,5-diglc than after only 30 days of storage.

In general, for the 2SM, 1WM and 2WM treatments some of the *p*-coumaroylated derivatives of the anthocyanins were more correlated with PC1 (positive loadings). Thus, with the increase in storage time, the proportion of *p*-coumaroylated anthocyanins in the anthocyanin profile increased. This trend was only observed for the temperature of 5 °C, but began to be apparent at a temperature of 25 °C and was clearly apparent after storage at 35 °C. This behavior regarding the variables more correlated to PC1 can be explained by the greater stability of the acylated anthocyanins, and certainly by the ability of the *p*-coumaroyl residues to be involved in the formation of inter- and intra-molecular co-pigmentation complexes (Lago-Vanzela et al., 2013, Patras, 2010), which could contribute to an improvement in the stability of the anthocyanins. These results were in agreement with those observed by Lago-Vanzela et al. (2014) in their study of the accelerated aging of wines from BRS Violeta grapes. The authors reported that the half-life values for *p*-coumaroylated anthocyanidin 3,5-glucosides, aged at 50 °C, almost doubled in relation to their corresponding non-acylated derivatives.

In the 2SM treatment some non-acylated derivatives of anthocyanidin-3,5-diglucosides and anthocyanidin-3-glucosides were more correlated with PC1 and presented negative loadings, thus, as the storage time increased, these anthocyanins decreased their proportion in the

anthocyanin profile. The *p*-coumaroylated anthocyanidin-3-glucosides and the non-acylated malvidin-3,5-diglucoside were more correlated to PC2, and presented similar behavior to 1SM, which did not show any logical differentiation with temperature.

In the 1WM treatment, the PC2 allowed one to evaluate the effect of storage temperature on the anthocyanins. It was observed that each group of samples corresponding to a determined storage temperature was in the same PC axis, regardless of storage time. The anthocyanins that could differentiate the control (0 days of storage) from the stored samples, and could differentiate the storage temperature, were minority anthocyanins with common structural features: they are 3-glucosides and belong to the series of B-ring tri-substituted anthocyanins (pt-3-glc and dp-3-glc), having at least one *o*-diphenol grouping (1 in petunidin; 2 in delphinidin). The proportion of these two anthocyanins decreased as compared to the control, the decrease being similar at 5 ºC and 25 ºC, and greater at 35 ºC.

For the 2WM treatment, the non-acylated anthocyanin derivatives (3,5-diglc and 3-glc) were more correlated with PC1 and presented negative loadings, thus their proportions decreased with storage time. The caffeoyl derivatives of both anthocyanidin-3,5-glucosides and 3-glucosides were more correlated with PC2 and presented negative loadings, their proportions reducing with increase in storage temperature. However the anthocyanins in the samples stored at 35 °C did not differ from those of the control, which seems to be anomalous behavior.

5.3.1.2 Flavonols

At zero time (0 days) seven flavonols were found in the microencapsulated grape juice: myricetin 3-glucoside, quercetin 3-galactoside, quercetin 3-glucuronide, quercetin 3-glucoside, free myricetin, laricitrin 3-glucoside, isorhamnetin 3-glucoside and syringetin 3-glucoside. The most important type of flavonol in the microencapsulated grape juice was based on myricetin (average of 77% at zero time), myricetin 3-glucoside being the main individual flavonol found (average of 70%), followed by the quercetin-based flavonols as the second most important type (average of 16%). The flavonol profile was the same at zero time and at the end of storage.

Table 5.2 shows the amounts of flavonols in the powdered grape juice at zero time and at the end of storage. The 1SM treatment showed a significant loss of flavonols at the end of storage at the three temperatures.

The treatments with 2SM and 2WM only showed significant losses when stored at 25 ºC. On the other hand, the 1WM treatment did not lead to a significant loss of flavonols at any of the three storage temperatures, which means that this formulation was the most efficient in protecting this phenolic compound during the shelf-life. An evaluation of the effect of temperature at the end of the storage period showed that temperature did not influence the amount of flavonols within the same treatment.

Table 5.2 Flavonol contents (mg/100 g powdered juice, expressed as quercetin 3-glucoside equivalents) in microencapsulated grape juice stored at 5, 25 and 35 ºC, at the start and end of storage.

