ELOÁ LOURENÇO DO CARMO

COENCAPSULAÇÃO POR SPRAY DRYING DE ANTOCIANINAS EXTRAÍDAS DE CASCAS DE UVA (*Vitis vinifera* var. Syrah) E α-TOCOFEROL: CARACTERIZAÇÃO E ESTABILIDADE

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Orientadora

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Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Carmo, Eloá Lourenço do.
Coencapsulação por spray drying de antocianinas extraídas de cascas de uva (*Vitis vinifera* var. Syrah) e α-tocoferol: caracterização e estabilidade / Eloá Lourenço do Carmo. - 2021.
72 p. : il.

Orientadora: Soraia Vilela Borges.
Coorientador: Diego Alvarenga Botrel.
Tese (Doutorado) - Universidade Federal de Lavras, 2021.
Bibliografia.

1. Vitamina E. 2. Compostos bioativos. 3. Aproveitamento de resíduos. 4.Corante natural. I. Borges, Soraia Vilela. II. Botrel, Diego Alvarenga. III. Título.

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CO-ENCAPSULATION BY SPRAY DRYING OF ANTHOCYANINS EXTRACTED FROM GRAPE SKINS (*Vitis vinifera* var. Syrah) AND α-TOCOPHEROL: CHARACTERIZATION AND STABILITY

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

APROVADA em 25 de Outubro de 2021.

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RESUMO GERAL

Neste trabalho, foi realizada a caracterização de micropartículas obtidas da coencapsulação por *spray drying* de antocianinas extraídas de cascas de uva (*Vitis vinifera* var. Syrah) e α-tocoferol. O extrato das cascas de uva foi homogeneizado com goma arábica (20%), Tween 80 (0,2%) e α-tocoferol, sendo este último adicionado nas concentrações de zero (controle), 0,5, 1,0, 1,5 e 2,0% (m/m) em relação à emulsão total. Foi avaliado o teor de antocianinas, atividade antioxidante, teor de fenólicos totais, concentração de α-tocoferol, cor, higroscopicidade, morfologia e espectros de FTIR das amostras de cada tratamento. Observou-se que maior concentração do composto proporcionou maior teor de fenólicos totais (8,69 mg ácido gálico equivalente/g pó em base seca), bem como menor higroscopicidade (21,21 g de umidade adsorvida/100 g de pó em base seca) e menor teor de antocianinas (0,917 mg malvidina-3-glucosídeo equivalente / g pó em base seca). Concentrações intermediárias de α-tocoferol proporcionou maior incidência de micropartículas de superfície lisa e maiores atividades antioxidante. Os espectros de FTIR confirmaram a eficácia do processo, apresentando bandas características dos compostos coencapsulados, como em 1600 cm^{-1}, relacionada a ligações de hidrogênio de grupos de OH dos carboidratos da uva, e 1210 cm^{-1}, que se refere ao éter aromático presente na estrutura do α-tocoferol. A partir dos resultados obtidos, foi possível verificar que concentrações entre 1,5 e 2,0% (m/m) de α-tocoferol resultaram em boas propriedades dos pós (menor higroscopicidade, maior teor de fenólicos totais e atividade antioxidante). A estabilidade das partículas adicionadas de zero (controle), 1,0 e 2,0% (m/m) de α-tocoferol também foi avaliada em relação a três fatores externos principais (umidade, temperatura e luz), por meio de três condições diferentes de armazenamento: em ambiente com umidade relativa (UR) de 75% (20°C, no escuro) por 47 dias; temperatura de 60°C (UR=65%, no escuro) durante 112 dias e com exposição à luz (25°C, UR=70%) por 105 dias. As partículas foram avaliadas ao longo do tempo em relação a atividade antioxidante, teor de antocianinas, fenólicos e coloração. Nos tempos inicial e final, foram determinados o teor de α-tocoferol e os espectros de FTIR das amostras. Em relação a coloração, teor e tempo de meia-vida das antocianinas, foi observado efeito sinergístico entre os compostos nas análises de termo e fotoestabilidade. Em contrapartida, efeito antagonista foi observado para as mesmas propriedades ao armazenar as partículas em ambiente com UR de 75%. Maior redução do teor de α-tocoferol foi observado na termoestabilidade e no armazenamento a 75% de UR. Na termo e fotoestabilidade, os espectros de FTIR demonstraram diminuição das bandas presentes em 2900 cm^{-1} (deformação axial de C-H) e 1210 cm^{-1} (aril-O-C) dos tratamentos adicionados de α-tocoferol, e das bandas na região entre 1600 e 900 cm^{-1} (estiramento de C-O-C e O-H) de todos tratamentos. No armazenamento das partículas a 75% de UR, foram observados espectros de FTIR levemente distorcidos e com maiores bandas de absorção. Com base nos resultados, observou-se que o α-tocoferol proporcionou melhor estabilidade às antocianinas, desde que as partículas sejam submetidas ao abrigo de oxigênio e umidade, representando assim uma contribuição potencial para o setor alimentício.

Palavras-chave: Vitamina E. Compostos bioativos. Aproveitamento de resíduos. Corante natural.

GENERAL ABSTRACT

The present study sought to characterize the microparticles obtained from the co-encapsulation of anthocyanins extracted from grape skins (*Vitis vinifera* var. Syrah) and α-tocopherol via spray drying. Briefly, grape skin extract was homogenized with gum arabic (20%), Tween 80 (0.2%), and different concentrations of α-tocopherol (0-control, 0.5, 1.0, 1.5, and 2.0%, w/w) relative to the total emulsion. Anthocyanin content, antioxidant activity, total phenolic content, α-tocopherol concentration, color, hygroscopicity, morphology, and the Fourier transform infrared spectroscopy (FTIR) results of each treatment were analyzed. The highest concentration of α-tocopherol led to the highest total phenolic content (8.69 mg gallic acid equivalent/g powder dry basis) and the lowest hygroscopicity (21.21 g adsorbed moisture/100 g powder dry basis) and anthocyanin content (0.917 mg malvidin-3-glucoside equivalent/g powder dry basis). Intermediate concentrations of α-tocopherol resulted in an increased incidence of microparticles with smooth surface and elevated antioxidant activity. The FTIR spectra verified the efficacy of the process, showing characteristic bands of the co-encapsulated compounds (at 1600 cm^{-1}, which is related to the hydrogen bonds of the OH groups in grape carbohydrates, and at 1210 cm^{-1}, which is related to the aromatic ether present in the structure of α-tocopherol). Overall, α-tocopherol concentrations between 1.5% and 2.0% (w/w) led to good powder properties (lower hygroscopicity, higher total phenolic content and antioxidant activity). The stability of the particles with zero (control), 1.0 and 2.0% (w/w) of α-tocopherol was also evaluated with respect to three main external factors (humidity, temperature, and light) via three different storage conditions: in an environment with relative humidity (RH) of 75% (20 °C, in the dark) for 47 days; a temperature of 60 °C (RH = 65%, in the dark) for 112 days; and with exposure to light (25 °C, RH = 70%) for 105 days. Moreover, the antioxidant activity, anthocyanin content, total phenolic content, and color parameters of these particles were assessed over time. The content of α-tocopherol and the FTIR spectra of the samples were determined at the initial and final times. A synergistic effect between compounds was observed under thermostability and photostability for color properties, content, and half-life time of anthocyanins. In contrast, an antagonistic effect was observed for the same properties when the particles were stored in an environment with RH of 75%. Greater reduction in α-tocopherol content was observed under thermostability and storage at 75% RH. Under thermostability and photostability, FTIR spectra exhibited decreased bands present at 2900 cm^{-1} (axial deformation of C–H) and 1210 cm^{-1} (aryl–O–C) in the α-tocopherol added treatments, and bands in the region between 1600 and 900 cm^{-1} (stretching of C–O–C and O–H) in all treatments. During storage of particles at 75% RH, slightly distorted FTIR spectra with higher absorption bands were observed. Based on these results, it was observed that α-tocopherol provided better stability to anthocyanins, as long as the particles are subjected to oxygen and humidity protection, which represents a potential contribution for the food sector.

Keywords: Vitamin E. Bioactive compounds. Waste utilization. Natural dye.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO

Durante as últimas décadas, tem sido crescente o número de consumidores em busca de produtos alimentícios sem aditivos artificiais. Com isso, a encapsulação tem se tornado bastante popular em indústrias alimentícias, devido ao interesse em adicionar nos alimentos ingredientes bioativos que não podem ser adicionados em sua forma livre.

Existem diversas técnicas para encapsulação de compostos, tais como spray drying, spray chilling, liofilização, coacervação, gelificação iônica, e outros (DIAS; FERREIRA; BARREIRO, 2015). O *spray drying* é uma das técnicas mais utilizadas para carreamento e encapsulação de ingredientes alimentícios. O processo consiste em aprisionar um composto ativo em uma matriz polimérica (material de parede) formada em virtude da rápida secagem a que o produto é submetido, o que resulta em uma rede tridimensional contendo a substância encapsulada (JANISZEWSKA, 2014). Existem diversos materiais que podem ser utilizados como material de parede, que devem ser devidamente selecionados levando em consideração suas características físico-químicas, uma vez existe uma interação direta entre material de parede e composto ativo.

A encapsulação de substâncias isoladas para enriquecer um alimento é uma técnica bastante eficaz. No entanto, a coencapsulação de compostos bioativos, que consiste na encapsulação de duas ou mais substâncias juntas em uma mesma matriz, é um campo emergente que demonstra abordagem promissora no desenvolvimento de alimentos funcionais. De acordo com Neunert *et al*. (2015), quando dois ou mais antioxidantes estão presentes em um sistema, atividades sinergísticas ou antagonistas podem ser observadas, indicando a importância de avaliar tais ensaios para antioxidantes coencapsulados.

O α-tocoferol, por exemplo, é um composto que apresenta atividade antioxidante reconhecida e, na literatura, podem ser observados estudos que já aplicaram o α-tocoferol coencapsulado (FU *et al.*, 2020; PRIOL *et al.*, 2021; TULINI *et al.*, 2017). O α-tocoferol é a forma mais ativa do grupo da Vitamina E (subconjunto de isoprenóides que reúne 4 tocoferóis e 4 tocotrienóis), sendo bastante utilizado tanto devido à sua importância para a nutrição humana como para auxiliar no aumento da vida útil de um produto alimentício em decorrência de sua elevada atividade antioxidante. Assim, a coencapsulação envolvendo antocianinas extraídas da casca de uvas tintas e α-tocoferol torna-se uma abordagem

interessante como forma de conferir maior estabilidade às antocianinas, uma vez que compostos de origem natural são bastante instáveis em determinadas condições de processamento e estocagem (MAHDAVI *et al.*, 2014). As antocianinas são reconhecidas por serem um dos pigmentos de maior distribuição no reino vegetal (STOLL *et al.*, 2016), e seus extratos tem se tornado bastante atrativo para a indústria alimentícia como alternativa para a substituição de corantes sintéticos e também por apresentarem diversos benefícios à saúde humana (MOSER *et al.*, 2017). Além disso, a extração de antocianinas de cascas de uvas, sendo estas oriundas do processo de produção de vinhos, representa uma forma viável de aproveitamento de resíduos, preocupação crescente por parte das indústrias nas últimas décadas (KALUSEVIC *et al.*, 2017).

Com base no exposto, torna-se importante a ampliação dos conhecimentos acerca do uso do α-tocoferol na coencapsulação, uma vez que sua propriedade antioxidante pode exercer um importante efeito nas características e estabilidade do material obtido ao final do processo. Além disso, estudos envolvendo a encapsulação de antocianinas extraídas da casca de uva tem crescido nos últimos anos, e a avaliação da coencapsulação desses dois compostos por *spray drying* representa uma contribuição potencial para o setor alimentício, possibilitando o aumento da estabilidade das antocianinas e, consequentemente, a substituição de corantes sintéticos.

Em particular, a avaliação da estabilidade do material obtido por meio do processo de *spray drying* submetido a diferentes condições de armazenamento é de grande importância, uma vez que diversas alterações podem ocorrer durante a estocagem do produto, resultando em modificações de suas propriedades físico-químicas. Assim, o estudo da estabilidade é essencial para compostos encapsulados como forma de prever possíveis alterações no material e garantir sua qualidade ao longo do armazenamento.

Portanto, o objetivo deste estudo foi realizar a coencapsulação de antocianinas extraídas de cascas de uva tinta e α-tocoferol por meio do *spray drying* e caracterizar as micropartículas obtidas, avaliando suas propriedades físico-químicas, bem como sua estabilidade quando submetidas a diferentes condições de armazenamento, verificando ação sinergista ou antagonista entre os compostos bioativos.

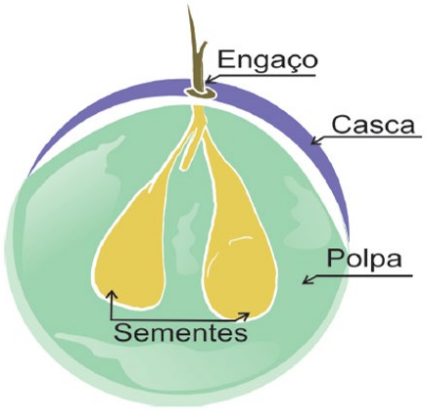
2 REFERENCIAL TEÓRICO

2.1 Resíduos de indústrias vitivinícolas

Em 2018, o Brasil foi o 14° maior produtor de uvas no mundo, sendo a uva a quarta fruta mais produzida no território brasileiro. De um total de quase 1,6 milhão de toneladas de uvas produzidas, 51,39% foram destinadas ao processamento, e o restante para consumo *in natura*. O Rio Grande do Sul foi o estado de maior produção, seguido de Pernambuco, São Paulo, Bahia, Santa Catarina, Paraná e Minas Gerais (CARVALHO; KIST; BELING, 2019; FAOSTAT, 2021).

Além da uva ser considerada uma das frutas mais produzidas no mundo, existe um interesse particular em relação às indústrias de vinho, as quais produzem grandes quantidades de resíduos. Estima-se que cerca de 18% a 20% (m/m) do total de uvas colhidas destinadas para produção de vinhos resultam em resíduos do processo (JELLEY *et al.*, 2016; TOLUN; ALTINTAS; ARTIK, 2016). O principal resíduo gerado no processo de vinificação é o bagaço de uva, que consiste em polpa residual dos bagos do fruto, cascas, sementes e engaços (TONON *et al.*, 2018). A Figura 1 ilustra as partes constituintes do bago de uva.

Figura 1 - Representação esquemática de um bago de uva.



Fonte: Tonon *et al.* (2018).

O crescente interesse na valorização de subprodutos de indústrias agrícolas e alimentícias é um assunto recorrente na literatura científica e a importância de estudos envolvendo fontes naturais de compostos bioativos, como bagaço de uva, são destaques (RUBIO *et al.*, 2020). O fato do bagaço de uva possuir potencial biotecnológico impulsionou

muitos trabalhos que abordam sobre a possibilidade de utilizá-lo como ingrediente em alimentos (IANNI *et al.*, 2019), os quais demonstraram que o bagaço de uva melhorou o perfil nutricional do produto final e aumentou seu valor (ANTONIC *et al.*, 2020). Assim, ao longo dos anos, tem-se buscado diferentes alternativas de aproveitamento de resíduos gerados pelas indústrias vitivinícolas, uma vez que este material é considerado como fonte de uma grande variedade de compostos bioativos, os quais apresentam numerosos benefícios à saúde humana (JELLEY *et al.*, 2016). Com isso, o aproveitamento desses resíduos por meio da recuperação destes compostos pode resultar na obtenção de produtos com alto valor agregado, reduzindo o impacto ambiental negativo causado pelo descarte inadequado dos resíduos (TONON *et al.*, 2018).

A composição do bagaço de uva é altamente dependente do tipo do próprio resíduo, variedade da uva, local de plantio, método de processamento e outros fatores (GARRIDO *et al.*, 2011). Como exemplo, enquanto a produção do vinho tinto envolve a fermentação da massa total da uva, os vinhos rosê e branco são feitos pela fermentação do suco, e isso resulta em uma grande variação na composição do resíduo. A composição aproximada do bagaço de uva obtida de estudos que incluíram uma análise de diferentes tipos de resíduos vitivinícolas é apresentada na Tabela 1 (ANTONIC *et al.*, 2020).

Tabela 1 - Composição aproximada do bagaço de uva.

Compostos	**Quantidade* (g / 100 g)**	**Compostos**	**Quantidade* (g / 100 g)**
Cinzas	1,73 a 9,10	**Na**	87 a 244
Proteína	3,57 a 14,17	**K**	1184 a 2718
Lipídeos	1,14 a 13,90	**Mg**	92 a 644
Fibra alimentar total	17,28 a 88,70	**Ca**	91 a 961
Fibra insolúvel	16,44 a 63,70	**Mn**	6 a 1356
Fibra solúvel	0,72 a 12,78	**Fe**	5 a 5468
Carboidratos	12,20 a 40,53	**Zn**	2 a 2254
Teor de fenólicos totais	0,28 a 8,70	**Cu**	39 a 130
Frutose	0,38 a 8,91	**P**	4 a 3157
Glicose	0,21 a 26,34		

* Em base seca.
Fonte: Antonic *et al.* (2020).

