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UNIVERSIDADE ESTADUAL PAULISTA – UNESP
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Maria Eduarda Carvalho Vargas

**Extrato fenólico de cálices de vinagreira (*Hibiscus sabdariffa* L.):
avaliação da estabilidade da cor e das antocianinas durante o armazenamento, e sua
aplicabilidade em iogurte**

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Área de Concentração: Alimentos e Nutrição

Orientadora: Prof^a. Dr^a. Ellen Silva Lago-Vanzela

Coorientador: Prof. Dr. Roberto da Silva

Coorientadora: Dr^a. Yara Paula Nishiyama-Hortense

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RESUMO

Os cálices de vinagreira (CV, *Hibiscus sabdariffa* L.) são fontes importantes de compostos fenólicos (CF), cujos extratos apresentam potencial para uso como bioingredientes na produção de alimentos. Este estudo teve como objetivo caracterizar físico-quimicamente os cálices *in natura* e desidratados (CVD) e desenvolver estratégias para melhorar a aplicabilidade dos extratos fenólicos obtidos a partir dos CVD. As abordagens empregadas incluíram a adição de açúcar e a desidratação por secagem em leito de espuma, com posterior aplicação em iogurtes. A caracterização físico-química dos CVD revelou uma umidade de 11,61% e uma atividade de água de 0,56, ambos em conformidade com a legislação brasileira para vegetais desidratados. Para a extração dos CF dos CVD, três soluções extratoras foram avaliadas: metanol acidificado, etanol 60% e água. A extração metanólica resultou na maior concentração de compostos fenólicos totais (CFT, mg EAG·g⁻¹ de CVD, em base seca) (17,48), seguida pela extração aquosa (16,15) e pela solução hidroalcoólica (14,51), evidenciando a viabilidade da extração aquosa para obtenção de extratos fenólicos alimentícios. A análise por cromatografia líquida de alta eficiência com detectores de arranjo de diodos e espectrometria de massas multidimensional (HPLC-DAD-ESI-MSⁿ) indicou que a concentração total de antocianinas (mg cy-3-glc·kg⁻¹) variou entre 2726,09 (extrato hidroalcoólico) e 4598,70 (extrato aquoso), sendo a delfinidina-3-sambubiosídeo e a cianidina-3-sambubiosídeo as principais antocianinas identificadas. Com base nesses resultados, os extratos aquosos foram empregados na formulação de xaropes e bioingredientes em pó (BIP). Os xaropes, preparados com diferentes concentrações de sacarose (5%, 15%, 25% e 35%), foram envasados em frascos herméticos, armazenados a 4 °C por 30 dias e avaliados quanto à estabilidade das antocianinas totais (ANT), dos CFT e dos parâmetros cromáticos (L^* , C^* e h°) nos tempos 0, 15 e 30 dias. O extrato controle (sem sacarose) apresentou retenção de 91,06% das ANT após o armazenamento, enquanto nos xaropes os valores variaram entre 78,41% e 91,20%, com maiores perdas em amostras com menor teor de sacarose. Em relação aos CFT, o extrato controle reteve 93,61%, enquanto nos xaropes a retenção mínima foi de 94,98%. A coloração foi mais estável nos xaropes com maior teor de sacarose, apresentando um ΔE de 5,48 após o armazenamento, indicando manutenção da tonalidade. Para a produção do BIP, a espuma formulada a partir dos extratos fenólicos e aditivos (emulsificante, estabilizante e maltodextrina) demonstrou propriedades adequadas para desidratação, incluindo densidade, estabilidade e percentual de expansão. O produto final apresentou concentração de antocianinas e CFT de 48,50 mg de mv-

3,5-diglc·g⁻¹ e 295 mg EAG·g⁻¹, respectivamente. Após análise dos parâmetros de cor foi possível constatar que o BIP produzido resultou em tonalidade de cor avermelhada, característica que foi preservada após sua incorporação ao iogurte. Visualmente, os iogurtes contendo BIP apresentaram coloração mais atrativa em comparação aos enriquecidos com xarope ou extrato controle. Os resultados demonstram que os extratos fenólicos de CVD, com ou sem adição de sacarose, podem ser armazenados sob refrigeração por até 30 dias, mantendo ainda importante concentração de antocianinas e CFT, assim como coloração vermelha atrativa. Além disso, a obtenção do BIP amplia o potencial de aplicação da vinagreira em diferentes matrizes alimentícias, representando uma alternativa promissora para a indústria de alimentos.