Days	**T (ºC)**	**Flavonols (mg/100 g)**			
		1SM	**2SM**	**1WM**	**2WM**
0	-	26.72 ± 0.68^{a}	29.68 ± 1.20^{a}	31.60 ± 1.62^{a}	29.89 ± 0.57^{a}
150	5	21.95 ± 0.40^{b}	25.06 ± 1.20^{ab}	25.95 ± 0.19^{a}	26.68 ± 1.48^{ab}
	25	20.76 ± 1.63^{b}	22.68 ± 0.80^{b}	26.79 ± 1.46^{a}	25.58 ± 0.85^{b}
	35	20.96 ± 1.62^{b}	26.38 ± 2.30^{ab}	25.29 ± 1.51^{a}	25.96 ± 1.99^{ab}

Means with different letters in the same column indicate significant differences ($p < 0.05$) between the beginning and end of the storage time according to the Student test.

5.3.1.3 Hydroxycinnamic acid derivatives (HCAD)

The following nine hydroxycinnamic acid derivatives (HCAD) were found in the microencapsulated grape juice before the storage period (0 days): *trans*-caftaric acid, three isomers of caffeoyl-glucose (caffeoyl-glucose-1, caffeoyl-glucose-2 and caffeoyl-glucose-3), *trans*-coutaric acid, three isomers of *p*-coumaroyl-glucose (*p*-coumaroyl-glucose-1, *p*-coumaroyl-glucose-2 and *p*-coumaroyl-glucose-3) and free caffeic acid. The most important types of hydroxycinnamic acid derivatives found in the microencapsulated grape juice were *trans*-caftaric acid and *p*-coumaroyl-glucose. The HCAD profile did not change during storage. All the samples showed significant losses of HCADs in the powder at the end of the storage period (Table 5.3).

When the 1WM treatment was stored at 25 ºC, a reduction of 56% was observed in the amount of hydroxycinnamic acid, showing that this carrier agent and temperature were not

suitable for preserving HCAD during the shelf-life. In addition, the storage temperature did not influence the amount of HCAD remaining within the same treatment.

Table 5.3 Hydroxycinnamic acid derivatives contents (mg/100 g powdered juice as caffeic acid equivalents) in microencapsulated grape juice stored at 5, 25 and 35 ºC, at the start and end of storage.

Days	T (ºC)	Hydroxycinnamic acid derivatives (mg/100 g)			
		1SM	**2SM**	**1WM**	**2WM**
0	-	32.75 ± 0.68^{a}	30.68 ± 1.18^{a}	24.26 ± 3.82^{a}	31.25 ± 1.51^{a}
150	5	22.54 ± 0.78^{b}	23.51 ± 1.57^{b}	14.72 ± 0.73^{b}	21.91 ± 2.03^{b}
	25	21.94 ± 0.92^{b}	22.56 ± 1.74^{b}	11.47 ± 0.78^{b}	22.82 ± 1.49^{b}
	35	19.30 ± 3.65^{b}	24.66 ± 1.06^{b}	15.27 ± 3.88^{b}	21.72 ± 1.99^{b}

Means with different letters in the same column indicate significant differences ($p < 0.05$) between the beginning and end of storage according to the Student t test.

5.3.1.4 Flavan-3-ol monomers and dimers

The total amount of flavan-3-ols in grape juice is expected to be low. This kind of phenolic compound can be found as monomers, B-type procyanidin dimers and oligomeric and polymeric proanthocyanidins (also called condensed tannins). The latter compounds are poorly soluble in water, mainly located in solid parts of the grapes, namely the seeds and skins, and the grape juices obtained using the steam extraction method usually contain low amounts of condensed tannins (YAMAMOTO et al., 2015). The main flavan-3-ol monomer found in microencapsulated grape juice was catechin, while the main flavan-3-ol dimer was procyanidin B1 (Table 5.4).

All the samples showed significant losses of catechin, epicatechin, procyanidin B1 and procyanidin B2 in the powder at the end of storage when stored at 35 ºC. In general, the 2SM treatment presented the greatest loss in the amount of flavan-3-ols as compared to the other treatments, showing that this carrier agent is not suitable for preserving this kind of phenolic compound during the shelf life.

On the other hand, the 1WM and 2WM treatments showed no significant losses of catechin at the end of the storage time when stored at 5 and 25 ºC. In addition, the 1WM treatment showed no loss of epicatechin under the same storage conditions and the 2WM treatment preserved the initial epicatechin content when stored at 5 ºC. Thus the samples

formulated with WM were more efficient in protecting the flavan-3-ol monomers during the shelf life than the samples formulated with SM.

Table 5.4 Flavan-3-ol monomer and dimer contents (B-type procyanidins) (mg/100 g powdered juice, expressed as catechin equivalents) in microencapsulated grape juice stored at 5, 25 and 35 ºC, at the start and end of storage.