Fibras, compostos fenólicos e minerais são os constituintes mais importantes do bagaço da uva. As fibras são os compostos marjoritários nos bagaços de uvas tintas, enquanto nos bagaços de uva branca o conteúdo deste componente é significativamente reduzido (ANTONIC *et al.*, 2020). Junto às fibras, os compostos fenólicos são os componentes mais

valiosos, sendo os principais responsáveis pelo potencial antioxidante do bagaço de uva (AVERILLA *et al.*, 2019). São constituídos principalmente por antocianinas (como malvidina, peonidina), flavan-3-ols (como catequina, proantocianidinas), flavonols (como quercetina, miricetina) e ácidos fenólicos (GOULA; THYMIATIS; KADERIDES, 2016; MAKRIS; BOSKOU; ANDRIKOPOULOS, 2007). Estes compostos apresentam propriedades benéficas à saúde (AVERILLA *et al.*, 2019), bem como características de interesse quando aplicados em alimentos, como atividade antioxidante, efeito antibacteriano e proteção contra oxidação lipídica (PEIXOTO *et al.*, 2018).

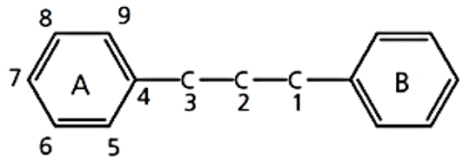
Em particular, o aproveitamento do bagaço de uvas tintas pode ser considerada uma alternativa de interesse para extração de antocianinas que, além de apresentarem coloração atrativa ao consumidor, possuem benefícios à saúde humana. Assim, o uso de antocianinas em alimentos possibilita a substituição de corantes sintéticos, resultando em produtos mais naturais e saudáveis, características cada vez mais valorizadas por parte dos consumidores (SHADDEL *et al.*, 2018; ZHANG, R. *et al.*, 2020).

2.2 Antocianinas

As antocianinas são consideradas um dos pigmentos de maior distribuição no reino vegetal, fazendo parte dos flavonoides, que por sua vez pertencem ao grupo dos compostos fenólicos (SCHWARTZ *et al.*, 2018). A palavra antocianina tem origem grega: *anthos* (flores) e *kyanos* (azul) (RIBEIRO; SERAVALLI, 2007). As antocianinas são pigmentos azuis, vermelhos ou roxos encontrados nas plantas, especialmente flores, frutas e tubérculos (KHOO *et al.*, 2017), e seus extratos têm se tornado bastante atrativos para a indústria alimentícia como alternativa para substituição de corantes sintéticos. Além disso, as antocianinas proporcionam benefícios à saúde humana devido à sua elevada atividade biológica, como atividade antioxidante, anti-inflamatória, antimicrobiana, dentre outras, atuando também na prevenção de doenças crônicas, como câncer, patologias cardiovasculares e neurodegenerativas (MOSER *et al.*, 2017).

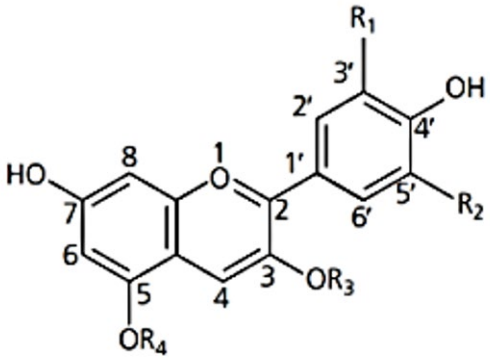
As antocianinas pertencem ao grupo dos flavonoides por apresentarem esqueleto carbônico do tipo C_6-C_3-C_6 (FIGURA 2) (XIE *et al.*, 2018). A estrutura base das antocianinas são derivados poli-hidroxi e/ou polimetóxi 2-fenilbenzopirona do sal flavilium (FIGURA 3) (SCHWARTZ *et al.*, 2018).

Figura 2 - Estrutura básica C_6-C_3-C_6 do grupo dos flavonóides.



Fonte: Schwartz *et al.* (2018).

Figura 3 - Cátion flavilium.

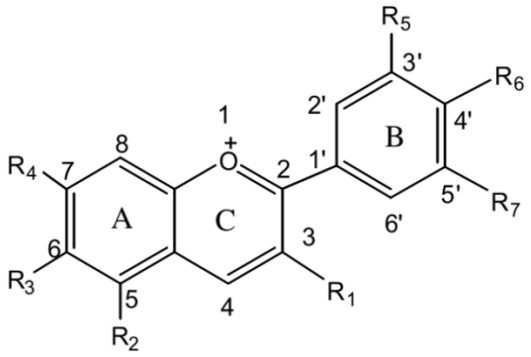


Legenda: R_1 e R_2 = -H, -OH, ou -OCH_3, R_3 = -glicosil, R_4 = -H ou -glicosil.
Fonte: Schwartz *et al.* (2018).

As antocianinas são antocianidinas ligadas a açúcares, sendo bastante comum conter ácidos ligados aos açúcares. Os açúcares mais comuns são glicose, galactose, ramnose e arabinose, e alguns dissacarídeos (rutinose, sambubiose, latirose e soforose). Em relação aos ácidos, normalmente são encontrados os ácidos p-cumárico e cafeico (RIBEIRO; SERAVALLI, 2007).

Das diversas antocianidinas (também chamadas de agliconas, FIGURA 4) que ocorrem naturalmente, apenas seis são mais frequentes (TABELA 2) (XIE *et al.*, 2018). Destas, apenas a pelargonidina não é encontrada nas uvas, além de existir muito mais variedades nos padrões de glicosilação e acetilação do que na maioria das plantas (COULTATE, 2004).

Figura 4 - Estrutura básica das antocianidinas.



Fonte: Castañeda-Ovando *et al.* (2009).

Tabela 2 - Principais antocianidinas encontradas em vegetais.

Nome	R_1	R_2	R_3	R_4	R_5	R_6	R_7
Pelargonidina	OH	OH	H	OH	H	OH	H
Cianidina	OH	OH	H	OH	OH	OH	H
Delfinidina	OH	OH	H	OH	OH	OH	OH
Malvidina	OH	OH	H	OH	OCH_3	OH	OCH_3
Peonidina	OH	OH	H	OH	OCH_3	OH	H
Petunidina	OH	OH	H	OH	OCH_3	OH	OH

Legenda: R_1 – R_7 são os substituintes de acordo com a Figura 4.
Fonte: Castañeda-Ovando *et al.* (2009).

A variação da cor das antocianinas se deve a diferenças estruturais em relação ao número de hidroxilas e/ou grupos metoxi presentes; tipos, números e sítios de ligação dos açúcares na molécula e tipos e números de ácidos ligados aos açúcares da molécula (GIUSTI; WROLSTAD, 2003). Outros fatores também influenciam na coloração, como respostas a mudanças no pH, formação de complexos metálicos e copigmentação (SCHWARTZ *et al.*, 2018).

Apesar de apresentarem maior estabilidade em meios ácidos (KHOO *et al.*, 2017), as antocianinas são consideradas pigmentos instáveis, que podem ser degradadas desde a sua extração até a estocagem. Os principais fatores que governam a degradação podem ser intrínsecos, como sua estrutura química e a copigmentação intramolecular, ou extrínsecos, como pH, temperatura, oxigênio, luz e composição da matriz (RIBEIRO; SERAVALLI, 2007; XUE *et al.*, 2019).

O íon flavilium corresponde à estrutura básica das antocianidinas, o qual é bastante reativo. Geralmente, a estabilidade das antocianidinas aumenta com o aumento da metoxilação, e diminui com o aumento da hidroxilação. O pH do meio é outro fator que influencia fortemente as antocianinas. Em valores entre 1,0 e 3,0, as antocianinas exibem coloração vermelha intensa, ao passo que um aumento no pH resulta em perda dessa coloração. A temperatura também afeta a estabilidade das antocianinas, sendo que elevadas temperaturas por tempo prolongado destroem as antocianinas. Em relação ao oxigênio, as antocianinas se tornam bastante vulneráveis à degradação devido à presença de insaturações em sua estrutura. A luz, por sua vez, é capaz de acelerar a degradação das antocianinas após o rompimento dos tecidos vegetais para extração. Algumas enzimas são capazes de degradar as antocianinas, como as glicosidases (hidrolisam ligações glicosídicas, resultando em açúcares e aglicona) e polifenoloxidases (oxidam as antocianinas). Outro fator de influência na cor é a copigmentação das antocianinas com outros flavonóides, certos ácidos fenólicos, alcalóides e outros compostos, o que aumenta a intensidade de sua coloração. Esse efeito depende de vários fatores, incluindo tipo e concentração de antocianinas e copigmentos, pH e temperatura, sendo que o aumento de temperatura reduz fortemente o efeito intensificador da cor (CASTAÑEDA-OVANDO *et al.*, 2009; KHOO *et al.*, 2017; RIBEIRO; SERAVALLI, 2007; SCHWARTZ *et al.*, 2018).

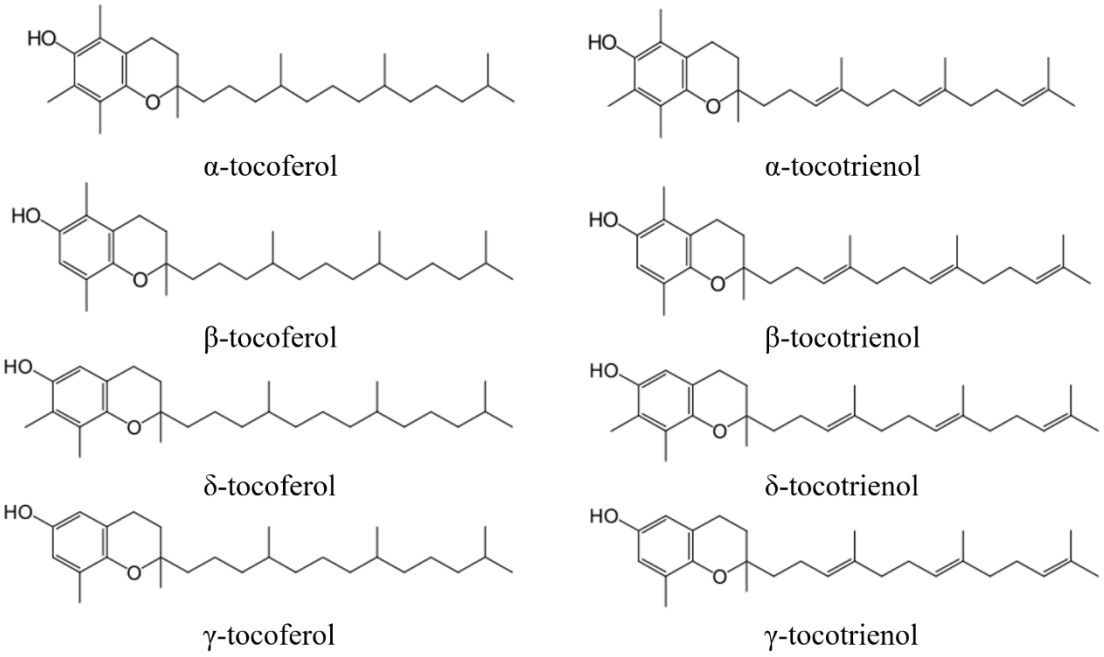
Apesar do grande interesse por parte das indústrias alimentícias, a aplicação das antocianinas é considerada um desafio por apresentarem instabilidade dependendo de diversos fatores físicos e químicos, conforme mencionados acima (TIWARI *et al.*, 2009). Assim, a estabilização desses pigmentos representa uma valiosa fonte de corantes naturais (BERES *et al.*, 2017), e a coencapsulação de antocianinas com α-tocoferol por *spray drying* pode ser considerada uma alternativa promissora para tal finalidade.

2.3 α-Tocoferol

O subconjunto de isoprenóides que reúne 4 tocoferóis e 4 tocotrienóis é conhecido como Vitamina E, os quais possuem atividade vitamínica semelhante à forma mais ativa do grupo: o α-tocoferol (GREGORY, 2018). Todos são derivados do 6-cromanol, sendo que os tocotrienois apresentam três ligações duplas na cadeia lateral da molécula. Os componentes da Vitamina E diferem entre si em relação ao número e posição de grupos metil no anel cromanol, que resultam em diferentes formas e atividades (α, β, γ ou δ-tocoferóis e

tocotrienóis) (REBOUL, 2017). As estruturas dos tocoferois e tocotrienois estão apresentados na Figura 5.

Figura 5 - Estruturas dos tocoferóis e tocotrienóis.



Fonte: Reboul (2017).

A Vitamina E é encontrada principalmente em óleos vegetais e produtos derivados, como maionese, temperos para salada, germe de trigo, gema de ovo, sementes e fígado (REBOUL, 2017), e destaca-se por sua atividade antioxidante, a qual pode doar facilmente um hidrogênio do grupo fenólico do anel cromanol para os radicais livres. Pelo fato de um par de elétrons do anel aromático deslocar-se, o radical resultante é estabilizado e torna-se não-reativo (GREGORY, 2018). Assim, a Vitamina E possui um importante papel na saúde humana por meio da proteção da integridade de tecidos. Sua ingestão está associada a menores riscos de doenças tais como doenças do coração, diabetes tipo 2, câncer e Alzheimer (PIERUCCI *et al.*, 2007).

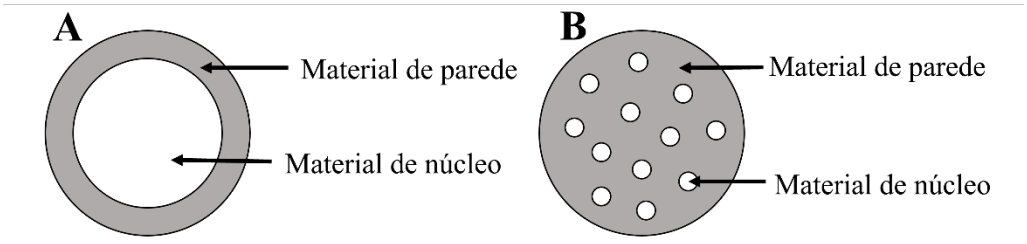
Os compostos vitamínicos E são bem estáveis ao calor, mas são sensíveis à luz ultravioleta, álcalis e oxigênio (RIBEIRO; SERAVALLI, 2007). O oxigênio afeta significativamente sua estabilidade, sendo que a degradação dos compostos torna-se ainda mais rápida na presença de radicais livres. A atividade de água também é um fator de influência, onde os compostos vitamínicos E degradam-se em taxas mínimas no valor de umidade da monocamada, e um aumento ou diminuição deste valor resulta em taxas maiores

de degradação. Uma caraterística interessante está relacionada ao fato de que a Vitamina E contribui para a estabilidade oxidativa de outros compostos enquanto é degradada (GREGORY, 2018). Assim, em tecnologia de alimentos, a Vitamina E desempenha um papel dualista pois, além da importância no que se refere à nutrição humana, essa vitamina pode ser utilizada como antioxidante na matriz alimentícia, como forma de aumentar a vida útil do produto (BATISTA; COSTA; PINHEIRO-SANT'ANA, 2007).

2.4 Encapsulação

O processo de encapsulação consiste no revestimento de compostos (sólidos, líquidos ou gasosos) como forma de protegê-los contra condições adversas tais como umidade, luz, oxigênio e interações com outros componentes. Esses compostos, chamados de material de núcleo, fase interna ou ativo, são envoltos por um material de parede (também conhecido como agente carreador ou matriz), o qual forma uma barreira física entre o composto encapsulado e o ambiente externo (FANG; BHANDARI, 2010; NEDOVIC *et al.*, 2011; VEIGA *et al.*, 2019). Assim, as partículas obtidas por meio da encapsulação podem apresentar diferentes morfologias, sendo que duas são mais comumente observadas: umas delas é a cápsula mononuclear (FIGURA 6A), na qual o núcleo é envolto por uma camada de material de parede, enquanto a outra consiste em uma matriz (FIGURA 6B), onde são observados diversos núcleos embutidos nesta matriz. Esses formatos são influenciados pelo processo utilizado para a encapsulação do composto, bem como pelas características dos materiais de parede e de núcleo a partir dos quais as partículas são obtidas (FANG; BHANDARI, 2010).

Figura 6 - Tipos principais de partículas obtidas da encapsulação de compostos (A-cápsula mononuclear; B-matriz).



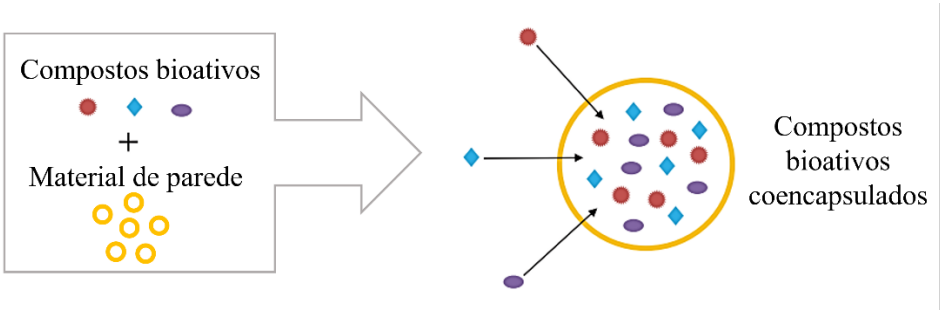
Fonte: Adaptado de Fang e Bhandari (2010).

Além de proteger um composto, os principais objetivos da encapsulação na indústria de alimentos são (EUN *et al.*, 2020): realizar liberação controlada do material de núcleo;

modificar as características físicas dos compostos facilitando o manuseio e sua incorporação em matrizes alimentícias; mascarar sabor ou aroma indesejável; facilitar e melhorar o transporte e armazenamento, evitando reações de degradação.