Palavras-chave: *Hibiscus sabdariffa L*; extratos fenólicos; bioingredientes.

ABSTRACT

The calyces of roselle (CV, *Hibiscus sabdariffa* L.) are important sources of phenolic compounds (PC), whose extracts have potential for use as bioingredients in food production. This study aimed to physicochemically characterize the fresh and dehydrated calyces (CVD) and develop strategies to improve the stability and applicability of the phenolic extracts obtained from CVD. The approaches employed included the addition of sugar and dehydration by foam-mat drying, followed by application in yogurts. The physicochemical characterization of CVD revealed a moisture content of 11.61% and a water activity of 0.56, both in compliance with Brazilian regulations for dehydrated plant materials. For the extraction of PC from CVD, three extracting solutions were evaluated: acidified methanol, 60% ethanol, and water. Methanolic extraction resulted in the highest concentration of total phenolic compounds (TPC, mg GAE·g⁻¹ of CVD, dry basis) (17.48), followed by aqueous extraction (16.15) and the hydroalcoholic solution (60% ethanol) (14.51), demonstrating the feasibility of aqueous extraction for obtaining food-grade phenolic extracts. High-performance liquid chromatography with diode-array detection and multidimensional mass spectrometry (HPLC-DAD-ESI-MSn) analysis indicated that the total anthocyanin concentration (mg cy-3-glc·kg⁻¹) ranged from 2726.09 (60% ethanol extract) to 4598.70 (aqueous extract), with delphinidin-3-sambubioside and cyanidin-3-sambubioside identified as the main anthocyanins. Based on these results, aqueous extracts were used in the formulation of syrups and powdered bioingredients (PBI). The syrups, prepared with different sucrose concentrations (5%, 15%, 25%, and 35%), were packaged in hermetically sealed bottles, stored at 4°C for 30 days, and evaluated for the stability of total anthocyanins (ANT), TPC, and color parameters (L^* , C^* , and h°) at 0, 15, and 30 days. The control extract (without sucrose) retained 91.06% of ANT after storage, while retention in the syrups ranged from 78.41% to 91.20%, with higher losses observed in samples with lower sucrose content. Regarding TPC, the control extract retained 93.61%, while in the syrups, the minimum retention was 94.98%. Color stability was greater in syrups with higher sucrose content, showing a ΔE of 5.48 after storage, indicating color maintenance. For PBI production, the foam formulated from phenolic extracts and additives (emulsifier, stabilizer, and maltodextrin) exhibited suitable properties for dehydration, including density, stability, and expansion percentage. Although drying at 80°C resulted in 13% retention of ANT and 24% retention of TPC, the PBI maintained an intense red color (15.61°),

which was preserved after incorporation into yogurt (25.44°). Visually, yogurts containing PBI presented a more attractive color compared to those enriched with syrup or control extract.

The results demonstrate that phenolic extracts from CVD, with or without sucrose addition, can be refrigerated for up to 30 days while maintaining their stability and technological functionality. Additionally, the production of PBI expands the potential application of roselle in various food matrices, representing a promising alternative for the food industry.

Keywords: *Hibiscus sabdariffa* L; phenolic extracts; bioingredients.

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LISTA DE ABREVIACOES E SIGLAS

