LAÍS BRUNO NORCINO

DEVELOPMENT OF FUNCTIONALIZED MICROPARTICLES CONTAINING ANTHOCYANINS FROM GRAPE EXTRACT (*VITIS* LABRUSCA L.) OBTAINED BY IONIC GELATION:

EVALUATION OF STABILITY, CONTROLLED RELEASE AND POTENTIAL USE AS A COLORIMETRIC INDICATOR

LAVRAS – MG
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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Engenharia de Biomateriais, área de concentração Produtos e Nanoprodutos Alimentícios para obtenção do título de Doutora.

Prof. Dr. Diego Alvarenga Botrel
Orientador

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LAÍS BRUNO NORCINO

DESENVOLVIMENTO DE MICROPARTÍCULAS FUNCIONALIZADAS CONTENDO ANTOCIANINAS DE EXTRATO DE UVA (VITIS LABRUSCA L.) OBTIDAS POR GELIFICAÇÃO IÔNICA: AVALIAÇÃO DA ESTABILIDADE, LIBERAÇÃO CONTROLADA E POTENCIAL USO COMO INDICADOR COLORIMÉTRICO

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APROVADA em 22 de novembro de 2022.
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LAVRAS – MG
2023

Aos meus pais, Claudio e Vera, pelo amor em seu pleno significado; à minha querida irmã Adriana, pela amizade e companheirismo; à minha avó Dalva (*in memoriam*), por todo carinho; ao meu namorado Daniel, por todo suporte e paciência.

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“Feeling my way through the darkness
Guided by a beating heart
I can't tell where the journey will end
But I know where to start.”

Avicii

RESUMO

Nos últimos anos, a busca da indústria alimentícia por compostos bioativos naturais para confecção de micropartículas multifuncionais capazes de colorir e informar o status de frescor dos alimentos em tempo real vem crescendo consideravelmente. Nesse contexto, o objetivo deste trabalho foi produzir micropartículas funcionalizadas com antocianinas extraídas da casca da uva, utilizando os métodos combinados de emulsificação e gelificação iônica ultrassônica, a fim de aplicá-las como corante alimentício e para monitorar a qualidade do leite in natura. Na primeira etapa do projeto, foram avaliados diferentes materiais gelificantes (alginato e pectina) para formação das emulsões múltiplas e sua influência nas propriedades físico-químicas, morfológicas e no perfil de liberação gastrointestinal das micropartículas. Em geral, o efeito da emulsificação combinado com gelificação foi mais pronunciada nas micropartículas de alginato e resultou em maior retenção das antocianinas, maior capacidade antioxidante, e também permitiu o melhor perfil de liberação durante a digestão intestinal. A partir da escolha do melhor sistema gelificante, foram incorporados diferentes materiais de parede complementares (amido modificado, inulina e maltodextrina) para otimizar a retenção dos compostos bioativos nas micropartículas. Ainda, as micropartículas produzidas na segunda etapa foram aplicadas em leite in natura na forma de corante natural e para o monitoramento da qualidade do produto, visando funcionar como indicadores colorimétricos comestíveis. A micropartícula combinada com amido modificado foi a que obteve melhor estabilidade em condições aceleradas e térmicas e maior retenção dos compostos bioativos quando comparadas as que continham inulina e maltodextrina. Além disso, as micropartículas à base de antocianinas foram capazes de discriminar entre leite fresco e impróprio para o consumo humano. Esses resultados nos mostram a possibilidade de utilizar essas micropartículas inteligentes como indicadores colorimétricos comestíveis, abrindo espaço para novas abordagens na indústria alimentícia, permitindo ao consumidor detectar facilmente a olho nu se o alimento está apto ou não para consumo, além de ser um ingrediente corante natural obtido de fontes de baixo custo.

Palavras-chave: Gelificação iônica ultrassônica. Antocianinas. Materiais de parede complementares. Indicador colorimétrico comestível.

ABSTRACT

In recent years, the food industry's search for natural bioactive compounds to produce multifunctional microparticles capable of coloring foods and providing real-time information about their freshness status has increased significantly. In this context, the aim of this work was to produce microparticles functionalized with anthocyanins extracted from grape skins, using the combined methods of emulsification and ionic gelation with ultrasound, in order to use them as food colorants and to monitor the quality of in-natura milk. In the first phase of the project, different gelling agents (alginate and pectin) were studied for the formation of multiple emulsions and their influence on the physicochemical and morphological properties, as well as on the gastrointestinal release profile of the microparticles. In general, the effect of emulsification combined with gelation was more pronounced in the alginate microparticles and resulted in greater retention of anthocyanins, greater antioxidant capacity, and also allowed the best release profile during intestinal digestion. Based on the choice of the best gelling system, different complementary wall materials were incorporated (modified starch, inulin and maltodextrin) to optimize the retention of bioactive compounds in the microparticles. In addition, the microparticles produced in the second phase were used in the form of a natural dye in milk to monitor the quality of the product and act as edible colorimetric indicators. The microparticles combined with modified starch were more stable under accelerated and thermal conditions compared to the microparticles containing inulin and maltodextrin, and retained the bioactive compounds better. Moreover, anthocyanin based microparticles were able to discriminate between fresh milk and milk unfit for human consumption. These results show us the possibility of using these smart microparticles as edible colorimetric indicators, opening up new approaches in the food industry and allowing consumers to tell with the naked eye whether the food is suitable for consumption or not, as well as being a natural colorant obtained from low-cost sources.