	Days	T (ºC)	Flavan-3-ol (mg/100 g)			
			1SM	2SM	1WM	2WM
Catechin	0	-	31.85 ± 3.47^{a}	89.63 ± 19.08^{a}	58.93 ± 6.37^{a}	37.20 ± 4.70^{a}
	150	5	24.13 ± 3.05^{b}	31.17 ± 2.35^{b}	58.13 ± 4.76^{a}	35.38 ± 1.35^{a}
		25	21.52 ± 5.89^{b}	22.88 ± 7.23^{b}	58.69 ± 3.74^{a}	36.32 ± 2.09^{a}
		35	19.78 ± 0.99^{b}	34.99 ± 1.77^{b}	42.74 ±5.76^{b}	26.04 ± 1.71^{b}
Epicatechin	0	-	7.15 ± 1.09^{a}	18.64 ± 2.99^{a}	12.91 ± 1.88^{a}	8.39 ±1.09^{a}
	150	5	5.99 ± 1.20^{ab}	7.99 ± 0.37^{b}	13.55 ± 1.27^{a}	8.61 ±0.61^{a}
		25	5.57 ± 1.58^{ab}	4.31 ± 1.61^{c}	11.69 ± 0.95^{a}	6.79 ±0.38^{b}
		35	4.87 ± 0.01^{b}	6.85 ± 0.80^{bc}	7.63 ±1.24^{b}	4.86 ±0.27^{c}
Procyanidin B1	0	-	19.62 ± 2.19^{a}	59.95 ± 9.09^{a}	43.67 ± 3.43^{a}	39.14 ± 5.19^{a}
	150	5	15.53 ± 2.02^{ab}	25.88 ± 2.57^{b}	47.49 ± 3.73^{a}	33.56 ± 0.64^{b}
		25	12.89 ± 3.95^{b}	15.06 ± 5.31^{c}	34.64 ± 1.87^{b}	26.88 ± 1.93^{c}
		35	11.06 ± 0.78^{b}	21.89 ± 2.31^{bc}	19.23 ± 2.74^{c}	17.50 ± 0.69^{d}
Procyanidin B2	0	-	6.31 ± 1.11^{a}	17.26 ± 2.08^{a}	13.69 ± 1.64^{ab}	12.68 ± 1.54^{a}
	150	5	5.68 ± 0.90^{ab}	9.24 ± 0.90^{b}	15.60 ± 1.14^{a}	11.48 ± 0.86^{a}
		25	4.48 ± 1.36^{ab}	4.66 ± 1.75^{c}	11.81 ± 0.19^{b}	8.67 ± 0.23^{b}
		35	4.21 ± 0.23^{b}	7.09 ± 0.93^{bc}	6.95 ± 0.77^{c}	5.51 ± 0.43^{c}

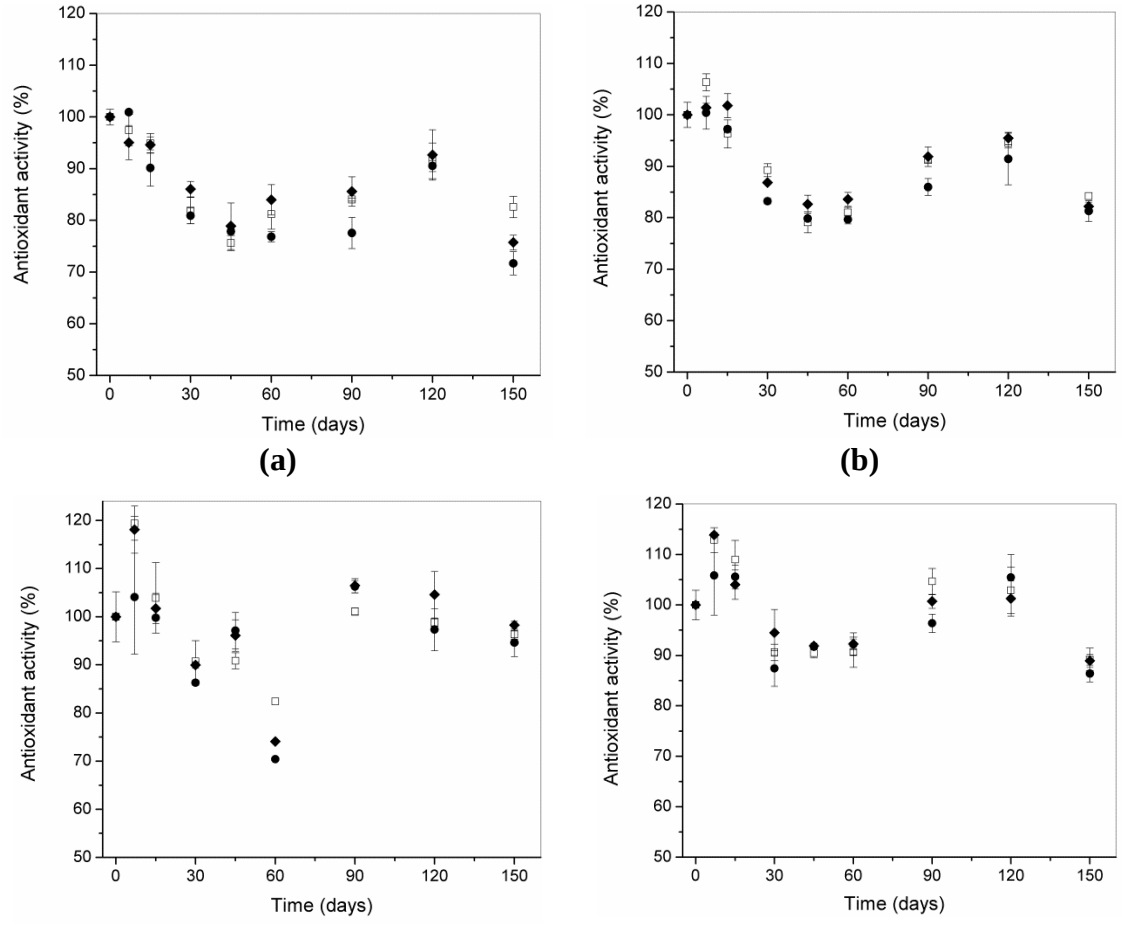
Means with different letters in the same column indicate significant differences ($p < 0.05$) between the beginning and end of storage according to the Student t test.