3-ACQ	cido 3-cafeoilqunico
3-AFQ	cido 3-feruloilqunico
3-<i>p</i>-ACoCQ	cido 3- <i>p</i> -cumaroilqunico
3,4-diACQ	cido 3,4-di-cafeoilqunico
3,5-diACQ	cido 3,5-di-cafeoilqunico
4-ACQ	cido 4-cafeoilqunico
4-AFQ	cido 4-feruloilqunico
4-<i>p</i>-ACoCQ	cido 4- <i>p</i> -cumaroilqunico
4,5-diACQ	cido 4,5-di-cafeoilqunico
5-ACQ	cido 5-cafeoilqunico
5-AFQ	cido 5-feruloilqunico
5-<i>p</i>-ACoCQ	cido 5- <i>p</i> -cumaroilqunico
A	Base quinoidal
ACP	Anlise de Componentes Principais
ANP	Antocianina Polimricas
ANT	Antocianinas Totais
AT	Acidez Titulvel
Aw	Atividade de gua
B	Hemicetal
B.S.	Base seca
BIP	Bioingrediente em p
C	Catequina
C*	Croma
CF	Compostos Fenlicos
CFT	Compostos Fenlicos Totais
CH	Chalcona
CIE	Commission Internationale de l'Eclairage
Cy	Cianidina
Cy-3-(2''-xyl)glic	Cianidina-3-(2''-xiloil)-glicosdeo
Cy-3-glc	Cianidina-3-glicosdeo
Cy-3-rutin	Cianidina-3-rutinsideo
Cy-3-samb	Cianidina-3-sambubiosdeo

Cy-3,5-diglic	Cianidina-3,5-diglicosídeo
CV	Cálices de vinagreira
CVD	Cálices de vinagreira desidratados
DAD	Detectores de arranjo de diodos
DCNTs	Doenças Crônicas Não Transmissíveis
Dp	Delfinidina
Dp-3-(2''-xyl)glic	Delfinidina-3-(2''-xiloil)-glicosídeo
Dp-3-glc	Delfinidina-3-glicosídeo
Dp-3-samb	Delfinidina-3-sambubiosídeo
E	Epicatequina
E0	Extrato sem sacarose
E5	Extrato com 5% de sacarose
E15	Extrato com 15% de sacarose
E25	Extrato com 25% de sacarose
E35	Extrato com 35% de sacarose
EA	Extrato adoçado com 35% de sacarose
EAG	Ácido Gálico
ECA	Enzima Conversora de Angiotensina
ECG	(-) galato-3-epigalocatequina
EGC	(-) – epigalocatequina
EGCG	(-)galato-3-epigalocatequina
EM	Emustab
ES	Extrato não adoçado
ESI-MSⁿ	Espectrometria de massas multidimensional
FDA	Food and Drug Administration
G	Gossipetina
GC	(+) -galocatequina
GCG	(+) galato-3-galocatequina
h(°)	Hue
HA+	Cátion Flavílio
HPLC	Cromatografia Líquida de Alta Eficiência
I-BIP	Iogurte adicionado com bioingrediente em pó
I-EA	Iogurte adicionado com extrato adoçado com 35% sacarose

I-ES	Iogurte adicionado com extrato sem sacarose
I-XC	Iogurte adicionado com xarope comercial de <i>hibicus</i>
IL-6	Interleucina-6
K	Kaempferol
K-3-cumglic	Kaempferol-3-cumarilglicosídeo
K-3-rutin	Kaempferol-3-rutinosídeo
K-3-samb	Kaempferol-3-sambubiosídeo
L*	Claridade
M	Miricetina
M-3-Ag	Miricetina-3- arabinogalactose
M-3-pent-7-hex	Miricetina-3-pentossil-7-hexosídeo
M-3-samb	Miricetina-3-sambubiosídeo
MA	Maltodextrina 10 DE
MCP-1	Proteína Quimiotática de Monócitos-1
Mv	Malvidina
Mv-3,5-diglic	Malvidina-3,5-diglicosídeo
NO	Óxido Nítrico
p-Co	ácido <i>p-cumárico</i>
PANC	Plantas Alimentícias Não Convencionais
PC1	Componente Principal 1
PC2	Componente Principal 2
Pg	Pelargonidina
Pn	Peonidina
Pt	Petunidina
Q	Quercetina
Q-3-(6-acetil-glic)	Quercetina-3- (6-acetil-glicosídeo)
Q-3-gal	Quercetina-3-galactosídeo
Q-3-glic	Quercetina-3-glicosídeo
Q-3-pent-7-hex	Quercetina-3-pentossil-7-hexosídeo
Q-3-rutin	Quercetina-3-rutinosídeo
Q-3-samb	Quercetina-3-sambubiosídeo
Q-galoil-hex	Quercetina-galoil-hexosídeo
SPN	Super Liga Neutra