Keywords: Ultrassonic-assisted ionic gelation. Anthocyanins. Complementary wall materials. Edible colorimetric indicator.

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PRIMEIRA PARTE

1 INTRODUÇÃO

O interesse dos consumidores por alimentos contendo compostos bioativos vem crescendo nos últimos anos devido as diversas evidências epidemiológicas que apontam pela necessidade de mudanças dos hábitos alimentares, visando a melhora da qualidade de vida e diminuição da incidência de doenças ligadas a uma alimentação inadequada.

A uva é uma das frutas mais cultivadas em todo o mundo e apresenta grande relevância econômica. Seu consumo é tanto na forma fresca ou como matéria-prima para a elaboração de sucos, geleias, passas e vinhos. No entanto, durante o seu processamento, são geradas toneladas de subprodutos incluindo sementes, talos e cascas que muitas vezes são descartados no meio ambiente ou destinados a outras atividades complementares tal como a adubação, por serem considerados produtos de baixo valor agregado.

A utilização desses subprodutos pela indústria alimentícia vem ganhando destaque, por representarem matérias-primas de baixo custo e estarem amplamente disponíveis. Além disso, possuem grandes quantidades de compostos fenólicos, incluindo as antocianinas, que são pigmentos e indicadores colorimétricos naturais, capazes de colorir e monitorar a qualidade de alimentos em tempo real. Entretanto, as antocianinas são moléculas instáveis e que se degradam facilmente quando em contato com o oxigênio, temperatura e exposição a luz, por exemplo. Assim, o grande desafio da indústria alimentícia está na busca por tecnologias capazes de preservar e incorporar esses compostos bioativos nos alimentos. Sendo assim, a microencapsulação vem como uma alternativa bastante promissora.

A microencapsulação tem como principal objetivo a proteção desses compostos bioativos e, com isso, aumentar sua estabilidade sob condições adversas, seja durante o processamento, armazenamento e aplicação em produtos alimentícios. Dentre as diversas técnicas de microencapsulação conhecidas, a gelificação iônica ganha destaque. É considerada uma tecnologia com condições brandas, simples, emergente e de baixo custo. Nesta é possível obter micropartículas que encapsulam e protegem os componentes ativos em uma rede tridimensional, aumentando a sua estabilidade. Os polímeros aniônicos alginato de sódio e pectina amidada são

materiais mais comumente utilizados no desenvolvimento de micropartículas por gelificação iônica.

No entanto, devido a porosidade das micropartículas obtidas, pode ocorrer uma liberação descontrolada e baixa proteção dos compostos bioativos sendo assim, faz-se necessário a combinação da gelificação iônica com outras técnicas complementares, como as emulsões múltiplas. Quando dispersos em sistemas emulsionados, os compostos bioativos presentes no extrato de uva são protegidos e imobilizados através da interação entre a emulsão e a rede tridimensional formada pelos polímeros e, portanto, apresentam melhora nas propriedades físico-químicas das micropartículas, aumentando a estabilidade e retenção dos compostos bioativos. Ainda, visando a melhora da retenção desses compostos no interior das micropartículas, pode-se utilizar materiais de parede complementares, tais como a inulina, maltodextrina e amido modificado. Esses materiais apresentam boas características tecnológicas e nutricionais e por isso nos últimos anos vêm sendo utilizados pela indústria alimentícia.

Desta forma, o desenvolvimento de micropartículas multifuncionais funcionalizadas com antocianinas de fontes naturais, com capacidade corante e de indicador colorimétrico, torna-se extremamente relevante, permitindo ao consumidor detectar facilmente a olho nu se o alimento está apto ou não para o consumo, além de ser um ingrediente corante natural, como forma de inovação no setor alimentício.