Similar results were found by Spanos and Wrolstad (1990) and by Ross, Hoye and Fernandez-Plotka (2011) for grape juice during processing and for grape seed flour, respectively. The authors observed that the procyanidins were sensitive to heat degradation. Procyanidins become sensitive to non-enzymatic condensation and polymerization at elevated temperatures, resulting in decreases in the contents (SPANOS; WROLSTAD, 1990).

5.3.2 Antioxidant activity

Since the different microencapsulation treatments led to differences in the phenolic composition of the powdered grape juices obtained, differences in their antioxidant activities (expressed as mmoles Trolox/100 g powdered juice) were also expected: 1SM = 9.83; 2SM = 11.06; 1WM = 7.94; and 2WM = 8.60. To study the effects of time and temperature separately, the antioxidant activity at the start of storage was considered as 100%, thus excluding the differences amongst the four treatments.

The storage temperature and time did not influence the antioxidant activity of the powdered grape juices. For the SM treatments there was a reduction in antioxidant activity (approximately 25%) after 45 days, followed by a recovery and then another fall at the end of the storage period (Figures 5.5A and 5.5B). For the WM treatments, no tendency for degradation with time was verified (Figures 5.5C and 5.5D) and the decrease in antioxidant activity was small, being only 6% for 1WM and 14% for 2WM at the end of storage.



(c) **(d)**

Figure 5.5 Antioxidant activity of the microencapsulated grape juice with (a) 1SM; (b) 2SM; (c) 1WM; and (d) 2WM and stored at □ 5 ºC, ● 25 ºC and ◆ 35 ºC for 150 days.

On the other hand, during storage the antioxidant activity appeared to have had no correlation with the observed decrease in the total anthocyanin content, which showed linear degradation with time. Fracassetti et al. (2013), studying the storage of freeze-dried wild blueberry powder, also found a reduction in total anthocyanins with maintenance of the antioxidant activity. According to these authors, this was probably due to the formation of antioxidant polymers, such as low molecular weight procyanidins, or the formation of degradation products of the anthocyanins or phenolic acids, which also show antioxidant activity.

It appears that the antioxidant activity has some correlation with the polymerized anthocyanins. The treatment with WM showed greater anthocyanin polymerization during storage, and also showed greater antioxidant activity. According to Laine et al. (2008), the phenolic compounds are typically "team players", meaning that they work synergistically by supporting each other's antioxidant activities. The loss of original phenolics might be compensated by newly formed phenolics with equal or improved antioxidant activities.

5.3.3 Color changes

Independent of the type of carrier agent used, the temperature and storage time did not influence the color of the corresponding reconstituted grape juice, the differences being smaller than 1.5 for any time/temperature combination, except for the 1WM treatment stored for 150 days at 35 °C (Table 5.5).