Durante as últimas décadas, observa-se um grande empenho pelo desenvolvimento de alimentos funcionais contendo compostos bioativos, que ofereçam benefícios à saúde humana (ZHANG *et al.*, 2019). Com isso, a encapsulação tem se tornado bastante popular em indústrias alimentícias devido ao interesse em adicionar ingredientes bioativos nos alimentos que não podem ser adicionados em sua forma livre, como vitaminas, óleos essenciais, extratos naturais, aroma e outros (MATOS-JR *et al.*, 2015; CHAWDA *et al.*, 2017). No entanto, uma abordagem que tem ganhado destaque é a coencapsulação de ingredientes, na qual dois ou mais compostos bioativos são encapsulados em conjunto. A partir dessa técnica, pesquisadores tem relatado efeito sinergístico entre os compostos, observando um aprimoramento da bioatividade e funcionalidade comparados aos componentes isolados (MISRA; PANDEY; MISHRA, 2021). O diagrama apresentado na Figura 7 ilustra a coencapsulação de múltiplos compostos bioativos.

Figura 7 - Coencapsulação de múltiplos compostos bioativos.



Fonte: Adaptado de Chawda *et al.* (2017).

O conceito de efeitos sinergísticos se encontra bastante presente no que se refere a alimentos funcionais, contribuindo estrategicamente para uma boa saúde e prevenção de doenças. O principal desafio está no desenvolvimento de uma mistura adequada de compostos bioativos, pois o sinergismo e a estabilidade do produto final depende da composição dessa mistura e da concentração de cada componente (CHAWDA *et al.*, 2017)

Várias técnicas podem ser empregadas para encapsulação de compostos bioativos, como liofilização, spray drying, spray chilling, coacervação, geleificação iônica, dentre outros (DIAS; FERREIRA; BARREIRO, 2015), sendo o *spray drying* um dos processos mais

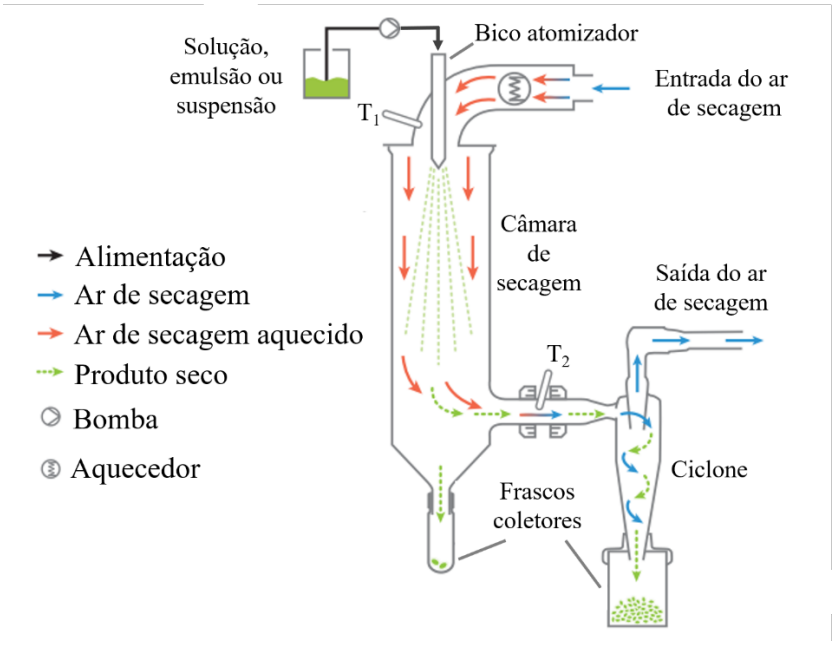
utilizados para encapsulação de compostos (FANG; BHANDARI, 2010), o qual apresenta diversas vantagens conforme descrito no item 2.5.

2.5 *Spray drying*

O *spray drying* é um dos métodos de encapsulação mais antigos, sendo utilizado desde 1930 para obtenção das primeiras substâncias aromáticas encapsuladas (VEIGA *et al.*, 2019), bem como é considerado um dos melhores métodos para preservar compostos bioativos (EUN *et al.*, 2020). O processo consiste em uma rápida secagem por meio do contato entre um gás aquecido (normalmente o ar) e gotículas de uma solução, emulsão ou suspensão aspergidas dentro de uma câmara, transformando o material do estado líquido para o estado sólido na forma em pó (BAKRY *et al.*, 2016; GHARSALLAOUI *et al.*, 2007; ISHWARYA; ANANDHARAMAKRISHNAN; STAPLEY, 2015).

O processo de *spray drying* envolve 4 etapas: preparo, homogeneização e aspersão do material fluido, e desidratação das gotículas aspergidas (BAKRY *et al.*, 2016). Geralmente, o preparo do material a ser desidratado envolve a mistura entre o material de parede, um solvente (geralmente a água) e o material de núcleo. Em seguida, é realizada a homogeneização (GERANPOUR; ASSADPOUR; JAFARI, 2020; JANISZEWSKA, 2014), e a solução resultante vai para o bico atomizador através da bomba de alimentação, que normalmente é uma bomba peristáltica. Dois tipos de bicos atomizadores são mais comuns: duplo fluido e disco giratório. O atomizador é responsável pela geração de inúmeras gotículas do material fluido a ser desidratado, aspergindo-o dentro da câmara de secagem. Ao mesmo tempo, o ar de secagem aquecido passa pela câmara no sentido concorrente ou contra-corrente em relação às gotículas aspergidas, as quais são secas rapidamente e vão para o fundo da câmara. Em seguida, as partículas secas vão para um ciclone, onde são separadas do ar de secagem e são depositadas em um frasco coletor (ASSADPOUR; JAFARI, 2019). O esquema geral do processo de *spray drying* está representado na Figura 8.

Figura 8 - Representação geral do processo de spray drying.



Legenda: T_1 e T_2 são as temperaturas de entrada e saída do ar de secagem, respectivamente.
Fonte: Assadpour e Jafari (2019).

O *spray drying* é considerado um dos métodos mais populares, simples, rápidos e econômicos para encapsulação de compostos (ASSADPOUR; JAFARI, 2019; GERANPOUR; ASSADPOUR; JAFARI, 2020). O processo pode ser utilizado inclusive em casos de substâncias termossensíveis (EUN *et al.*, 2020), pois apesar do uso de elevadas temperaturas (geralmente maiores que 150 °C), a rápida evaporação de água das gotículas mantem a temperatura em seu interior menor comparada à temperatura do ar de secagem (VEIGA *et al.*, 2019). Assim, o processo não resulta em danos consideráveis aos compostos durante o processo devido ao curto tempo de contato entre o material aspergido e o calor (OLIVEIRA; PETROVICK, 2010). Além disso, a técnica do *spray drying* possibilita produção em larga escala e de forma contínua, e permite o uso de uma variedade relativamente grande de materiais de parede. Os produtos obtidos apresentam boa retenção de compostos voláteis, e a remoção de umidade durante o processo e a atividade de água reduzida das partículas proporcionam maior estabilidade do material (VEIGA *et al.*, 2019). Outras vantagens do processo de *spray drying* incluem boas propriedades de reconstituição, maior facilidade, praticidade e menor custo de armazenamento e transporte por transformar materiais líquidos em materiais em pó (ASSADPOUR; JAFARI, 2019; EUN *et al.*, 2020). No entanto, algumas limitações da técnica que podem ser citadas é a probabilidade de

aglomeração dos pós (EUN *et al.*, 2020), e uma certa perda de energia em termos de calor durante o processo de *spray drying* (GHARSALLAOUI *et al.*, 2007).

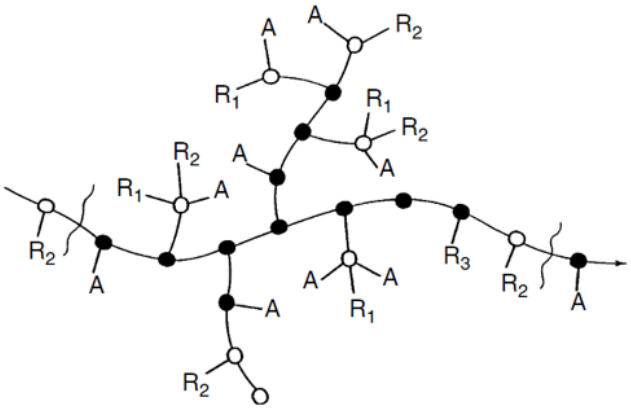
É importante ressaltar que a qualidade das partículas obtidas por meio do *spray drying* dependem dos parâmetros de operação e composição da solução que será submetida ao processo de secagem (AGHBASHLO *et al.*, 2013). Assim, fatores como a temperatura de entrada e saída do ar, taxa que o material fluido passa pelo processo, fluxo do ar de secagem, temperatura e composição da solução anterior à secagem influenciam diretamente nas propriedades das partículas e devem ser otimizados como forma de garantir a qualidade do material em pó obtido no final do processo (BAKRY *et al.*, 2016; ESTEVINHO *et al.*, 2013).

Em relação aos materiais de parede, existem diversos tipos aplicáveis ao processo de encapsulação, como carboidratos (amidos, maltodextrinas, quitosana), gomas (arábica, carragena), proteínas (isolado proteico de soro de leite e soja) e outros (VEIGA *et al.*, 2019). No entanto, suas características físicas e químicas devem ser levadas em consideração, bem como as interações entre o material e o composto bioativo (ROCHA; NOREÑA, 2020).

2.6 Goma arábica

As gomas de exsudato natural são tipicamente polissacarídeos que são liberados por determinadas plantas em condições de estresse, como lesões físicas (cortes e incisões) e ataque de fungos. Essas gomas possuem aplicações alimentícias como estabilizante, espessante, gelificação e emulsificação. A goma arábica ou goma acácia é uma goma de exsudato produzida marjoritariamente (80%) pela *Acacia Senegal* (BARAK; MUDGIL; TANEJA, 2020), e seu uso principal é como material de parede no desenvolvimento de produtos encapsulados, como aromas, óleos, componentes bioativos e outros, e como agente carreador na secagem de suco de frutas (EUN *et al.*, 2020; KRISHNAN; BHOSALE; SINGHAL, 2005). Uma representação da estrutura molecular da goma arábica é apresentada na Figura 9.

Figura 9 - Representação estrutural da goma arábica.



Legenda: A = arabinosil, {●} = β-1,3 galactose, {○} = β-1,6 galactose 6-ligada, R_1 = raminose-ácido galacturônico, R_2 = galactose-1,3-arabinose, R_3 = arabinose-1,3-arabinose-1,3-arabinose.
Fonte: Stephen e Churms (1995).

A goma arábica é um biopolímero que consiste em ácido D-glucurônico, L-ramnose, D-galactose e L-arabinose, com aproximadamente 5% de proteína. Apresenta boa solubilidade em água, baixa viscosidade e temperatura de transição vítrea elevada, o que viabiliza bastante seu uso em processos de encapsulação. Entretanto, esse material possui custo elevado e disponibilidade limitada (COMUNIAN *et al.*, 2011; GUPTA *et al.*, 2015; TONON *et al.*, 2009). A goma arábica é um dos materiais de parede mais utilizados na encapsulação por spray drying, sendo que os produtos obtidos geralmente apresentam boa estabilidade em baixas atividades de água e longa vida útil. A porção proteica favorece o uso da goma arábica como estabilizante e emulsificante, o que é considerado importante em relação à encapsulação de óleos (COIMBRA; CARDOSO; GONÇALVES, 2021; EUN *et al.*, 2020; RAMÍREZ; GIRALDO; ORREGO, 2015).

A goma arábica apresenta em sua constituição moléculas com diferentes polaridades, que podem interagir tanto com substâncias hidrofílicas como lipofílicas (RAMAKRISHNAN *et al.*, 2018). Assim, a goma arábica pode ser uma indicação interessante para uso como material de parede na coencapsulação de α-tocoferol e antocianinas devido à diferente natureza destes compostos.

2.7 Coencapsulação por *spray drying*

Conforme já ressaltado, o *spray drying* é um dos processos mais empregados para a encapsulação de compostos bioativos, e a coencapsulação é uma técnica bastante estabelecida

no setor farmacêutico, que vem se tornando cada vez mais popular também no setor alimentício. Assim, a coencapsulação consiste em uma área que necessita ser explorada como forma de obtenção de alimentos funcionais com as vantagens de múltiplos componentes bioativos (CHAWDA *et al.*, 2017).

A coencapsulação de óleos de girassol e linhaça com antioxidantes hidrofílico (propilgalato) e lipofílico (α-tocoferol) foi realizada por meio do *spray drying* utilizando isolado proteico de ervilha como material de parede. Foi observado que a adição de propilgalato proporcionou maior estabilidade oxidativa dos óleos, enquanto o α-tocoferol apresentou efeito pró-oxidante e resultou em maior oxidação do óleo de girassol. Assim, a coencapsulação de óleos de girassol e linhaça com antioxidantes se mostrou uma técnica eficaz para o aumento da estabilidade dos óleos, desde que seja empregada a combinação e o tipo de antioxidante adequados para retardar a oxidação do material (PRIOL *et al.*, 2021).

A deficiência das vitaminas B12 e D3 são relatadas em todo o mundo e a coencapsulação dessas vitaminas pode representar uma solução combinada para este problema. Assim, Bajaj *et al.* (2021) empregaram a técnica de *spray drying* para a coencapsulação de vitamina B12 e D3 objetivando a seleção do melhor material de parede para as partículas. O uso de goma arábica:amido de milho modificado:maltodextrina na proporção de 38:60:2 foi a composição do material de parede que resultou em partículas com melhor estabilidade e biodisponibilidade. A partir deste estudo, foi possível disponibilizar uma vitamina hidrofílica (B12) e outra lipofílica (D3) em um único sistema de forma prática e eficiente, o que torna interessante no que se refere à fortificação de produtos alimentícios.

Olga *et al.* (2015) avaliaram a coencapsulação por *spray drying* de dois compostos fenólicos: ácido trans-ferúlico (AF) e ácido gálico (AG), utilizando 2-hidroxipropil-b-ciclodextrina como material de parede. Os autores observaram que a eficiência de encapsulação para o AG foi menor, enquanto para o AF foi praticamente estável. As partículas produzidas no estudo apresentaram formato mais arredondado para os fenólicos coencapsulados comparado aos mesmos compostos encapsulados separadamente. Por fim, os dados obtidos por meio de espectroscopia de infravermelho com Transformada de Fourier (FTIR) indicaram possível interação entre AF e AG. A partir dos resultados, os autores ressaltam a importância de se realizar estudos mais aprofundados abordando a liberação dos compostos das partículas e sua atividade antioxidante em diferentes condições que comprovam sua funcionalidade e potencial para uso em produtos alimentícios.

Três compostos bioativos lipofílicos (óleo de peixe, fitoesteróis e limoneno) foram coencapsulados por *spray drying* utilizando isolado proteico de soro de leite (IPS) e caseinato de sódio (NaCA) como material de parede na proporção de 4:1 (IPS:NaCA). Neste trabalho, não foi observada diferença entre partículas contendo apenas óleo de peixe e contendo os três compostos bioativos no que se refere à eficiência de encapsulação e oxidação. No entanto, as partículas contendo os compostos coencapsulados apresentaram melhor perfil de sabor/odor e maior retenção de ácidos graxos (ácido eicosapentaenoico – EPA- e ácido docosa-hexaenoico - DHA) comparado às partículas contendo somente óleo de peixe após secagem e armazenamento. Os resultados obtidos foram capazes de demonstrar que a coencapsulação de óleo de peixe proporcionou melhores propriedades às partículas comparados às outras contendo apenas óleo de peixe encapsulado (CHEN *et al.*, 2013).

A partir dos estudos encontrados na literatura, é possível perceber o crescente interesse pela coencapsulação de compostos bioativos e a importância de dar continuidade em trabalhos que abordam o tema, resultando em produtos cada vez mais diversificados com uso potencial em alimentos, que agregam valor e representam inovação no setor alimentício.

3 CONSIDERAÇÕES GERAIS

A encapsulação de compostos bioativos vem sendo bastante explorada ao longo dos anos e na literatura encontra-se ampla abordagem do tema, principalmente nos setores farmacêutico e alimentício. As antocianinas são pigmentos naturais que despertam grande interesse não só pela sua coloração atrativa, como também devido aos diversos benefícios à saúde humana, embora apresente grande instabilidade depois de serem extraídas de suas fontes vegetais. Assim, a encapsulação de antocianinas pode ser considerada uma das formas de tornar as antocianinas mais estáveis e também é bastante estudada. No entanto, ainda não foi aplicada a coencapsulação envolvendo as antocianinas, como forma de aumentar ainda mais sua estabilidade, além da vantagem de um único sistema apresentar vários benefícios oriundos de diferentes componentes bioativos. O α-tocoferol, principal representante da Vitamina E, pode ser considerado um grande aliado na coencapsulação das antocianinas, o qual pode proporcionar maior estabilidade às antocianinas, proporcionando assim mais uma inovação por se tratar de um produto natural e funcional, com maior potencial de aplicação em alimentos.