- SS** Sólidos Solúveis
- TNF- α** Fator de Necrose Tumoral Alfa
- UV** Ultravioleta
- UV-Vis** Ultravioleta-visível
- XC** Xarope comercial de *Hibiscus*

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1 INTRODUÇÃO GERAL

A busca por sistemas alimentares mais sustentáveis e seguros tem impulsionado o interesse por espécies vegetais subutilizadas, especialmente em países com rica biodiversidade, como o Brasil. Nesse contexto, as Plantas Alimentícias Não Convencionais (PANC), incluindo a vinagreira (*Hibiscus sabdariffa* L.), emergem como alternativas promissoras para diversificação alimentar e desenvolvimento de produtos inovadores. A valorização dessas espécies negligenciadas não apenas amplia as opções nutricionais disponíveis para a população, mas também fortalece práticas agrícolas sustentáveis e estimula o aproveitamento de recursos naturais de forma mais eficiente (Kinupp; Lorenzi, 2014; Mariutti *et al.*, 2021). Paralelamente, mudanças nos padrões de consumo têm levado à crescente demanda por alimentos mais saudáveis, livres de aditivos sintéticos e visualmente atrativos, impulsionando a substituição de corantes artificiais por alternativas naturais (Albuquerque *et al.*, 2020; Gordillo *et al.*, 2018).

Nesse cenário, os pigmentos naturais pertencentes ao grupo dos flavonoides, como as antocianinas, destacam-se por sua versatilidade, conferindo coloração intensa e propriedades bioativas aos alimentos. Entretanto, desafios associados à estabilidade desses compostos durante a extração, o processamento e o armazenamento ainda limitam sua ampla utilização na indústria de alimentos (Cheng *et al.*, 2023; Gordillo *et al.*, 2018; Sampaio *et al.*, 2021; Teixeira *et al.*, 2023; Wijesekara; Xu, 2024).

A vinagreira se sobressai como uma cultura anual de fácil cultivo e amplamente disseminada no Brasil, inclusive no estado de São Paulo. Seus cálices vermelhos são ricos em compostos fenólicos, principalmente antocianinas, conferindo-lhes potencial para aplicação como corante natural em produtos alimentícios (Mariod; Tahir; Mahunu, 2021). Além da função corante, esses compostos apresentam atividade antioxidante, estando associados à redução do risco de doenças não transmissíveis, como enfermidades cardiovasculares e câncer (Coelho; Amorim, 2019; Da-Costa-Rocha *et al.*, 2014; Nowicka; Wojdylo, 2019; Teixeira *et al.*, 2023).

Apesar do seu potencial, a incorporação da vinagreira em formulações alimentícias apresenta desafios significativos. Nos cálices, os compostos fenólicos (CF) estão naturalmente protegidos pela complexa estrutura celular da planta. Durante o processamento, a ruptura dessas estruturas leva à descompartimentalização das organelas, desencadeando reações químicas e bioquímicas que afetam a estabilidade desses compostos. A estrutura química das antocianinas, que lhes confere propriedades funcionais, também as torna vulneráveis a processos oxidativos, térmicos e enzimáticos, comprometendo sua retenção e bioatividade ao longo da cadeia produtiva (Da-Silva; Lago-Vanzela; Baffi, 2015). Como consequência, ocorre uma redução progressiva dos CF originalmente presentes na matriz vegetal, acompanhada da formação de

derivados poliméricos que impactam tanto as características sensoriais quanto a funcionalidade dos produtos elaborados (Debelo; Ferruzi, 2020). Além disso, em formulações contendo múltiplos ingredientes, interações entre macronutrientes e micronutrientes podem influenciar a estabilidade desses compostos, afetando diretamente suas propriedades tecno-funcionais e sua eficácia como bioativos (Da-Silva; Lago-Vanzela; Baffi, 2015). Dessa forma, fatores como a estrutura química, a concentração e as interações com a matriz alimentar são determinantes para a viabilidade de aplicação da vinagreira em produtos diferenciados (Nagar *et al.*, 2021; Ribas-Agustí *et al.*, 2018).