This result is important because it demonstrates that microencapsulated grape juice can maintain the color of the original product during its shelf-life, and hence its sensory aspects. According to Obón et al. (2009), a total color difference from 0 to 1.5 indicates that, visually the sample is almost identical to the original one, whilst in the range from 1.5 to 5, the difference in color can be distinguished.

Reconstituted grape juice from the 1WM treatment presented a color difference value of 1.79 at the end of the storage time at 35 ºC. The lightness (L*) increased slightly from 26.75 at zero time to 28.05 at the end, implying in a brighter juice with less color. Changes in the

contribution of different color components were observed. Thus the red color component (a*, positive value) increased during storage at 35 ºC from 4.35 (0 days) to 4.53 (150 days). This behavior could be related to the formation of anthocyanin-derived pigments that stabilize the flavylium red-colored form (LAGO-VANZELA et al., 2014).The blue color component (b*, negative value), associated with purplish nuances, decreased its initial negative value from -3.28 to -2.07 at the end of storage. The evolution from negative to positive or less negative b* values is associated with the loss of copigmentation effects accompanied by the formation of anthocyanin-derived red-orangish pigments such as pyranoanthocyanins (LAGO-VANZELA et al., 2014), even though the latter compounds were not detected in the samples of reconstituted grape juices analyzed.

Table 5.5 Total color difference of reconstituted grape juices with respect to microencapsulated juice color at initial time, stored at 25 and 35 ºC during 150 days.

Time (days)	1SM	2SM	1WM	2WM
	ΔE* (25 ºC)			
0	-	-	-	-
7	0.20 ± 0.03	0.06 ± 0.09	0.25 ± 0.04	0.34 ± 0.06
15	0.28 ± 0.17	0.05 ± 0.03	0.25 ± 0.05	0.44 ± 0.04
30	0.24 ± 0.09	0.3 ± 0.07	0.59 ± 0.10	0.51 ± 0.06
45	0.32 ± 0.13	0.28 ± 0.10	0.95 ± 0.12	0.73 ± 0.02
60	0.38 ± 0.07	0.19 ± 0.06	0.72 ± 0.06	0.54 ± 0.07
90	0.29 ± 0.08	0.22 ± 0.04	0.79 ± 0.16	0.87 ± 0.06
120	0.70 ± 0.07	0.3 ± 0.11	0.76 ± 0.13	1.17 ± 0.23
150	0.91 ± 0.07	1.29 ± 0.11	1.26 ± 0.10	0.95 ± 0.14
	ΔE* (35 ºC)			
0	-	-	-	-
7	0.13 ± 0.10	0.08 ± 0.04	0.22 ± 0.12	0.35 ± 0.04
15	0.07 ± 0.08	0.10 ± 0.02	0.32 ± 0.05	0.59 ± 0.10
30	0.2 ± 0.00	0.36 ± 0.05	0.72 ± 0.06	0.6 ± 0.13
45	0.31 ± 0.11	0.32 ± 0.02	1.26 ± 0.16	0.87 ± 0.13
60	0.09 ± 0.03	0.21 ± 0.07	1.13 ± 0.22	0.69 ± 0.03
90	0.35 ± 0.08	0.23 ± 0.04	1.04 ± 0.08	0.73 ± 0.00
120	0.7 ± 0.01	0.37 ± 0.04	1.17 ± 0.10	1.28 ± 0.11

150	1.14 ± 0.13	1.22 ± 0.07	1.79 ± 0.03	1.21 ± 0.03

The greatest loss of total anthocyanins was already observed in the 1WM treatment during storage as compared to the other treatments, and this was accompanied by the same behavior in the color of the reconstituted juice. Thus the 1WM treatment does not appear to be a good option for the microencapsulation of anthocyanins. Idham, Muhamad & Sarmidi (2012), studying the color of spray-dried roselle powder, observed that the storage period and encapsulating agent significantly affected the color change, whereas the storage temperature had no effect.

5.4 CONCLUSIONS

The 1SM treatment stored at 5 ºC presented the lowest anthocyanin degradation rate and consequently the longest half-life time, whereas the 1WM treatment stored at 35 ºC presented the highest degradation rate and the shortest half-life time, suggesting that this carrier agent is not effective in protecting anthocyanins. It is very likely that the largest amounts of carrier agent and highest ratios of maltodextrin also improved the stability of the anthocyanins. The anthocyanins polymerized more in WM than in SM. The WM treatments presented a positive linear correlation between the polymerized anthocyanin content and the storage time. The individual anthocyanin profile changed slightly during storage. In the 2SM, 1WM and 2WM treatments an increase in storage time resulted in a greater proportion of *p*-coumaroylated anthocyanins. This behavior can be explained by the greater stability of the acylated anthocyanins, especially those acylated with *p*-coumaroyl residues. The 1SM treatment led to significant losses of flavonols after 150 storage days, but this did not happen with the 1WM treatment. All the samples showed significant losses of HCAD in the powdered juice during storage. The loss of flavan-3-ols occurred in the powdered juice at the end of storage when stored at 35 ºC and the 2SM treatment presented the greatest loss of this kind of phenolic compound.