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SEGUNDA PARTE - ARTIGOS

ARTIGO 1 – CO-ENCAPSULATION OF A-TOCOPHEROL AND ANTHOCYANINS EXTRACTED FROM GRAPE SKINS (*Vitis vinifera* var. SYRAH) VIA SPRAY DRYING

Artigo publicado no **Journal of Food Processing and Preservation, apresentado na íntegra da publicação.**

Received: 29 June 2021 | Revised: 13 September 2021 | Accepted: 29 September 2021

DOI: 10.1111/jfpp.16038

Journal of Food Processing and Preservation | ifst | WILEY

ORIGINAL ARTICLE

Co-encapsulation of anthocyanins extracted from grape skins (*Vitis vinifera* var. Syrah) and α-tocopherol via spray drying

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Funding information
Conselho Nacional de Desenvolvimento Científico e Tecnológico, Grant/Award Number: 141824/2017-4; Fundação de Amparo à Pesquisa do Estado de Minas Gerais, Grant/Award Number: CAG-APQ - 02487-16

Abstract

The present study sought to characterize the microparticles obtained from the co-encapsulation of anthocyanins extracted from red grape skins and α-tocopherol via spray drying. Briefly, grape skin extract was homogenized with gum arabic, Tween 80, and different concentrations of α-tocopherol. Anthocyanin content, antioxidant activity, total phenolic content, α-tocopherol concentration, color, hygroscopicity, morphology, and the Fourier transform infrared spectroscopy (FTIR) results of each treatment were analyzed. The highest concentration of α-tocopherol led to the highest total phenolic content, and the lowest hygroscopicity and anthocyanin content. Intermediate concentrations of α-tocopherol resulted in an increased incidence of microparticles with smooth surface and elevated antioxidant activity. The FTIR spectra verified the efficacy of the process, showing characteristic bands of the co-encapsulated compounds. Overall, α-tocopherol concentrations between 1.5% and 2.0% (w/w) led to good powder properties (hygroscopicity, total phenolic content and antioxidant activity), generating further research interest to evaluate the use of α-tocopherol in foods.

Practical applications

The development of products containing bioactive compounds that offer benefits to human health have gained a lot of attention. In this study, the co-encapsulation of anthocyanins extracted from grape skin and α-tocopherol by spray drying proved to be interesting as an interaction occurred between compounds, which enables the attainment of an added-value natural product, representing an innovation in the food sector.

1 | INTRODUCTION

Color is one of the most important characteristics of food. Moreover, color is considered as an indicator of product quality and plays a crucial role in food acceptance (Syamila et al., 2019). Many products are colored using synthetic food dyes (Guneser, 2016). However, the use of natural dyes is trending in the current market because of consumer concerns regarding the safety of artificial dyes, which is reinforced by the potential health benefits of natural pigments (Rodriguez-Amaya, 2016). Thus, anthocyanins have attracted increasing interest from food-processing industries as an alternative for synthetic dyes (Shaddel et al., 2018; Zhang et al., 2020). Anthocyanins are widely distributed in nature, namely in plant seeds, barks, and fruits (Shaddel et al., 2018). Although the general purpose of food colorants is to provide color, anthocyanins are considered to be value-added colorants as they are potent antioxidants

and have displayed several beneficial effects on human health (Schwartz et al., 2018).

Anthocyanins are very unstable when exposed to adverse conditions, such as high temperature, light, oxygen, and enzymes. As a result, its application in food matrices is a challenge (Mahdavi et al., 2016; Zhang et al., 2020). In the past few decades, the microencapsulation of bioactive compounds has received considerable attention and is considered a practical and effective strategy for increasing the stability of anthocyanins (Huang et al., 2019; Zhang et al., 2020). The goal of microencapsulation is to protect and preserve bioactive compounds in a core encased by a membrane or dispersed within an enclosure (wall material); these compounds are then released at the appropriate place and time (Huang et al., 2019). Co-encapsulation is a concept that is associated with microencapsulation and has gained prominence recently. Co-encapsulation involves the encapsulation of two or more bioactive compounds, thereby enhancing their bioactivity and functionality compared to that of the individual compound (Chawda et al., 2017).

The main form of vitamin E, α-tocopherol, may be of interest for co-encapsulation with anthocyanins, as it contributes to the oxidative stability of other compounds while undergoing degradation (Gregory, 2018). In food technology, α-tocopherol plays a dual role; in addition to its importance in human nutrition, this vitamin can be used as an antioxidant in the food matrix to increase the shelf life of the product (Batista et al., 2007).

Several techniques exist for compound encapsulation, including spray drying, spray chilling, lyophilization, coacervation, and ionic gelation (Dias et al., 2015). Spray drying is considered a very efficient method for the stabilization of bioactive compounds (Carvalho et al., 2016) and is one of the most used techniques for encapsulation of substances (Fang & Bhandari, 2010). Thus, components that have health benefits, including antioxidants, vitamins, essential oils, flavors, and antimicrobials can be made available in functional foods through encapsulation within a suitable wall material (Chawda et al., 2017).

Several wall materials are applicable to the microencapsulation process. However, their physical and chemical characteristics should be considered, in addition to their interaction with the bioactive compound (Rocha & Noreña, 2020). Gum arabic is one of the most used wall materials in microencapsulation by spray drying. It is composed of molecules of differing polarity that interact with both hydrophilic and lipophilic substances (Ramakrishnan et al., 2018). Thus, gum arabic may be employed as a wall material in the co-encapsulation of α-tocopherol and anthocyanins because of the different nature of these compounds.

As presented in the literature, co-encapsulation involving α-tocopherol has shown promising results. For example, a study using α-tocopherol co-encapsulated with *Cinnamomum zeylanicum* proanthocyanidins revealed synergism in relation to the antioxidant activity of the microparticles obtained by spray chilling (Tulini et al., 2017). Another study that addressed the co-encapsulation of α-tocopherol and coenzyme Q10 also revealed a synergistic effect on the oxidative stability of microparticles obtained by spray drying (Fu et al., 2020). It is important to expand the current knowledge on the use of α-tocopherol in co-encapsulation, as its antioxidant property may exert a beneficial effect on the characteristics of the material obtained at the end of the process. Moreover, the number of works on the encapsulation of anthocyanins extracted from grape skin (i.e., to utilize grape waste) has increased in recent years, and the co-encapsulation of these two compounds by spray drying may lead to innovation in the food sector. Thus, the objective of the present study was to evaluate the physicochemical properties of microparticles obtained by co-encapsulation of anthocyanins extracted from grape skins and α-tocopherol via spray drying.

2 | MATERIAL AND METHODS

2.1 | Material

α-Tocopherol was purchased from DSM Nutritional Products Ltd (Basel, Switzerland; 1,000 IU per gram). Gum arabic (Alland & Robert, Paris, France) was employed as wall material in the spray drying process. The grape bunches (*Vitis vinifera* var. Syrah) were donated by a producer in Campos Gerais-MG, Brazil; they were sorted, washed in water, packed in polyethylene bags, and frozen (−18°C) until use.

2.2 | Extract preparation

Grape skin extracts were obtained using the method described by Kuck et al. (2017), with some modifications. Using a blender, the skins were homogenized with water acidified with citric acid (2%, w/v) at a ratio of 1:3 (skin:acidified water). The liquid extract (without the residue) for spray drying was obtained by filtering the mixture through organza after a resting time of 20 hr in the dark at 22 ± 1°C.

2.3 | Preparation of the solutions and spray drying

The solutions were kept on a magnetic stirrer (Go-Stirrer MS-H-S) at 750 rpm with heating (45 ± 5°C) throughout the preparation and spray drying process to achieve better homogenization of α-tocopherol (which is highly viscous). Different amounts of α-tocopherol [0 (control), 0.5, 1.0, 1.5, and 2.0%, w/w], 0.2% (w/w) of Tween 80 (used as emulsion stabilizer), and 20% (w/w) of gum arabic (determined in preliminary trials, data not shown) were added to the extract (in relation to the final solution); the mixture was subsequently homogenized with a homogenizer (Ultra-Turrax IKA T18 Basic, Wilmington, USA) for 20 min at 1,200 rpm. Finally, the solutions were placed in the spray dryer (model MSD 1.0; Labmaq do Brasil, Ribeirão Preto, Brazil) with the following drying parameters: inlet and outlet drying air temperature of 170 ± 5°C and 115 ± 5°C, respectively, feed flow rate of 0.5 L/h, and drying air flow rate of 35 L/min. The spray drying parameters were determined in preliminary tests (data not shown), which yielded a free-flowing powder.

2.4 | Experimental design

The experimental design was completely randomized with three repetitions. The effect of different concentrations of α-tocopherol on the physicochemical properties of the microparticles obtained by spray drying was evaluated.

2.5 | Characterization of the microparticles

To evaluate the antioxidant activity, the anthocyanin and phenolic content of the powders, an extract was prepared by diluting 0.6 g of sample in 11 ml of an ethanol solution (water:ethanol, 1:1). The mixture was vortexed for 1 min, placed in an ultrasonic bath for 1 hr, and centrifuged at 1,260× *g* for 5 min at 25°C; then, the supernatant was retrieved for analysis.

2.6 | Anthocyanin content

Anthocyanin content was expressed as mg malvidin-3-glucoside equivalent/g powder on dry basis (mg mv-3-glu/g powder d. b.). An aliquot of 0.5 ml of the extract was added to 3 ml of acidified ethanol (HCl 0.1%, v/v) and the mixture was vortexed (30 s) and centrifuged (1,570 × *g*, 5 min at 25°C). Absorbance of the supernatant was measured at 520 nm and the anthocyanin content was calculated according to Equation (1) (Lee et al., 2005):

$$\mathrm{AC} = \frac{A \times MM \times V \times 1000}{\varepsilon \times l \times m \times \mathrm{TSC}}, \quad (1)$$

where AC is the anthocyanin content (mg mv-3-glu/g powder d. b.), *A* is the absorbance of the sample at 520 nm, MM is the molar mass of malvidin-3-glucoside (493.2 g/mol), *V* is the volume of the acidified ethanol used in the analysis (L), 1,000 is the conversion factor from grams to milligrams, *Ɛ* is the molar absorptivity for malvidin-3-glucoside (28,000 L/(mol.cm)), *l* is the width of the cuvette (1 cm), m is the mass of the sample (g) used in the analysis, and TSC is the total solids content of the sample (g solids/100 g sample).

2.7 | Antioxidant activity

The 2.2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was used to determine the antioxidant activity of the samples according to the method proposed by Dima et al. (2014), with some modifications. Approximately 4 ml of extract was added to 4 ml of ethanol, homogenized in a vortex mixer for 30 s, and centrifuged at 1,260× *g* for 5 min at 25°C. Subsequently, 1.5 ml of supernatant was added to 1.5 ml of DPPH ethanolic solution (0.1 mg/ml), vortexed for 5 s, and kept in the dark for 1 hr at 25°C. Absorbance was measured at 515 nm and antioxidant activity (AA %) was calculated using Equation (2) (Siripatrawan & Harte, 2010):

$$\mathrm{AA}(\%) = \left(\frac{A_c - A_s}{A_c}\right) \times 100, \quad (2)$$

where A_s and A_c are the absorbance values obtained at 515 nm for the sample and control, respectively. The control is the solution containing 1.5 ml of ethanol and 1.5 ml of ethanolic DPPH solution.

2.8 | Phenolic content

The method described by Babbar et al. (2011), with some modifications, was used to determine the total phenolic content of the samples. A mixture of 4 ml of extract and 4 ml of ethanol was homogenized in a vortex mixer for 30 s and centrifuged at 1,260× *g* for 5 min at 25°C. The solution was diluted by adding 1 ml of supernatant to 3 ml of distilled water, and 1 ml of this dilution was added to 1 ml of Folin–Ciocalteu reagent (0.2 N). After 3 min, 1 ml of sodium carbonate solution (4% w/v) was added and the mixture was allowed to stand in the dark for 2 hr at 25°C. The absorbance of the samples was measured at 760 nm and quantification of total phenolic content was performed using a gallic acid (GA) standard curve generated with concentrations of 1, 5, 10, 20, 30, 40, 50, and 60 μg/ml. The result was expressed as mg of equivalent GA/g powder on dry basis (mg GAE/g powder d. b.).

2.9 | α-tocopherol content

High-performance liquid chromatography (HPLC) was used to determine the α-tocopherol content (Gimeno et al., 2000). Briefly, 0.1 g of each sample was homogenized in 2.5 ml of methanol and kept in an ultrasonic bath for 2 hr. Then, the samples were filtered through a 0.45-μm PTFE membrane filter and stored in amber vials for subsequent injection into the chromatograph (Shimadzu Corp., Japan). An injection volume of 20 μl was used, the mobile phase consisted of methanol:water (96:4 v/v), and the rate of elution was 1 ml/min. The analytical column (C18) was kept at 45°C and detection was performed at 295 nm (UV-Vis detector SPD 10Ai).

2.10 | Color

The powdered samples were placed in Petri dishes and transferred to the colorimeter (Spectrophotometer CM-5, Konica Minolta, Japan); Illuminant D65 and an observation angle of 10° were used. The values of chroma (C^*) and hue angle ($h°$) were calculated using Equations (3) and (4) respectively, based on the CIE $L^*a^*b^*$ parameters obtained. The color of the samples was described by means of the parameters L^* (0 = black, 100 = white, which indicates the lightness of the sample), C^* (color saturation), and $h°$ (hue: from 0° or 360° = red color, 90° = yellow, 180° = green, and 270° = blue).

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

$$h° = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (4)$$

2.11 | Hygroscopicity

Hygroscopicity was determined according to the method described by Viana et al. (2019), using the static gravimetric technique. Briefly, 0.6 g of powder from each treatment was placed in a desiccator containing saturated NaCl solution. The samples were kept in direct contact with an environment that had a relative humidity of 75% until equilibrium, at 20°C. Hygroscopicity was expressed as g of adsorbed moisture/100 g powder on dry basis (g/100 g powder d. b.).

2.12 | Morphology

Samples of each treatment were mounted on aluminum stubs with double-sided carbon tape, gold coated by evaporation (SCD 050 apparatus), and observed under the scanning electron microscope (LEO EVO 40 XVP, Carl Zeiss) at the Laboratory of Electron Microscopy and Ultrastructural Analysis of UFLA.

2.13 | Fourier-transform infrared (FTIR) spectroscopy

Infrared vibrational spectroscopy analyses were performed using a Fourier-transform infrared (FTIR) Varian 600-IR spectrometer (Agilent, Santa Clara, USA) equipped with a GladiATR zinc selenide crystal accessory (Pike Technologies) for measurement in attenuated total reflectance (ATR) mode at 45°. The spectral range was 400–4,000 cm^{-1}; and 32 scans were recorded at a resolution of 4 cm^{-1}.

2.14 | Statistical analysis

The software Statistica (ver. 8.0, Stat. Soft. Inc., Tulsa, USA) was used for analysis of variance, and Duncan's test of means was used to assess the differences between the results at a 5% probability level ($p \leq .05$).

3 | RESULTS AND DISCUSSION

3.1 | Anthocyanin content

The content of anthocyanins (Table 1) differed significantly between the treatments ($p \leq .05$) and was inversely proportional to the concentration of α-tocopherol in the samples. This finding reveals the antagonistic effect occurring between the compounds. The intermolecular interaction between co-encapsulated bioactive compounds is a crucial factor that determines the synergistic effects between substances. Therefore, it should be properly evaluated (Chawda et al., 2017). Because α-tocopherol is highly nonpolar (Gregory, 2018), incompatibility may have existed between compounds within the matrix of the microparticles, resulting in competition for space. Therefore, α-tocopherol may have expelled the anthocyanin molecules because of the difference in polarity.

Temperature is a factor that has considerable influence on the stability of anthocyanins in foods. Moreover, thermal degradation rates are influenced by the presence or absence of oxygen and other substances in the matrix, which can interact with anthocyanins (Schwartz et al., 2018). Therefore, oxidation of α-tocopherol during spray drying may have been an influencing factor. Because drying occurs in the presence of oxygen, α-tocopherol may have been degraded during the process as its degradation rate increases in the presence of oxygen (Gregory, 2018). Furthermore, the high temperature used in spray drying may have accelerated this degradation rate (Syamila et al., 2019) and anthocyanins may have acted as a protective agent for α-tocopherol instead of vice versa.

Another possible cause of the antagonism between anthocyanins and α-tocopherol is related to the ability of α-tocopherol to exhibit pro-oxidant activity depending on its concentration and conditions of the medium to which it is added (Martin-Rubio et al., 2018; Sotler et al., 2019). Thus, α-tocopherol can induce oxidative stress through the formation of reactive oxygen species or by inhibiting the antioxidant system (Sotler et al., 2019). In the present study, α-tocopherol may have contributed to the oxidation of anthocyanins, consequently reducing their concentration in the treatments to which the compound was added. α-Tocopherol co-encapsulated with sunflower oil also had a pro-oxidant effect and reduced the oxidative

TABLE 1 Characterization of the microparticles based on antioxidant activity and anthocyanin, phenolic, and α-tocopherol content

Treatments[1]	AC (mg mv-3-glu/g powder d.b.)	AA (%)	TPC (mg GAE/g powder d.b.)	α-Tocopherol (mg/g powder d.b.)
C	1.015 ± 0.004^{a}	86.93 ± 0.313^{b}	8.33 ± 0.059^{b}	–
1	0.975 ± 0.002^{b}	88.03 ± 0.224^{a}	8.08 ± 0.112^{c}	9.12 ± 0.55^{d}
2	0.937 ± 0.002^{c}	87.67 ± 0.136^{a}	8.24 ± 0.026^{b}	15.01 ± 1.31^{c}
3	0.922 ± 0.013^{d}	87.91 ± 0.186^{a}	8.32 ± 0.054^{b}	20.51 ± 1.66^{b}
4	0.917 ± 0.007^{d}	86.07 ± 0.224^{c}	8.69 ± 0.020^{a}	36.72 ± 2.30^{a}

Note: ^{a,b,c,d}Means followed by the same letters in the same column do not differ significantly ($p > .05$) using Duncan's test.