Para contornar esses desafios e viabilizar a aplicação dos extratos fenólicos obtidos da vinagreira em alimentos, este estudo adotou duas abordagens principais: o desenvolvimento de xaropes adoçados e a obtenção de extratos desidratados por secagem em leito de espuma. A formulação de xaropes adoçados busca facilitar a incorporação dos compostos bioativos em diferentes produtos alimentícios, tornando-os mais acessíveis à indústria. No entanto, a estabilidade desses xaropes ao longo do armazenamento deve ser avaliada para garantir a preservação da cor e das concentrações dos compostos bioativos presentes (Aaby; Amundsen, 2023). Paralelamente, a secagem do extrato tem como objetivo a obtenção de pós solúveis e estáveis, e com elevada importante concentração de CF. Dentre as técnicas de secagem, o leito de espuma se destaca como uma alternativa viável, de fácil execução e baixo custo, permitindo a ampliação do uso desses extratos em diversas formulações alimentares sem comprometer sua funcionalidade (Tavares *et al.*, 2015; 2017; 2019).

CONCLUSÃO GERAL

Os resultados obtidos neste trabalho evidenciam a viabilidade e o potencial dos uso dos CV para a produção e aplicação de bioingredientes. A caracterização físico-química dos CV *in natura* e desidratados revelou a influência do processo de secagem, o qual proporcionou parâmetros seguros de umidade (11,61%) e A_w (0,56). Além disso, foram observadas significativas concentrações de AT (18,50 g ácido málico·100 g⁻¹), SS (1,93 °Brix), CFT (21,90 mg EAG·g⁻¹), ANT (6,10 mg de mv-3,5-diglc·g⁻¹) e ANP (3,30 mg de mv-3,5-diglc·g⁻¹), destacando a potencialidade dos CVD como matéria-prima rica em CF.

Para validar o potencial dos CVD na obtenção de extratos fenólicos, a extração com metanol acidificado (controle) apresentou maior retenção de CFT (17,48 mg EAG·g⁻¹, B.S.). Por outro lado, a extração com água demonstrou um bom potencial, alcançando valores médios de 16,15 mg EAG·g⁻¹ (B.S.). A análise por HPLC-DAD-ESI-MSⁿ revelou que a concentração de total antocianinas (mg cy-3-glc·kg⁻¹) variou entre 2726,09 (extrato 60% etanol) e 4598,70 (extrato aquoso), sendo identificadas como majoritárias a delfinidina-3-sambubiosídeo e a cianindina-3-sambubiosídeo. Esses resultados reforçam a extração aquosa como uma alternativa viável, segura e sustentável para a obtenção de extratos fenólicos e seus bioingredientes.

Os xaropes apresentaram retenções de CFT entre 94,94% (E15) e 113,86% (E35) e de ANT entre 78,41% (E15) e 91,20% (E35), mantendo os parâmetros cromáticos, especialmente nas formulações com maiores concentrações de sacarose (E25 e E35). Os extratos sem sacarose (E0) também demonstraram boa estabilidade desses compostos, com retenções finais de 93,61% para CFT e 91,06% para ANT. Ambos os tipos de extrato mantiveram sua estabilidade durante 30 dias de armazenamento refrigerado (4 °C). A secagem em leito de espuma, utilizada para a obtenção do BIP, resultou em uma coloração vermelha preservada (15,61°), mesmo após a incorporação ao iogurte (25,44°). Dessa forma, as estratégias adotadas neste estudo – extração com água, adição de sacarose e secagem em leito de espuma – mostraram-se eficazes na preservação dos compostos bioativos e das propriedades cromáticas dos extratos de CVD. Essas abordagens ampliam as possibilidades de aplicação dos CV como bioingrediente em diversos produtos alimentícios, consolidando-se como uma alternativa sustentável para a indústria alimentícia.

Buscou-se a partir de tecnologias acessíveis, oferecer soluções para preservar a funcionalidade tecnológica e a estabilidade dos CF dos extratos de CVD, promovendo alimentos inovadores que pudessem atender às demandas do consumidor moderno.

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