Considering a single treatment, the time/temperature combination did not influence the antioxidant activity of the powdered grape juice after 150 days of storage. The storage time and temperature did not influence the color of the reconstituted grape juice, the color difference being less than 1.5 at the end of storage, except for the 1WM treatment stored at 35 °C for 150 days.

The most remarkable result of this study was the thermal stability of the 1SM treatment. Microencapsulation was an effective way of stabilizing phenolic compounds, ensuring a longer shelf life as well as a range of possible industrial applications.

ACKNOWLEDGMENTS

The authors are grateful to CAPES (Grants PDSE 99999.013844/2013-000), FAPESP (Grants 2012/09074-4 and 2010/09614-3) and EMBRAPA Grape and Wine (Jales, Brazil). S.G.A. is grateful to the Fondo Social Europeo and the Junta de Comunidades de Castilla-La Mancha for co-funding his contract through the INCRECYT program.

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CONCLUSÕES GERAIS

6 CONCLUSÕES GERAIS

Ao avaliar os parâmetros de secagem em *spray dryer* observou-se que baixa vazão de alimentação resultou em pós com menor umidade, enquanto o elevado fluxo de ar resultou em maior rendimento, embora tenha afetado a coloração do produto. A concentração de maltodextrina também influenciou na coloração do suco em pó. Com base nos resultados dos ensaios inciais, as condições escolhidas para a produção de suco de uva em pó foram: temperatura do ar de secagem de 140ºC, vazão de alimentação de 2 mL/min e baixa concentração de carreador.

Ao avaliar a influência da concentração de carreador (CAC, g de agente carreador/g sólidos solúveis do suco) e proporção de proteína (g proteína/100 g de carreador) no processo de secagem e nas características das microesferas formadas com maltodextrina e proteína de soja (SM) ou maltodextrina e proteína de soro (WM), observou-se que maior CAC resultou em maior rendimento do processo. Nos sucos em pó produzidos com formulações WM, o aumento de CAC resultou na maior retenção das antocianinas (95%), enquanto nos sucos produzidos com formulações SM, maior CAC melhorou a eficiência de encapsulação (99%). As microesferas produzidas com WM apresentaram maior aglomeração, enquanto o uso de SM resultou em partículas mais esféricas. O aumento da concentração de carreador e proporção de proteínas resultou na maior claridade dos pós. Entretanto, após a reconstituição dos pós em água, os sucos de uva reconstituídos apresentaram coloração muito semelhante ao suco natural.

As concentrações de agente carreador e proporções de proteína que apresentaram os melhores resultados globais foram escolhidas para realizar o estudo das propriedades físico-químicas e estabilidade das antocianinas durante o armazenamento. Os quatro melhores tratamentos foram: CAC 1.25, 10.00%; CAC 1.00, 5.86%; CAC 0.75, 30.00%; e CAC 0.85, 20.00%, sendo codificados como 1SM, 2SM, 1WM e 2WM, respectivamente.

O modelo de Guggenheim, Anderson and De Boer (GAB) apresentou bom ajuste às isotermas de sorção das amostras produzidas com os diferentes agentes carreadores. A temperatura de transição vítrea diminuiu com o aumento da atividade de água, o que confirma o efeito plasticizante da água sobre o suco de uva em pó. O tratamento 1SM resultou em menor higroscopicidade do pó e maior força de compressão, o que demonstra que foi menos pegajoso que os outros pós avaliados.

No estudo da estabilidade das antocianinas frente à luz, observou-se que o tratamento 1SM ofereceu a melhor proteção para as antocianinas, enquanto as antocianinas microencapsuladas com 2SM sofreram maior degradação. Após 30 dias de exposição à luz, houve uma queda na quantidade de antocianinas nos quatro tratamentos (perdas entre 27 e 44%), que foi seguido por uma estabilização durante os próximos 60 dias de armazenamento (perdas de 1%) O tratamento 1SM não apresentou mudanças significativas na sua coloração durante armazenamento com exposição a luz por 90 dias.