Abbreviations: AA, antioxidant activity; AC, anthocyanin content; TPC, total phenolic content.

[1]C corresponds to the control treatment (no α-tocopherol added), whereas 1, 2, 3, and 4 are the treatments with 0.5, 1.0, 1.5 and, 2.0% (w/w) α-tocopherol, respectively.

stability of the oil in the study conducted by Priol et al. (2021). Gagneten et al. (2019) obtained an anthocyanin concentration in a spray-dried blackcurrant extract that was similar to that found in the present study. However, decreased concentrations were obtained by Oliveira et al. (2018) when grape skin extract encapsulated by spray drying was analyzed.

3.2 | Antioxidant activity

No proportionality was observed between the antioxidant activity values and α-tocopherol concentration. Furthermore, the treatment with the highest α-tocopherol content and the control treatment exhibited the lowest antioxidant activities (Table 1). Thus, a dose-dependent relationship may exist, resulting in a synergistic effect on the antioxidant activity of the powders at intermediate concentrations of α-tocopherol. Co-encapsulation of *Cinnamomum zeylanicum* proanthocyanidins and α-tocopherol was found to result in synergism in the antioxidant activity of microparticles obtained by spray chilling (Tulini et al., 2017).

Although anthocyanins and α-tocopherol are recognized as antioxidant compounds, other factors may influence the antioxidant activity of the treatments in general. One hypothesis is that the protein fraction of gum arabic contributes to the occurrence of Maillard reactions during the spray drying process, resulting in compounds that have antioxidant activity (Ferrari et al., 2013). Degradation products of anthocyanins or phenolic acids, or even the formation of antioxidant polymers, such as low molecular weight procyanidins, may also influence the values obtained for the antioxidant activity of the powders (Fracassetti et al., 2013). Lower antioxidant activities were observed by Tolun et al. (2016) who evaluated the microencapsulation of polyphenols extracted from grapes using spray drying. Similarly, in the study by Bazaria and Kumar (2016) in which they evaluated beetroot juice dried by spray drying, lower values for antioxidant activity were observed.

3.3 | Phenolic content

The addition of α-tocopherol resulted in elevated phenolic content in the samples when the concentration of the compound was 2% (w/w; Table 1). According to Becker et al. (2004), antioxidants in varying amounts and combinations can interact with each other owing to different mechanisms, such as regeneration (one for the other) and sacrificial oxidation (one antioxidant protects another by capturing free radicals). Thus, several studies have been conducted to elucidate the interaction between the evaluated compounds and to understand how these interactions lead to synergistic or antagonistic effects. In a study on lipid oxidation, Neunert et al. (2015) observed a synergistic effect between α-tocopherol and three phenolic compounds (ferulic, caffeic, and chlorogenic acid). However, with regard to chlorogenic acid, synergism was observed when the highest total concentration of antioxidants was used, whereas an antagonistic effect was obtained at the lowest concentration of antioxidants. Yin et al. (2012) also observed synergism when they investigated the effect of the combination of α-tocopherol and green tea extract on lipid oxidation. With regard to the phenolic content in spray-dried grape skin extract, Tolun et al. (2016) obtained values similar to those in the present study, whereas Abrahão et al. (2019) reported higher values in spray-dried extract samples of espresso coffee grounds.

3.4 | α-tocopherol content

The concentration of α-tocopherol in the samples was proportional to the amount of compound initially added; in other words, treatments with higher amounts of α-tocopherol had higher concentrations of the compound in the microparticles (Table 1). However, the difference in content between treatments 4 and 3 was greater than that between treatments 3 and 2 and between treatments 2 and 1. Some researchers reported that α-tocopherol was regenerated or protected against oxidation by the presence of phenolic compounds that provide greater stability to the compound under certain conditions. In a study involving liposome oxidation, the interaction between α-tocopherol and *Aronia melanocarpa* juice was evaluated (Graversen et al., 2008). According to their findings, the concentration of α-tocopherol throughout the initial period of oxidation was practically constant, which indicated that the phenolic compounds in the juice protected the compound from oxidation. Such finding may be explained by two mechanisms: (a) the phenolic compounds present in the juice are more easily oxidized than α-tocopherol or (b) α-tocopherol is initially oxidized but subsequently regenerated by fast reduction by phenolic compounds in the juice. Regeneration of α-tocopherol by phenolics present in coffee was also suggested by Chew et al. (2020). Thus, in the present study, α-tocopherol in treatment 4 may have been more protected by phenolics in the grapes against oxidation during the spray drying process because this treatment had a higher phenolic content than the other treatments. While investigating the microencapsulation of α-tocopherol by spray drying, Carmo et al. (2017) obtained higher α-tocopherol concentrations, and Pierucci et al. (2007) reported values similar to those of treatment 4.

3.5 | Color

The parameters, L^*, C^*, and $h°$, obtained for the treatments are shown in Table 2. The L^* parameter was inversely proportional to the concentration of α-tocopherol, which indicated that the samples were lighter in the absence of this compound. The same behavior was observed in relation to color saturation (i.e., higher α-tocopherol contents resulted in lower chroma (C^*) values). The results of the hue angle ($h°$) revealed that the addition of α-tocopherol to the samples provided a coloration tendency toward shades of red.

A direct correlation was observed between anthocyanin concentration and two color parameters (L^* and C^*; i.e., lower values

TABLE 2 Characterization of the microparticles based on color and hygroscopicity

Treatments[1]	Color			Hygroscopicity (g/100 g powder d.b.)
	L^*	C^*	$h°$	
C	62.33 ± 0.32^{a}	29.05 ± 0.16^{b}	341.20 ± 0.07^{e}	22.45 ± 1.17^{a}
1	60.79 ± 0.38^{b}	29.56 ± 0.11^{a}	341.70 ± 0.19^{d}	22.06 ± 1.03^{a}
2	61.03 ± 0.52^{b}	29.00 ± 0.15^{b}	342.74 ± 0.12^{c}	21.82 ± 0.02^{a}
3	60.82 ± 0.37^{b}	28.75 ± 0.07^{b}	343.35 ± 0.15^{b}	21.21 ± 0.68^{ab}
4	59.95 ± 0.29^{c}	27.86 ± 0.27^{c}	345.94 ± 0.15^{a}	19.78 ± 0.77^{b}

Note: [a,b,c]Means followed by the same letters in the same column do not differ significantly ($p > .05$) using Duncan's test.

[1]C corresponds to the control treatment (no α-tocopherol added), whereas 1, 2, 3, and 4 are the treatments with 0.5, 1.0, 1.5 and, 2.0% (w/w) α-tocopherol, respectively.

of these parameters were observed for the treatments with lower levels of anthocyanins, which indicated lower lightness and saturation of the color of the samples). The reverse behavior was observed for $h°$, with higher values of $h°$ obtained in samples with lower concentration of anthocyanins. Notably, $h°$ values from 270° to 360° (or 0°) indicate shades from blue to red, respectively. Among these colors are purple, a characteristic color of the grapes used in the present study, and after red, there is a transition to yellow. Therefore, because $h°$ increased with decreasing anthocyanin content, the color of the samples went from purple to red and then from red to yellow, which correlates with the degradation of anthocyanins.

Lower values of the L^* parameter were observed in powders obtained by microencapsulation via spray drying of anthocyanins extracted from grapes, using brewer's yeast (*Saccharomyces cerevisiae*) waste as wall material (Rubio et al., 2020). In the microencapsulation of *Porphyridium cruentum* microalgae biomass by spray drying, values of C^* were found to be similar to those in the current study (Toker, 2019). Moreover, Santiago et al. (2016) obtained values of $h°$ in spray-dried pomegranate juice that were similar to those in the present work.

3.6 | Hygroscopicity

The hygroscopicity results of the powders are presented in Table 2. Significant differences were found between the treatments ($p \leq .05$), with the highest concentration of α-tocopherol causing the lowest hygroscopicity and the control treatment exhibiting the highest hygroscopicity. As α-tocopherol is a highly nonpolar compound (Gregory, 2018), the microparticles containing α-tocopherol adsorbed less moisture, resulting in less hygroscopic materials. In contrast, the higher moisture adsorption by the control treatment is related to the low molecular weight sugars and organic acids, with low glass transition temperatures, that are normally present in fruit extracts and provide higher hygroscopicity to the powdered material (Ferrari et al., 2012).

The hygroscopicity of powdered food products is one of the most important properties in determining the shelf life and stability of these materials. Lower hygroscopicity results in lower susceptibility to product deterioration (Vergara et al., 2014; Viana et al., 2019). The addition of α-tocopherol was found to result in less hygroscopic powders, which is interesting because it directly contributes to the stability of the powders. In the study conducted by Andrade et al. (2018), the hygroscopicity of three varieties of powdered propolis was lower than that in the present study. On the other hand, Ribeiro et al. (2019) obtained values similar to the current work in acerola and seriguela juice mix powder, with the powders being obtained by spray drying in both studies.

3.7 | Morphology

Figure 1 shows the images of the microparticles using scanning electron microscopy. The same magnification was used for all samples (3,000×). Thus, it was ascertained that, in general, the size of the microparticles did not markedly differ between the treatments. All treatments presented microparticles that were predominantly of spherical shape and varying sizes, which is typical of spray-dried products (Carvalho et al., 2016). Furthermore, most had rough surfaces, without cracks. However, the treatments with lower percentages of α-tocopherol (0.5% and 1.0%) had a higher incidence of smooth surface microparticles. This is a characteristic of interest for the controlled release and stability of encapsulated substances (Osorio et al., 2010), which may translate into greater protection of anthocyanins against oxidative degradation during storage (Laokuldilok & Kanha, 2015). A similar microparticle morphology was found by Carvalho et al. (2016) and Kuck and Noreña (2016) through an analysis of jussara pulp (*Euterpe edulis* Martius) and grape skin extract (*Vitis labrusca* var. Bordo) encapsulated by spray drying, respectively.

3.8 | Fourier-transform infrared (FTIR) spectroscopy

The FTIR spectra obtained for the powdered samples are presented in Figure 2a. Broad bands in the region between 3,500 and 3,000 cm^{-1} correspond to the OH groups (Espinosa-Andrews et al., 2010; Kalusevic et al., 2017), whereas bands at 2,900 cm^{-1}

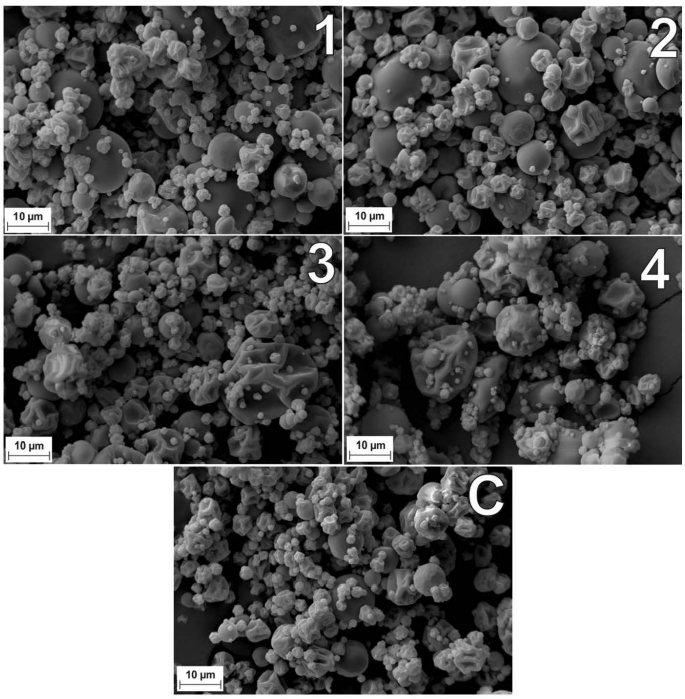

FIGURE 1 Micrographs of the microparticles obtained using scanning electron microscopy. C corresponds to the control treatment (no α-tocopherol added), whereas 1, 2, 3 and 4 are the treatments with 0.5, 1.0, 1.5, and 2.0% (w/w) α-tocopherol, respectively

represent axial deformation of the C–H bond (Silverstein et al., 2019). The small band at 1,710 cm^{-1} could be assigned to C=O stretching (He et al., 2007), whereas those observed at 1,640 cm^{-1} might be due to angular deformation of H-O-H, indicating the presence of residual water in the samples. The hydrogen bonds of the OH groups of carbohydrates may have also contributed to this band (He et al., 2007; Souza et al., 2015). Stretching bands of C-O-C and O-H are observed between 1,600 cm^{-1} and 900 cm^{-1}, which are characteristic of the phenolic compounds and carbohydrate monomers present in grape extracts, while C=C stretching bands (region from 1,800 to 1,520 cm^{-1}) are typical of aromatic compounds (Rocha & Noreña, 2020). Bands from 1,420 cm^{-1} to 1,320 cm^{-1} represent the angular deformation of O-H. Specifically, absorption bands from 1,100 cm^{-1} to 900 cm^{-1} can be assigned to C–O and C–O–C bond vibrations of carbohydrates (Fragoso et al., 2011). Thus, all samples showed a strong band at 1,040 cm^{-1} that can be attributed to carbohydrates (C–O bond stretching), whereas small bands around this peak are because of the presence of organic acids (Kalusevic et al., 2017).

Through a comparison of the FTIR spectra of the samples (Figure 2a) and the individual constituents of the microparticles (Figure 2b), specific bands were revealed, which demonstrate the interaction of the bioactive compounds (anthocyanins and α-tocopherol) with the wall material (gum arabic); such finding established the occurrence of co-encapsulation. The main band observed in the spectrum of gum arabic (at 1,040 cm^{-1}) was also found in the spectra of the treatment samples and was related to carbohydrates (C–O bond stretching; Kalusevic et al., 2017). The grape extract exhibited a band at 1,600 cm^{-1}, which could be related to the hydrogen bonds of the OH groups in carbohydrates (He et al., 2007; Souza et al., 2015). This band might have been slightly displaced in the spectra of the samples due to molecular interactions among compounds. Finally, two bands in the spectra of the samples should be noted, which were also detected in the spectrum of α-tocopherol. The first band, at 2,900 cm^{-1}, is characteristic of axial deformation of the C-H bond and can be associated with the long saturated side chain of the molecule as well as other methyl groups along its structure. The second band, at 1,210 cm^{-1}, is related to the aromatic ether (aryl-O-C) present in the structure of α-tocopherol. Although the bands were smaller in the spectra of the samples, their size in the spectra is proportional to the amount of α-tocopherol added to the samples (i.e., these bands were not present in the sample that does not contain α-tocopherol). Such finding aligns with the characteristics of infrared spectroscopy (i.e., a higher concentration of a given substance translates into a higher absorption band that is characteristic of a group present

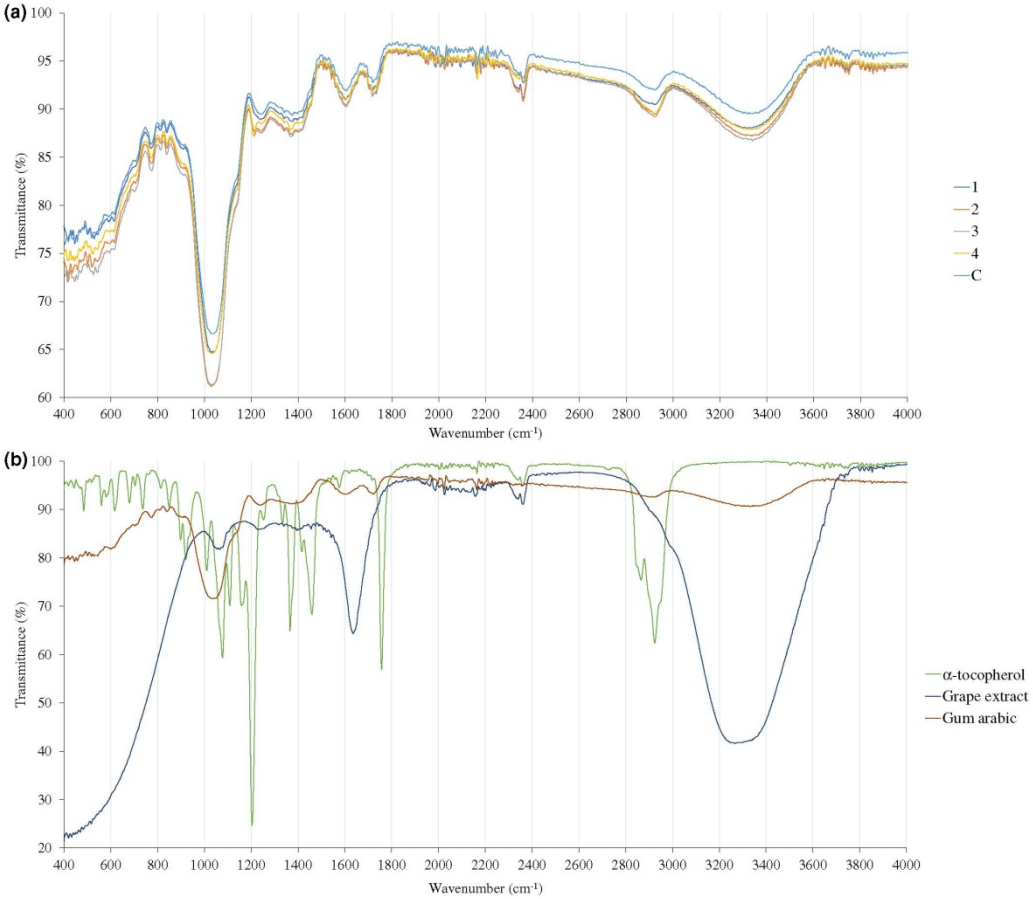

FIGURE 2 (a) FTIR spectra of the samples of each treatment and (b) the individual constituents of the microparticles (grape extract, α-tocopherol, and gum arabic). C corresponds to the control treatment (no α-tocopherol added), whereas 1, 2, 3, and 4 are the treatments containing 0.5, 1.0, 1.5 and 2.0% (w/w) α-tocopherol, respectively

in the chemical structure of the analyzed compound; Silverstein et al., 2019).

4 | CONCLUSION

In the present study, intermediate concentrations of α-tocopherol resulted in higher antioxidant activity and microparticles with smoother surfaces, whereas higher concentrations caused higher total phenolic content, and lower hygroscopicity and anthocyanin content. Substances from grape skins may have exerted a protective effect on α-tocopherol rather than vice versa. Thus, co-encapsulation proved to be interesting as an interaction occurred between the compounds, which directly influenced the final properties of the microparticles. α-tocopherol concentrations between 1.5% and 2.0% (w/w) resulted in good powder properties, such as decreased hygroscopicity and increased total phenolic content and antioxidant activity, thereby garnering interests to conduct studies to assess the use of anthocyanins and α-tocopherol co-encapsulated by spray drying in foods. Further studies should be conducted to better understand the synergistic or antagonistic effects of combinations of several antioxidant compounds in encapsulated systems.

ACKNOWLEDGEMENTS

The authors would like to thank: the National Council for Scientific and Technological Development (CNPq) for their financial support (Process: 141824/2017-4) and the Foundation for Research of the State of Minas Gerais – FAPEMIG (CAG-APQ - 02487-16) for the

financial support. The authors also thank the Chemical Analysis and Prospection Center of the Federal University of Lavras; the Laboratory of Electronic Microscopy and Ultrastructural Analysis of the Federal University of Lavras; and Finep, Fapemig, CNPq, and Capes for providing the equipment and technical support for the experiments involving chromatographic analysis and electron microscopy.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest with respect to the research, authorship, and/or publication of this article.

AUTHOR CONTRIBUTIONS

Eloá Lourenço do Carmo: Conceptualization; Formal analysis; Investigation; Writing-original draft. **Mariá Andrade Teixeira:** Methodology; Validation. **Isadora Simão e Souza:** Data curation; Methodology. **Jayne de Abreu Figueiredo:** Methodology. **Regiane Victória de Barros Fernandes:** Resources. **Diego Alvarenga Botrel:** Resources; Supervision; Writing-review & editing. **Soraia Vilela Borges:** Project administration; Resources; Supervision; Writing-review & editing.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: do Carmo, E. L., Teixeira, M. A., Souza, I. S., Figueiredo, J. A., Fernandes, R. V. B., Botrel, D. A., & Borges, S. V. (2021). Co-encapsulation of anthocyanins extracted from grape skins (*Vitis vinifera* var. Syrah) and α-tocopherol via spray drying. *Journal of Food Processing and Preservation*, 00, e16038. https://doi.org/10.1111/jfpp.16038

ARTIGO 2 – STABILITY OF PARTICLES CONTAINING α-TOCOPHEROL AND ANTHOCYANINS EXTRACTED FROM GRAPE SKINS (*Vitis vinifera* var. Syrah) CO-ENCAPSULATED BY SPRAY DRYING

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Artigo formatado com base na NBR 6022 (ABNT, 2018), conforme é orientado no Manual de Normalização da UFLA.

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Stability of particles containing α-tocopherol and anthocyanins extracted from grape skins (*Vitis vinifera* var. Syrah) co-encapsulated by spray drying

ABSTRACT

The present study evaluated the influence of humidity, temperature, and light on the stability of particles obtained from co-encapsulation of anthocyanins extracted from red grape skins and α-tocopherol by spray drying. α-Tocopherol was added at different concentrations (0-control; 1 and 2% w/w). The stability of the particles was evaluated via three different storage conditions: in an environment with relative humidity (RH) of 75% (20 °C, in the dark) for 47 days; a temperature of 60 °C (RH = 65%, in the dark) for 112 days; and with exposure to light (25 °C, RH = 70%) for 105 days. Antioxidant activity, anthocyanin content, total phenolic content, and color properties of the particles were assessed over time. Moreover, the α-tocopherol content and the FTIR spectra of the samples were determined at the initial and final times. A synergistic effect between compounds was observed under thermostability and photostability for color properties, half-life time and anthocyanin content. In contrast, an antagonistic effect was observed for the same properties when the particles were stored in an environment with RH of 75%. Greater reduction in α-tocopherol content was observed under thermostability and storage at 75% RH. Under thermostability and photostability, FTIR spectra exhibited decreased bands in all treatments, and particles stored under 75% RH condition showed distorted FTIR spectra. The α-tocopherol provided better stability to anthocyanins when particles were not in direct contact with oxygen and humidity during storage.

Keywords: vitamin E, natural dye, bioactive compounds, waste utilization.

1 INTRODUCTION

Over the last decades, the development of functional foods containing bioactive compounds that offer benefits to human health have gained immense interest industrial applications (ZHANG *et al.*, 2019). As a result, encapsulation, a process in which a material is surrounded by a membrane or dispersed in a matrix (COMUNIAN *et al.*, 2016; HUANG *et al.*, 2019), has gained popularity in food industries due to its property of adding bioactive ingredients (vitamins, essential oils, natural extracts, flavoring, among others) that cannot be added in their free form to foods (CHAWDA *et al.*, 2017; MATOS-JR *et al.*, 2015). Thus, the encapsulation of isolated substances to enrich a food is a technique that has gained attention (HUANG *et al.*, 2019).

Nevertheless, the combination of different bioactive compounds may result in better stability or bioactivity in comparison to individual compounds (ZHANG *et al.*, 2019). Thus, co-encapsulation of bioactive compounds is a promising approach in developing functional foods, which encapsulate two or more bioactive substances together (CHAWDA *et al.*, 2017). According to Neunert *et al.* (2015), when two or more antioxidants are present in a system, synergistic or antagonistic activities can be observed, thereby indicating the importance of evaluating such assays for co-encapsulated antioxidants.

α-Tocopherol, the principal component of the vitamin E group, is a compound that exhibits remarkable antioxidant activity and has beneficial effects on human health (MARTIN-RUBIO *et al.*, 2018). Moreover, α-tocopherol can indirectly contribute to the oxidative stability of other substances while being degraded simultaneously (GREGORY, 2018). Consequently, previous studies have reported the application of encapsulated α-tocopherol together with other compounds (TAVANO *et al.*, 2014; TULINI *et al.*, 2017; ZHANG, F. *et al.*, 2019). Thus, co-encapsulation of anthocyanins extracted from red grape skins and α-tocopherol by spray drying can be considered as a promising approach. Spray drying is widely used for encapsulation of compounds (FANG; BHANDARI, 2010), thereby allowing production of a powder material in an economical and efficient way (SEMYONOV *et al.*, 2010). Anthocyanins are pigments widely distributed in the plant kingdom (STOLL *et al.*, 2016), and possess antioxidant, anti-inflammatory, antimicrobial, and other activities (MOSER *et al.*, 2017). Their extracts have wide applications in the food industry as they contain components with bioactive potential and can be used as alternatives for synthetic dyes (MOSER *et al.*, 2017). Furthermore, the extraction of anthocyanins from grape skins

represents an interesting way of waste utilization, a growing concern for industries in recent decades (KALUSEVIC *et al.*, 2017); however, natural compounds are quite unstable under certain processing and storage conditions, and α-tocopherol together with anthocyanins may confer greater stability to these compounds (GREGORY, 2018; MAHDAVI *et al.*, 2014; SCHWARTZ *et al.*, 2018).

In this scenario, the main challenge in the production of powdered materials by spray drying is the achievement of products with desirable characteristics, under reduced costs (FERNANDES *et al.*, 2017), although several changes may occur during storage of the product, resulting in modifications to their physical and chemical properties. Therefore, it becomes essential to determine the stability of encapsulated compounds, while evaluating their behavior under certain conditions, as a way to predict possible changes, and to ensure quality control of the powders throughout their storage. Based on the aforementioned topics, the present study aimed to evaluate the influence of humidity, temperature, and light on the stability of anthocyanins extracted from red grape skins (*Vitis vinifera* var. Syrah) encapsulated with different concentrations of α-tocopherol via spray drying, studying the effects between the compounds over time in different storage conditions.

2 MATERIAL AND METHODS

2.1 Material

The grapes (*Vitis vinifera* var. Syrah) were donated by a grape cultivator from Campos Gerais-MG, Brazil. The grape bunches were selected, washed in water, packed in polyethylene bags, and frozen (−18 °C) until use. α-Tocopherol was purchased from DSM Nutritional Products Ltd. (Basel, Switzerland) in oil form, containing 1000 IU per gram, and gum arabic (Alland & Robert, France) was used as wall material in the spray drying process.

2.2 Preparation of extracts

Grape peel extracts were obtained using water acidified with citric acid (2%, w/v), according to the methodology described by Kuck, Wesolowski e Noreña (2017), with some modifications. After separating the pulp and seeds from the grape skins, the extracts were obtained by mixing the skins with acidified water at a ratio of 1:3, and were homogenized using a blender. The mixture was kept in the dark for 20 h at 22 ± 1 °C, then it was filtered using organza fabric to separate the residue, and the liquid extract was spray dried.

2.3 Preparation of solutions and spray drying

Throughout the solution preparation, as well as during the spray drying process, the solutions were kept under agitation (750 rpm, Go-Stirrer MS-H-S magnetic stirrer) and heated (45 ± 5 °C) for better homogenization of α-tocopherol due to its high viscosity. Thereafter, 0.2% (w/w) Tween 80, 20% (w/w) gum arabic, and different amounts of α-tocopherol (1.0 and 2.0%, w/w) were added to the extract relative to the final solution. The control treatment consisted of the extract homogenized only with gum arabic and Tween 80, without addition of α-tocopherol. Subsequently, the solutions were homogenized using a homogenizer (Ultra-Turrax IKA T18, basic, Wilmington, USA) for 20 min at 1200 rpm, and then was fed into spray drying process (model MSD 1.0; Labmaq do Brasil, Ribeirão Preto, Brazil) configured with the following drying parameters determined in preliminary tests: inlet and outlet drying

air temperatures of 170 ± 5 °C and 115 ± 5 °C, respectively; feed flow rate of 0.5 L/h; drying air flow rate of 35 L/min.

2.4 Experimental design

The experiment was conducted in an entirely randomized design with two repetitions, and the results of the analyses were obtained in triplicate. The powder stability was evaluated in relation to three main external factors (humidity, temperature, and light), under three different conditions: an environment with a relative humidity (RH) of 75%, at 20 °C, protected from light, for 47 days; at a temperature of 60 °C, RH of 65%, protected from light, for 112 days; and exposed to light, at 25 °C, with an RH of 70% for 105 days. It was studied the effect of different concentrations of α-tocopherol (0-control, 1%, and 2%) on the properties of the particles throughout the storage.

2.5 Evaluation of powder stability

The powders were evaluated over time in relation to their antioxidant activity, anthocyanins content, total phenolic content, and color properties. Additionally, the half-life time ($t_{1/2}$) was calculated for the anthocyanins in the treatments under each storage condition, as well as α-tocopherol content and the Fourier transform infrared (FTIR) spectra of the samples were determined at the initial and final times of the storages.

2.5.1 Thermal Stability

To evaluate the thermal stability of the powders, 0.6 g sample in each treatment was incubated at 60 °C (RH of 65%) in polyethylene bags wrapped with aluminum foil. The temperature determined for the study corresponds to one of the most recommended for accelerated stability studies (TSALI; GOULA, 2018). The samples were analyzed after 7, 14, 28, 56, 84, and 112 days of storage.

2.5.2 Stability under Light

The photostability of the powders was evaluated according to Moser *et al.* (2018), with modifications. Samples of each treatment (0.6 g) were packed in polyethylene bags and stored at 16 cm from incident light (three 15W fluorescent lamps), at a temperature of 25 °C and RH of 70%. Thereafter, the samples were evaluated after 7, 14, 28, 49, 77, and 105 days of storage.

2.5.3 Stability under Humidity

The samples (0.6 g) were arranged in small plastic dishes, spread for maximum surface exposure, and stored in desiccators containing a saturated NaCl solution (75% RH) at 20 °C, protected from light, according to the procedure performed by Souza *et al.* (2014) with slight modifications. The samples were then analyzed after 2, 4, 7, 12, 22, and 47 days of storage.

2.6 Physicochemical analyses

To evaluate the antioxidant activity, anthocyanin content, total phenolic content, and color properties of powders, the same extract was used, which involved diluting 0.6 g of sample in 11 mL of ethanolic solution (water:ethanol, 1:1). The mixture was vortexed for 1 min, then kept in an ultrasonic bath for 1 h, and centrifuged at 1260 × g for 5 min at 25 °C. The supernatant was used to perform the analyses.

2.6.1 Antioxidant activity

The antioxidant activity of the samples was determined by monitoring the consumption of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical according to the methodology described by Dima *et al.* (2014), with modifications. 4 mL of extract was added in 4 mL of ethanol, and this solution was homogenized in vortex for 30 s and then centrifuged at 1260 × g for 5 min at 25 °C. Thereafter, 1.5 mL of the supernatant was added in 1.5 mL of ethanolic solution of DPPH (0.1 mg/mL), which was again agitated in vortex for 5 s. This mixture was kept in the dark for 1 h at room temperature (25 °C) and the absorbance at 515

nm was obtained. The antioxidant activity (AA, %) was calculated using Equation 1 (SIRIPATRAWAN; HARTE, 2010):

$$\text{AA (\%)} = \left(\frac{A_c - A_s}{A_c}\right) * 100 \quad (1)$$

where A_s and A_c are the absorbance values obtained at 515 nm from the sample and control, respectively. The control corresponds to the solution containing 1.5 mL of ethanol and 1.5 mL of ethanolic solution of DPPH.

2.6.2 Anthocyanin content and half-life time

A 0.5 mL aliquot of extract was added to 3 mL of acidified ethanol (HCl 0.1%, v/v), followed by vortex agitation (30 s), and centrifugation (1570 × g, 5 min at 25 °C). The absorbance of the supernatant was read at 520 nm and the anthocyanins content (AC) was calculated, expressed as mg of malvidin-3-glucoside equivalent/g powder on dry basis (mg mv-3-glu/g powder d.b.) according to Equation 2 (LEE; DURST; WROLSTAD, 2005):

$$\text{AC} = \frac{AxMMxVx\ 1000}{\text{Ɛ}\ xlxmxTSC} \quad (2)$$

where A is the absorbance of the sample at 520 nm, MM is the molecular mass of malvidin-3-glucoside (4932 g/mol), V is the volume of acidified ethanol used for the analysis (L), 1000 corresponds to the conversion of the result from grams to milligrams, Ɛ is the molar absorptivity for malvidin-3-glucoside (28,000 L/(mol·cm)), l is the cuvette width (1 cm), m is the sample mass (g) used in the analysis, and TSC is the total solids content of the sample (g solids/100 g sample).

For each stability evaluated, the half-life time ($t_{1/2}$, in days) of the anthocyanins was calculated following the first-order kinetics model, as described in Equations 3 and 4 (TONON; BRABET; HUBINGER, 2010):

$$kt = -ln\left(\frac{AC_t}{AC_0}\right) \quad (3)$$

$$t_{1/2} = \frac{ln2}{k} \quad (4)$$

where k is the first order reaction rate constant, t is the storage time (in days), AC_0 and AC_t are the anthocyanin contents at the initial time and at time t, respectively.

2.6.3 Total phenolic content

Total phenolic content of the samples was determined according to the methodology described by Babbar *et al.* (2011), with some modifications. 4 mL of extract was homogenized with 4 mL of ethanol by vortexing (30 s), followed by centrifugation (1260 × g for 5 min at 25 °C). Thereafter, 1 mL aliquot of the supernatant was diluted in 3 mL of distilled water, and from this dilution, 1 mL was added to 1 mL of Folin–Ciocalteu reagent (0.2 N). After 3 min, 1 mL of sodium carbonate solution (4%, w/v) was added, the mixture was kept in the dark for 2 h at room temperature (25 °C), and the absorbances of the samples were obtained at 760 nm. The quantification of total phenolic content was performed from a standard curve of gallic acid (GA) constructed using concentrations of 1, 5, 10, 20, 30, 40, 50, and 60 µg/mL, and the result was obtained in mg of GA equivalent /g of powder on dry basis (mg GAE/g powder d.b.).