No estudo da estabilidade dos compostos fenólicos armazenado em diferentes temperaturas verificou-se que o uso da formulação 1SM como material de parede resultou na maior proteção das antocianinas, enquanto as amostras microencapsuladas com 1WM não apresentaram boa estabilidade térmica. O perfil das antocianinas individuais apresentou pequenas mudanças, sendo que os tratamentos 2SM, 1WM e 2WM apresentaram aumento na proporção das antocianinas *p*-cumarilas durante o armazenamento. Após 150 dias de armazenamento, somente o tratamento 1SM apresentou perdas de flavonóis. Todos os tratamentos tiveram perdas de ácidos hidroxicinâmicos, independente da temperatura de armazenamento, e perdas de flavan-3-óis quando o suco foi armazenado a 35 ºC. Por outro lado, o tempo e a temperatura não influenciaram a atividade antioxidante do suco de uva microencapsulado e nem a coloração do suco de uva reconstituído.

No estudo da estabilidade, o resultado mais marcante foi a estabilidade das antocianinas microencapsuladas com a matriz 1SM (mistura de proteína de soja e maltodextrina com CAC = 1,25 e 10% de proteína), as quais se mostraram mais estáveis em relação aos outros três tratamentos durante o armazenamento em diferentes temperaturas e sob a incidência de luz.

Diante do exposto, pode-se concluir que as misturas de maltodextrina com proteínas de soro ou de soja auxiliam na secagem por atomização do suco de uva e, além disso, podem ser utilizadas para encapsular antocianinas e outros compostos fenólicos, os quais se mantêm relativamente estáveis durante a vida de prateleira. O suco de uva em pó obtido por atomização pode ser considerado um aditivo promissor para a incorporação de antocianinas de uva BRS Violeta em alimentos, assim como o desenvolvimento de produtos inovadores com alto potencial colorante e diversos benefícios para a saúde.

SUGESTÕES PARA TRABALHOS FUTUROS

- Verificar se há interação físico-química entre a maltodextrina e as proteínas, o que pode afetar o processo de formação das microesferas. Esse estudo poderia ajudar a explicar as diferenças de estabilidade das antocianinas observadas em função da formulação das matrizes encapsulantes.

- Avaliar o efeito dos outros constituintes do suco na estrutura e, consequentemente, na proteção das microesferas.

- Estudar a estabilidade das matrizes proteicas ao longo do tempo, em diferentes condições de umidade relativa e temperatura, uma vez que o processo de envelhecimento das proteínas pode afetar as propriedades funcionais e sensoriais das microcápsulas.

- Aplicar o suco de uva em pó como corante em alimentos (iogurtes, sorvetes, geleias, sobremesas, etc.) e avaliar a estabilidade das antocianinas nesses produtos.

- Aplicar o suco de uva em pó como corante em alimentos e realizar análise sensorial para verificar se os agentes carreadores causam alterações no sabor do produto e a sua aceitabilidade.

- Avaliar a viscosidade do suco de uva reconstituído com misturas de maltodextrina e proteínas, e avaliar a viscosidade do suco utilizando apenas maltodextrina. Dessa forma será possível observar a influência das proteínas nas propriedades finais da bebida.

APÊNDICE

7 APÊNDICE

7.1 EXTRAÇÃO DO SUCO DE UVA

O suco de uva foi elaborado em um equipamento designado “extrator de suco” (Figura 7.1). Esse equipamento é formado por três partes principais: um recipiente perfurado onde é colocada a baga (Figura 7.1 A); um recipiente com abertura cônica no centro, para passagem do vapor e abertura lateral para o engarrafamento do suco (Figura 7.1 B); e um depósito de água para gerar o vapor necessário para extração do suco (Figura 7.1 C).

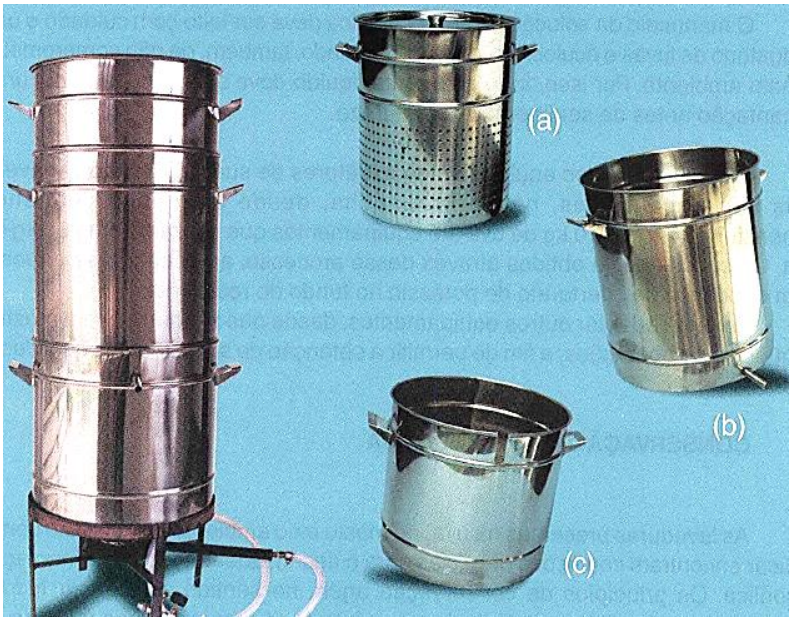

Figura 7.1 Equipamento extrator de suco de uva (RIZZON; MANFROI; MENEGUZZO,1998).

Para elaboração do suco de uva, inicialmente foram descongeladas 7 kg de uva BRS Violeta (Figura 7.2 A). Posteriormente as frutas foram acomodadas na panela extratora contendo água em ebulição (Figura 7.1 B). A extração foi realizada durante 1 hora e a temperatura do suco foi mantida entre 75 e 85 ºC. Ao terminar a extração, o suco foi mantido a 85 ºC por 20 minutos, para garantir a estabilidade biológica e a conservação da bebida sem aditivos químicos.

O envase foi realizado à quente em recipientes de vidro com capacidade para 500 mL (Figura 7.1 C). O suco foi armazenado em câmara fria à-18 ºC, ao abrigo da luz, sendo descongeladas apenas as quantidades necessárias para cada ensaio.

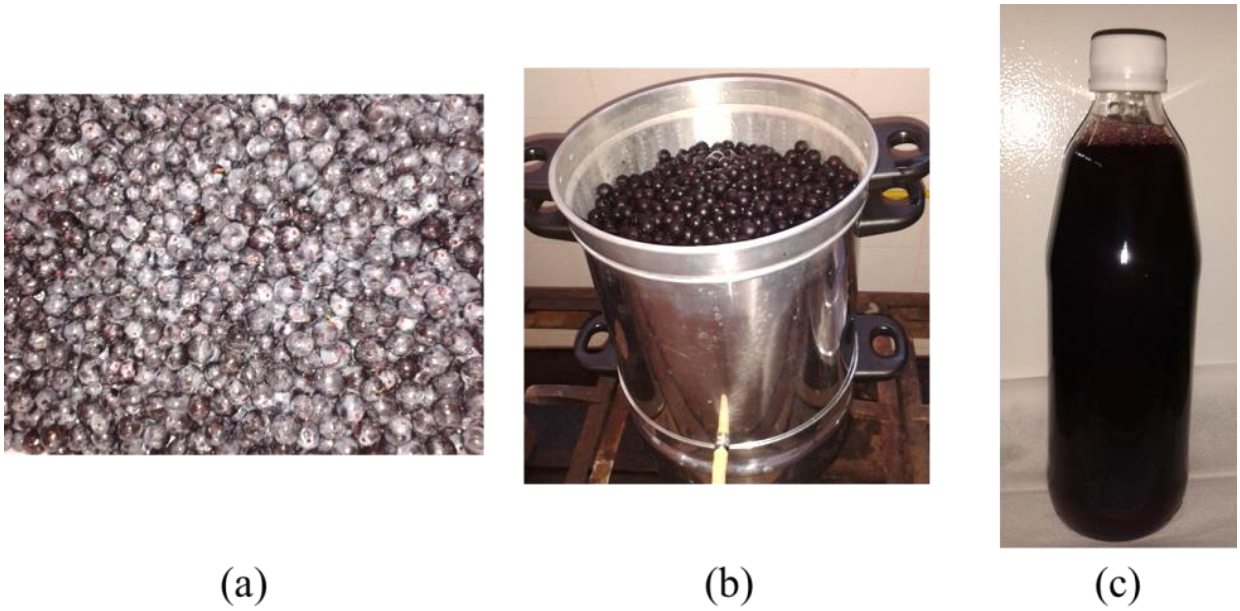

Figura 7.2 Processo de elaboração do suco de uva, (a) uva da cultivar BRS Violeta; (b) equipamento extrator do suco de uva; e (c) produto envasado.

TERMO DE REPRODUÇÃO XEROGRÁFICA

Autorizo a reprodução xerográfica do presente Trabalho de Conclusão, na íntegra ou em partes, para fins de pesquisa.

São José do Rio Preto, 25/02/2016

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Assinatura do autor