2.6.4 Color properties

Color properties were evaluated by means of the color difference (ΔE*) among the parameters L* (luminosity), a* (green to red), and b* (blue to yellow) obtained using a colorimeter (Spectrophotometer CM-5, Konica Minolta, Japan) at the time studied in relation to the initial time, according to Equation 5 (ESTUPIÑAN; SCHWARTZ; GARZÓN, 2011):

$$\Delta E^* = \sqrt{[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]} \quad (5)$$

2.6.5 α-Tocopherol content

α-Tocopherol content was quantified via high-performance liquid chromatography (HPLC). Initially, the samples were homogenized (0.1 g in 2.5 mL of methanol) and kept in an ultrasonic bath for 2 h. The samples were then filtered through a 0.45 µm PTFE membrane filter and stored in amber vials for subsequent injection into the chromatograph equipped with

a diode array detector (LC20AD, Shimadzu, Japan). An injection volume of 20 μL was used, with a mobile phase comprising methanol:water (96:4, v/v), and an elution rate of 2 mL/min. The analytical column (C18) was kept at 45 °C, and detection was performed at 295 nm (GIMENO *et al.*, 2000).

2.6.6 FTIR Analysis

Infrared vibrational spectroscopy analyses were performed using a Fourier transform infrared (FTIR) Varian 600-IR spectrometer (Agilent, Santa Clara, USA), with a coupled GladiATR accessory from Pike Technologies for measuring total attenuated reflectance (ATR) at 45° with a zinc selenide crystal. The spectral range analyzed was 400 to 4000 cm^{-1}, at 4 cm^{-1} resolution, with 32 scans.

2.7 Statistical analysis

The software Statistica (ver. 8.0, Stat. Soft. Inc., Tulsa, USA) was used to perform an ANOVA, and differences between the mean values obtained were calculated at 5% probability level ($p \leq 0.05$) using Duncan's test.

3 RESULTS E DISCUSSION

3.1 Thermal Stability

The stability study of the samples stored at 60 °C showed that the antioxidant activity of the powders decreased over time (FIGURE 1a), although it exhibited an oscillatory behavior. Moreover, the treatment with intermediate concentration of α-tocopherol (1.0%) revealed higher antioxidant activity value when compared to the others for most of the time, thereby demonstrating synergistic effect between the bioactive compounds and a possible relationship of dose-dependence between the content of α-tocopherol and antioxidant activity during storage.

According to Fracassetti *et al.* (2013), the oscillatory behavior may be a consequence of the antioxidant polymer formation, such as low molecular weight procyanidins, or due to degradation products of anthocyanins or phenolic acids, which also present antioxidant activity. Another factor that may have influenced the oscillatory behavior of the antioxidant activity is related to Maillard reactions, since this reaction is favored during food processing at high temperatures or during the storage of the food product for a long period (TONON; BRABET; HUBINGER, 2010). As a result, compounds that present antioxidant activity are formed along the reaction (GU *et al.*, 2009). Tonon *et al.* (2010) reported the possible occurrence of Maillard reactions when they observed an increase in the antioxidant activity in açai powders obtained via spray drying when stored at higher temperatures.

Anthocyanin content in the powders decreased over time (FIGURE 1b), although they presented a lesser decrease compared to the other evaluated stabilities (light and humidity). All treatments revealed a more pronounced reduction in the first 7 days of storage. This may be related to the anthocyanins present on the surface of the particles, which are more readily exposed to the external conditions, thereby, presenting greater susceptibility to degradation (DAS; GOUD; DAS, 2019). The treatments in which α-tocopherol was added revealed similar anthocyanin concentrations, whereas the control treatment presented greater degradation over time, and consequently, a shorter half-life time. This behavior suggests a synergistic effect between anthocyanins and α-tocopherol, since the addition of α-tocopherol resulted in lower degradation of anthocyanins over the evaluated time, as well as a longer half-life time for anthocyanins (TABLE 1). In the study conducted by Mahdavi *et al.* (2016), lower values of

half-life time were found for microparticles of spray-dried barberry extract when evaluating the stability of samples stored at different temperatures.

In general, the stability of anthocyanins is influenced by factors such as temperature, light, oxygen, pH, enzymes, and others (MAHDAVI *et al.*, 2016; SOUZA *et al.*, 2014). Furthermore, the degradation of anthocyanins during storage may occur due to several reaction mechanisms, such as condensation reactions, oxidation, and covalent bond breaking, making it difficult to establish the exact mechanism by which the degradation of anthocyanins occurred during storage (PATRAS *et al.*, 2010).

An increase in the total phenolic content was observed in the samples stored at 60 °C, followed by a slight decrease at the end of the evaluated time (FIGURE 1c). Higher content of α-tocopherol resulted in a lower variation in phenolic content compared to that of the other treatments, demonstrating a synergistic effect between the compounds. The fact that phenolic compounds do not exhibit a well-established behavior when subjected to different storage conditions can be attributed to structural changes in their molecules, since they present the ability to react among themselves and hydrolyze into small molecules, or even carry out polymerization that forms larger molecules, resulting in phenolic compounds with higher or lower antioxidant activity (KUCK; WESOLOWSKI; NOREÑA, 2017; SUN-WATERHOUSE; WATERHOUSE, 2015; TSALI; GOULA, 2018). Oxidation reactions over storage time also affect the degree of polymerization of phenolics, which can influence the quantification of these compounds via colorimetric methods (SOUZA *et al.*, 2020). Flores *et al.* (2014) also observed an increase in the total phenolic content when storing blueberry pomace extract powder obtained by spray drying at higher temperatures.

For color properties, the control treatment exhibited the highest ΔE* value, that is, a greater variation in color parameters over time, thereby indicating less stability compared to the other treatments, and greater addition of α-tocopherol led to less color variation (FIGURE 1d). Again, the addition of α-tocopherol demonstrates a synergistic effect, thus protecting the samples against color variation when exposed to higher temperatures. At the final time of stability evaluation, the treatments presented ΔE* between 21 and 25. These values were higher compared to those at the final time of the other storage conditions evaluated (light and humidity), thereby demonstrating greater color variation in the powders exposed to higher temperatures. According to Obón *et al.* (2009), when the difference in coloration is less than 5, this difference cannot be visually distinguished. Indeed, color plays an important role in the acceptance of a food product, and color changes during storage can affect the visual quality

and reflect the nutritional value (SYAMILA *et al.*, 2019). In the present study, ΔE* values less than or equal to 5 were observed up to 14 days of storage while exposed to a temperature of 60 °C. Carmo *et al.* (2018) observed ΔE* between 3 and 9 in beetroot extract powder also at 60 °C after 105 days of storage.

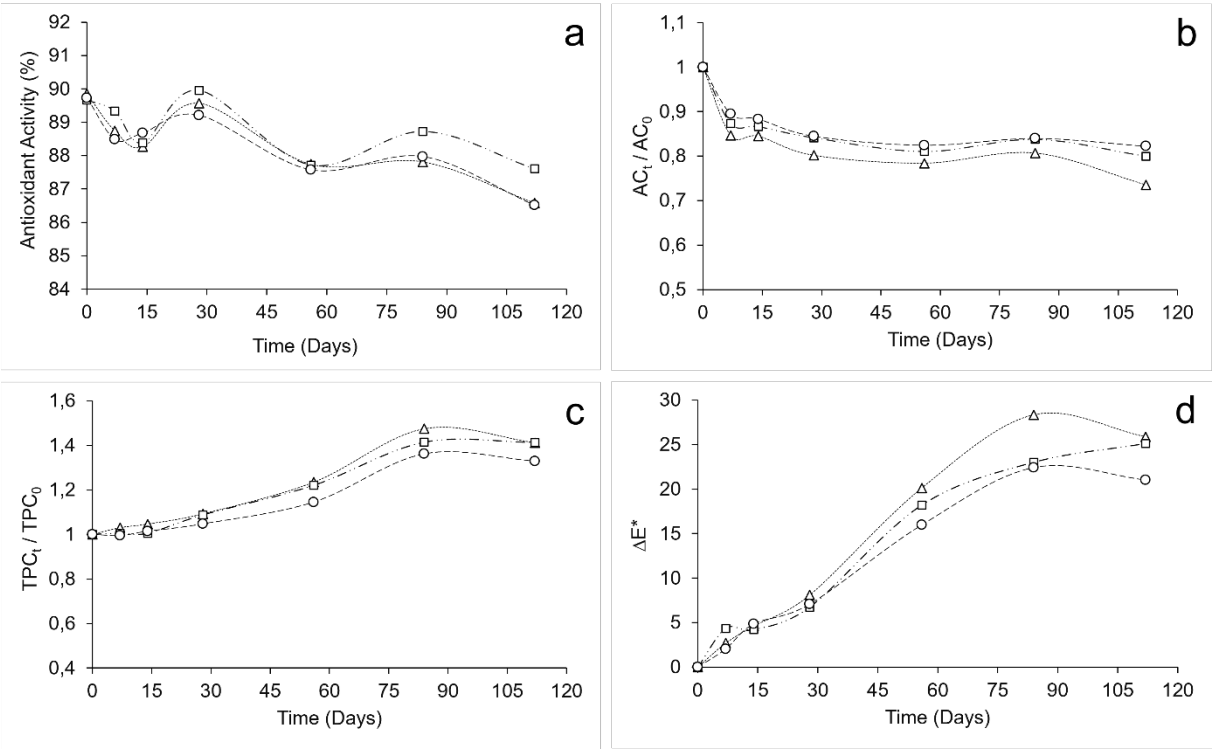
The color stability is directly related to the degradation reactions of anthocyanins (MORENO; COCERO; RODRÍGUEZ-ROJO, 2018), which can occur at different speeds, and cause various changes in coloration, with the most common being the red color becoming brown (RIBEIRO; SERAVALLI, 2007). Temperature is a factor that considerably influences the stability of anthocyanins in foods, and thermal degradation rates are influenced by the presence or absence of oxygen and other substances in the matrix that can interact with anthocyanins. As an example, compounds resulting from the Maillard reaction easily condense with anthocyanins, forming brown compounds. Another widely observed effect is the copigmentation of anthocyanins with other flavonoids, certain phenolic acids, alkaloids, and other compounds, which increases the intensity of their color. This effect depends on several factors, including type and concentration of anthocyanins and copigments, pH, and temperature, wherein the increasing temperature strongly reduces the color-intensifying effect (RIBEIRO; SERAVALLI, 2007; SCHWARTZ *et al.*, 2018). Therefore, it can be observed that temperature markedly influences the stability of anthocyanins and, consequently, coloration, which corroborates with the results observed in the present study, in which higher values of ΔE* were observed for the powders stored at 60 °C.

When the α-tocopherol content was evaluated at the end of storage, it was observed that high temperature caused greater degradation of α-tocopherol compared to the other stabilities evaluated (TABLE 1). The oxidative degradation of α-tocopherol is influenced by the same factors that influence the oxidation of unsaturated fatty acids. Thus, its degradation rate increases in the presence of oxygen, and can remarkably increase in presence of free radicals (GREGORY, 2018). The results of the present study were in accordance with those performed by Syamila *et al.* (2019), wherein they evaluated spray-dried spinach juice. The authors highlighted the fact that higher temperature increases free radical activity, thus resulting in greater degradation of α-tocopherol.

While evaluating the FTIR spectra of the samples after storage at 60 °C (FIGURE 2b), a decrease was observed in the intensity of the bands compared to that at the initial time (FIGURE 2a). The bands present at 2900 cm^{-1} in the α-tocopherol added treatments were reduced, which represent axial C–H deformation (SILVERSTEIN *et al.*, 2019). This may be

related to the bond breaking of the long saturated side chain of this compound due to thermal degradation, in addition to other methyl groups present along its structure. Another characteristic band of α-tocopherol that exhibited decrease can be observed at 1210 cm^{-1}, which refers to the aromatic ether (aryl–O–C) present in the structure of α-tocopherol. The decrease in the bands between 3500 and 3000 cm^{-1} (which corresponds to –OH groups), and around 1640 cm^{-1} (angular deformation of H–O–H) can be indicative of a decrease in the residual water in the samples (ESPINOSA-ANDREWS *et al.*, 2010; KALUSEVIC *et al.*, 2017). Moreover, a reduction in bands was also observed in the region between 1600 and 900 cm^{-1}, which corresponds to C–O–C and O–H stretching bands, characteristic of phenolic compounds and carbohydrate monomers present in grape extracts, as well as characteristic C=C stretching bands (region from 1800 to 1520 cm^{-1}) typical of aromatic compounds (ROCHA; NOREÑA, 2020), thus indicating thermal degradation of these compounds.

Figure 1 - Antioxidant activity (a), anthocyanins (b), total phenolic content (c), and color difference (d) during storage at 60 °C.



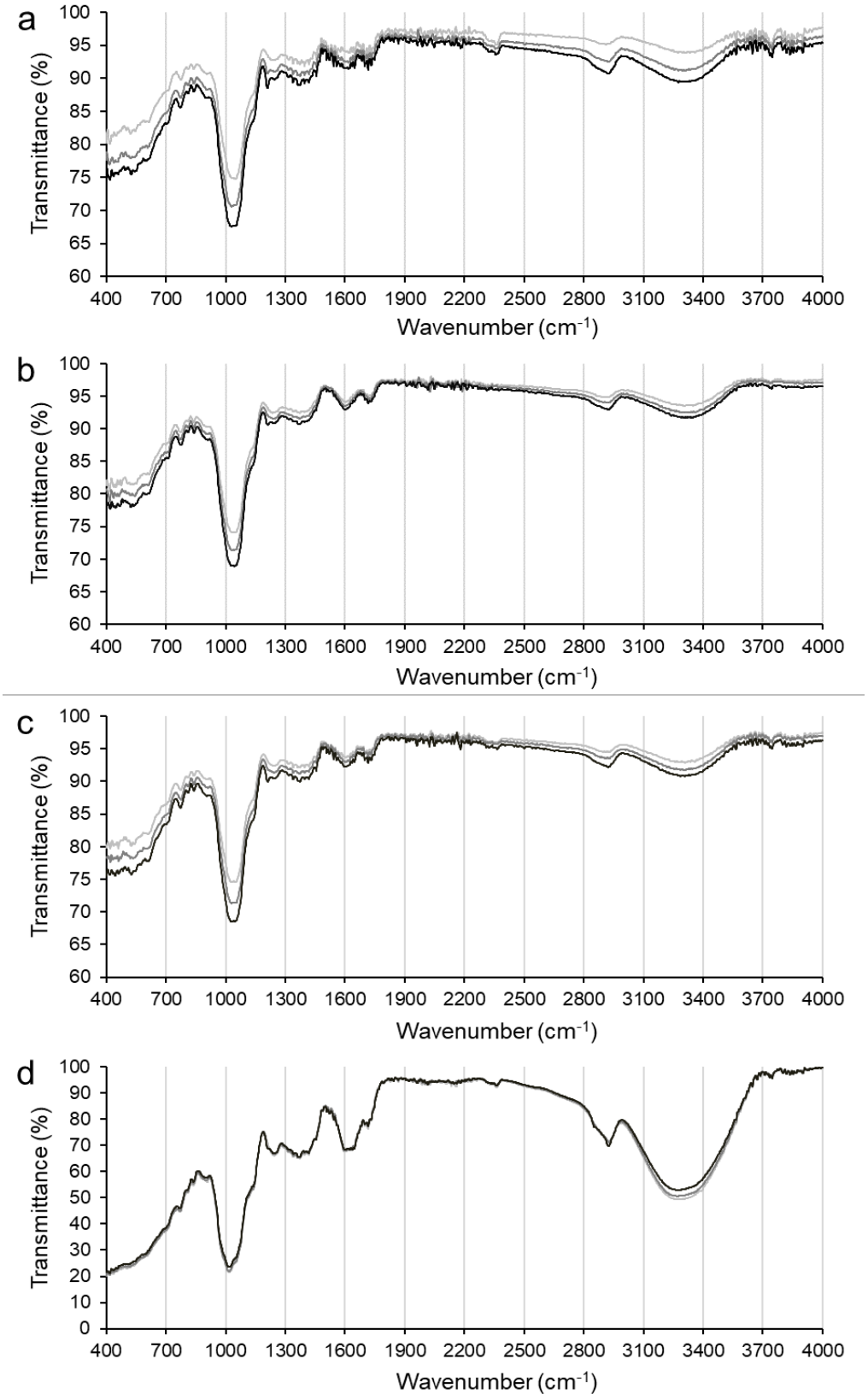
Legend: AC_t/AC_0 corresponds to the ratio between the anthocyanin content at time t (AC_t) and the initial time (AC_0). TPC_t/TPC_0 refers to the ratio between the total phenolic content at time t (TPC_t) and the initial time (TPC_0). Treatments: C (-△-) = control; 1% (– □ –) and 2% (--○--) = addition of 1.0 and 2.0% (w/w) of α-tocopherol, respectively.

Table 1 - Half-life time (t1/2) of anthocyanins, and reduction of α-tocopherol content of treatments after storage under different conditions.

Stability*	Treatments**	$t_{1/2}$ (days)	α-tocopherol Reduction (%)
Light	**C**	90.72 ± 3.45 [b]	-
	1%	100.54 ± 2.76 [a]	26.72 ± 0.56 [a]
	2%	104.78 ± 0.82 [a]	18.64 ± 0.58 [b]
T = 60 °C	**C**	252.37 ± 5.80 [c]	-
	1%	347.45 ± 17.46 [b]	42.99 ± 1.69 [a]
	2%	398.22 ± 31.74 [a]	40.51 ± 0.35 [a]
RH = 75%	**C**	57.91 ± 1.57 [a]	-
	1%	51.53 ± 1.30 [b]	20.92 ± 1.81 [a]
	2%	43.54 ± 0.94 [c]	24.57 ± 2.44 [a]

[a, b, c] Values followed by the same letters in the same column, under the same storage conditions, do not differ according to Duncan's test (p> 0.05). * T = temperature; RH = relative humidity. ** C = control; 1% and 2% = addition of 1.0 and 2.0% (w/w) of α-tocopherol, respectively.

Figure 2 - FTIR spectra of the treatments before (a) and after storage at 60 °C (b), exposed to light (c), and at 75% relative humidity (d).



Legend: Treatments: C (———) = control; 1% (———) and 2% (———) = addition of 1.0 and 2.0% (w/w) of α-tocopherol, respectively.

3.2 Stability under Light

As observed in thermostability, the samples exposed to light also presented an oscillatory behavior in relation to the antioxidant activity, revealing a decrease at 28 days of storage, followed by an increase. At the end of storage, the antioxidant activity of the samples was relatively similar to that at the initial time (FIGURE 3a). Starting from 28 days of storage, the treatment with higher concentration of α-tocopherol exhibited higher antioxidant activity compared to the others, and this may be related to the higher content of α-tocopherol in the sample (MARTIN-RUBIO *et al.*, 2018). Moser *et al.* (2017) also observed oscillatory behavior in antioxidant activity when evaluating the stability of grape juice microencapsulated via spray drying.

Exposure of the treatments to light resulted in a decrease in anthocyanins, and the control treatment presented a slightly greater degradation over time (FIGURE 3b). Ramakrishnan *et al.* (2018) evaluated the storage of samples of tamarillo juice powder obtained through spray drying, and also observed reduction of anthocyanins content when exposed to light. Regarding the half-life time (TABLE 1), the addition of α-tocopherol resulted in a longer half-life time for anthocyanins, demonstrating a synergistic effect between the compounds.

The phenolic content exhibited a slight decrease, followed by an increase, returning to the initial value (FIGURE 3c). Moreno *et al.* (2018) also did not observe remarkable increase or decrease in the phenolic content in samples of grape peel extract powder while stored in presence of light.

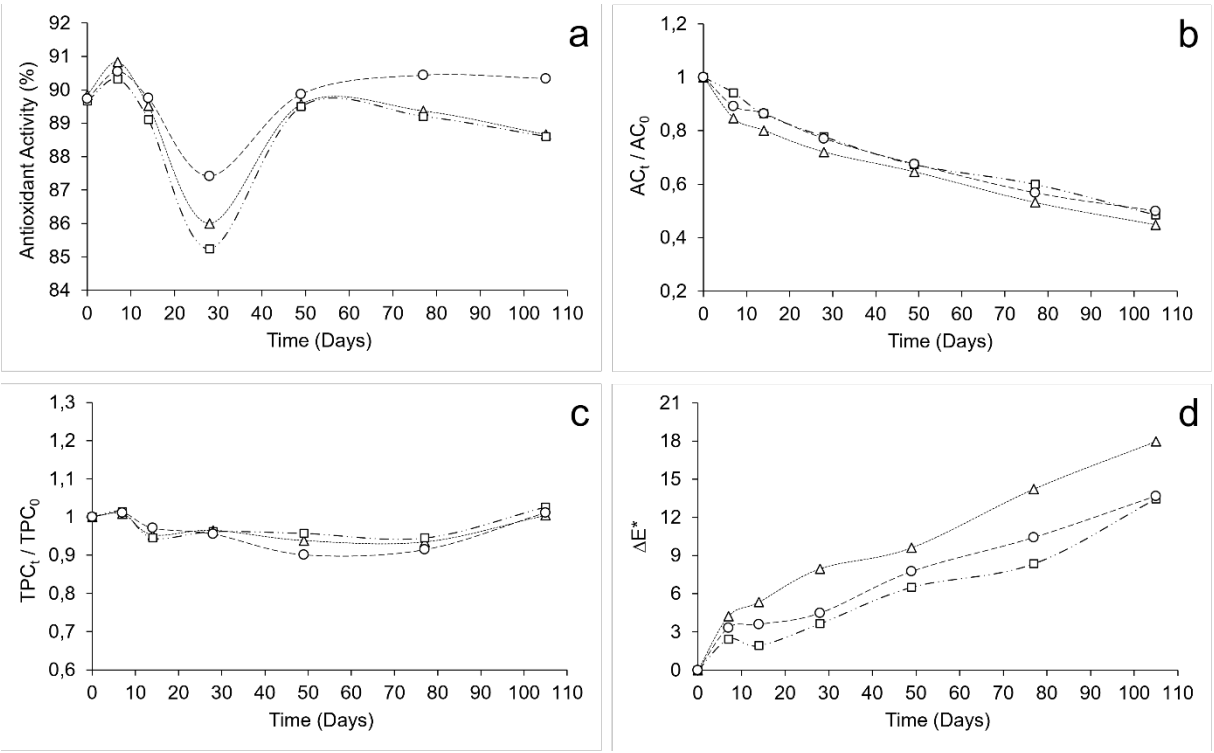
Regarding color properties, a behavior similar to that in thermostability was observed. The control treatment revealed a higher ΔE* number, demonstrating lower stability when compared to the treatments with α-tocopherol (FIGURE 3d). Thus, there was a synergistic effect between the compounds, protecting the samples against color variation when exposed to light. Furthermore, a dose-dependent relationship can be observed, in which a higher concentration of α-tocopherol resulted in an intermediate color variation. $\Delta E^* \leq 5$ was observed up to 14 days of storage. Moser *et al.* (2018) evaluated grape juice powder obtained via spray drying, and observed $\Delta E^* = 12$ after 30 days of storage under light exposure.

While evaluating the α-tocopherol content, the light seemed to be less effective in reducing this compound, considering that the observed reduction was lower than that found in thermostability, as the storage time under both conditions was similar to each other (TABLE

1). The higher molecular weight of arabic gum compared to other wall materials discussed in the literature (maltodextrin, for example) may have contributed to a greater stability in α-tocopherol, since higher molecular weight materials form a thicker polymeric layer on the particles, thus allowing less light penetration, and consequently less degradation of compounds during storage, as highlighted in the study by Ramakrishnan *et al.* (2018). The authors observed that gum arabic was better for the photostability of hydrophilic and lipophilic compounds when compared to lower molecular mass wall materials.

Analyzing the FTIR spectra, the changes in the bands were similar to those observed in thermostability, but in lower intensity (FIGURE 2c). The bands related to the residual water content (between 3500 and 3000 cm^{-1}, which corresponds to –OH groups, and around 1640 cm^{-1}, referring to the angular deformation of H–O–H) decreased in lower intensity, presumably due to the fact that in photostability, the temperature was not as high as in thermostability, which did not substantially affect the moisture content of the particles.

Figure 3 - Antioxidant activity (a), anthocyanins (b), total phenolic content (c), and color difference (d) during storage with light exposure.



Legend: AC_t/AC_0 corresponds to the ratio between the anthocyanin content at time t (AC_t) and the initial time (AC_0). TPC_t/TPC_0 refers to the ratio between the total phenolic content at time t (TPC_t) and the initial time (TPC_0). Treatments: C (-△-) = control; 1% (- □ -) and 2% (--○--) = addition of 1.0 and 2.0% (w/w) of α-tocopherol, respectively.

3.3 Stability under Humidity

In the treatments exposed to 75% RH, the antioxidant activity decreased in general, although oscillation over time was observed (FIGURE 4a). The treatment with the highest concentration of α-tocopherol exhibited the lowest value during most of the storage time. Since these particles have higher α-tocopherol content, they may have a larger amount of the compound on their surface, which makes them more susceptible to contact with oxygen and moisture, promoting their degradation (SOUZA *et al.*, 2020).

The anthocyanin content in all treatments was reduced over the storage time, with a higher concentration of α-tocopherol resulting in a greater degradation of anthocyanins (FIGURE 4b). Consequently, it was observed that higher the concentration of α-tocopherol, the shorter the half-life time for anthocyanins (TABLE 1), which demonstrates an antagonistic effect between the compounds. Furthermore, all treatments revealed shorter half-life time compared to that under other storage conditions evaluated in this study (thermostability and photostability). In the work performed by Tonon *et al.* (2010), higher values of half-life time were observed for spray-dried açai juice when stored in environments with RH of 33% and 53%, at 25 °C and 35 °C.

Importantly, anthocyanins are easily oxidized during several stages of processing and storage. Thus, the presence of oxygen can significantly accelerate the degradation of anthocyanins through a direct oxidation mechanism and/or through the action of oxidative enzymes (PATRAS *et al.*, 2010). Therefore, the treatments stored in an environment with RH of 75% may have exhibited greater degradation of anthocyanins, or even, a shorter half-life time, because the powders under these conditions were stored in direct contact with oxygen. In the other storage conditions evaluated, the powders were packaged, which drastically reduces the contact with the oxygen in the environment around the samples. Another characteristic that makes the powders more susceptible to the degradation of anthocyanins stored under these conditions is related to the adsorption of moisture from the environment by the samples, which results in greater mobility of components in the particle matrix, facilitating the occurrence of physicochemical degradation reactions (VERGARA *et al.*, 2014).

The phenolic content of all treatments was predominantly constant over time (FIGURE 4c). In the study conducted by Kuck *et al.* (2017), no major changes were observed in the phenolic content of samples of grape peel powder extract exposed to environments having different temperatures and relative humidities.

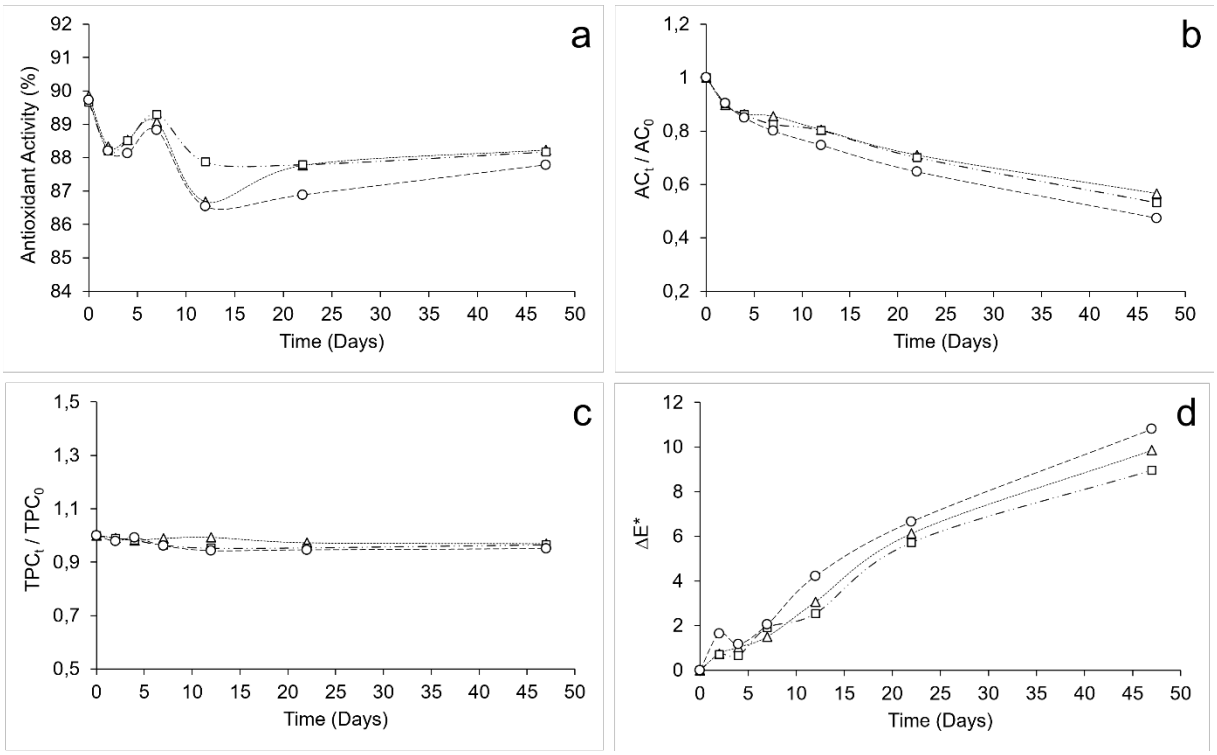
While evaluating the color properties, it was observed that a greater addition of α-tocopherol resulted in higher ΔE* compared to that of the other treatments (FIGURE 4d). Another dose-dependent relationship may also have occurred under this storage condition, where the absence of α-tocopherol resulted in intermediate ΔE* values. ΔE* ≤ 5 was observed up to 12 days of storage.

Comparing the three stabilities evaluated, the reduction in α-tocopherol in the treatments stored at 75% RH was relatively similar to the values found in photostability (TABLE 1); however, notably, the samples were stored at 75% RH for only 47 days, whereas photostability was evaluated for 105 days. This indicates that a shorter time of storage at 75% RH resulted in a decreased α-tocopherol content comparable to a longer time under another type of storage (with light exposure), indicating high degradation of the compound under the first condition mentioned. This behavior may be related to the direct contact of the samples with surrounding oxygen, since α-tocopherol is quite susceptible to oxidation (CHEW; TAN; NYAM, 2018). Another factor worth mentioning is the influence of water activity on the degradation of α-tocopherol, which reveals similar behavior to that of unsaturated fatty acids. Thus, degradation of the compound can occur at minimal rates considering the monolayer moisture value, and higher and faster rates are observed for both higher and lower water activities (GREGORY, 2018). When the samples were stored at 75% RH, the samples adsorbed moisture, which results in an increased water activity in the particles, leading to greater degradation of the compound under these conditions. With the aforementioned results, α-tocopherol seemed to confer less protection on anthocyanins when the powders were stored in direct contact with oxygen and moisture, since the compound exhibited high degradation in this condition, which justifies the greater degradation of anthocyanins and higher ΔE* value in the treatments in which α-tocopherol was added in higher concentrations.

The FTIR spectra obtained for the samples stored in an environment at 75% RH indicated a peculiar behavior (FIGURE 2d). The water molecules adsorbed by the particles seem to have exerted great influence on the structure of the compounds present in the samples, resulting in an increase in the infrared absorption, and a slight distortion in the spectra. In particular, water is markedly absorbed in the infrared (LUCASSEN; VEEN; JANSEN, 1998), which may have resulted in larger bands in the spectra. Furthermore, when samples are diluted in polar solvents, the spectra may be more distorted compared to that when samples are diluted in apolar solvents (SILVERSTEIN *et al.*, 2019). Since the samples stored in an environment with RH of 75% have abundant adsorbed water, hydrogen bonding

effects may have occurred between the molecules present in the particles and water, resulting in small distortions in the FTIR spectra. In the literature, it can be observed spectra with higher infrared absorption in samples with higher water content. Das, Goud and Das (2019) evaluated FTIR spectra of liquid and spray-dried anthocyanin extract, and the spectrum for the liquid extract exhibited higher infrared absorption when compared to that of the dry samples. The same behavior was observed for flexirubin extract in liquid and powder forms (VENIL *et al.*, 2016).

Figure 4 - Antioxidant activity (a), anthocyanins (b), total phenolic content (c), and color difference (d) during storage at 75% relative humidity.



Legend: AC_t/AC_0 corresponds to the ratio between the anthocyanin content at time t (AC_t) and the initial time (AC_0). TPC_t/TPC_0 refers to the ratio between the total phenolic content at time t (TPC_t) and the initial time (TPC_0). Treatments: C (-△-) = control; 1% (- □ -) and 2% (--○--) = addition of 1.0 and 2.0% (w/w) of α-tocopherol, respectively.

4 CONCLUSION

Based on the data obtained in the present study, a synergistic effect between α-tocopherol and anthocyanins was observed when particles were stored without direct contact with oxygen and humidity. In particular, packaging with good barrier properties against oxygen and moisture are commonly used for powdered food products, ensuring good product stability. Thus, it can be concluded that the addition of α-tocopherol (2.0 %, w/w) provided better stability to anthocyanins which is an interesting contribution for the food sector. Future studies involving the application of these particles are important as a way to evaluate the performance of the material in different types of food matrices, thus promoting the addition of bioactive compounds and the use of natural origin ingredients in foods.

Acknowledgments

The authors thank the National Council for Scientific and Technological Development (CNPq) for financial support (Process: 141824/2017-4); the Central of Analysis and Chemical Prospecting of the Federal University of Lavras, and Financier of Studies and Projects (FINEP), Minas Gerais State Research Foundation (FAPEMIG), CNPq e Improvement Coordination of Higher Level Personnel (CAPES) for supplying the equipment and technical support for experiments involving chromatographic analyzes.

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CONCLUSÃO GERAL

A coencapsulação de α-tocoferol e antocianinas extraídas de cascas de uva representa um grande potencial de inovação, visto que ainda não foi abordado na literatura e apresentou resultados promissores. Os compostos demonstraram interação entre si, e embora tenha sido observado alguns efeitos antagonistas, em condições adequadas de armazenamento a adição de α-tocoferol proporcionou maior estabilidade às antocianinas. Estudos futuros envolvendo a aplicação dessas partículas são importantes como forma de avaliar o desempenho do material em diferentes tipos de matrizes alimentícias, favorecendo assim a adição de compostos bioativos e o uso de ingredientes de origem natural nos alimentos.