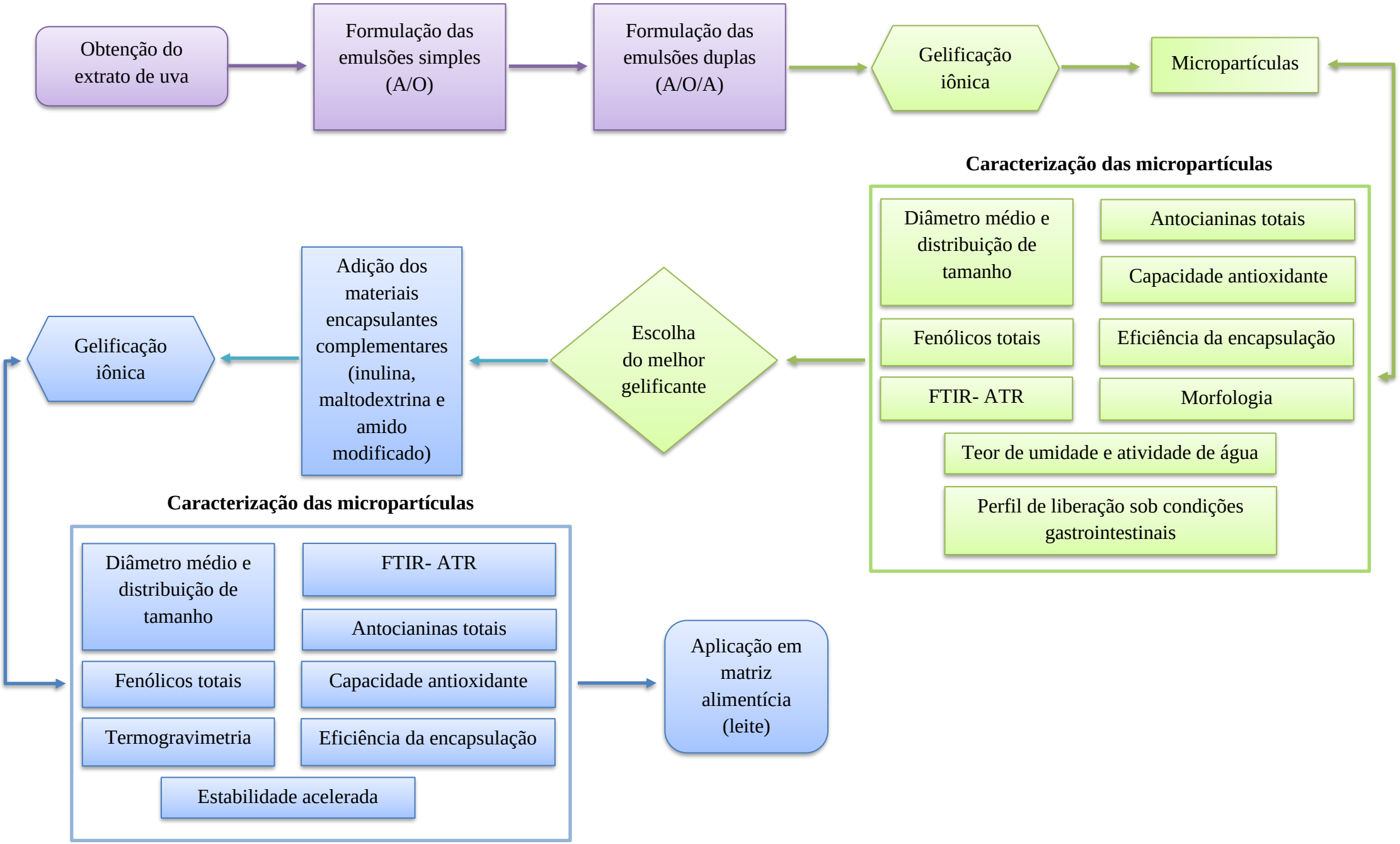
Diante do exposto, o presente trabalho tem como objetivo a produção, caracterização e avaliação de micropartículas contendo antocianinas extraídas da casca de uva, empregando a técnica de gelificação iônica ultrassônica, combinada com emulsões múltiplas e materiais de parede complementares a fim de melhorar as propriedades físico-químicas, térmicas, morfológicas e o perfil de liberação gastrointestinal (*in vitro*) das antocianinas. Ainda, foi avaliada a aplicação das micropartículas em matriz alimentícia como corante natural e indicador colorimétrico comestível.

2 ESTRUTURAÇÃO DO PROJETO

Para facilitar a compreensão da proposta de trabalho do Doutorado, este projeto foi dividido em três etapas, conforme descrição resumida seguinte. A primeira etapa visou a obtenção do extrato de casca de uva e otimização da melhor formulação das emulsões simples e múltiplas (dados não apresentados), investigando a concentração ideal do extrato de casca de uva, óleo de soja, emulsificante (PGPR - polirricinoleato de poliglicerol) e materiais gelificantes. Após a otimização, prosseguiu-se para a segunda etapa. Nessa etapa foi realizada a gelificação iônica e caracterização das micropartículas, também foi escolhido o melhor material gelificante baseado nos resultados obtidos das caracterizações. Após escolha do melhor material gelificante, foram estudados os efeitos dos diferentes materiais encapsulantes complementares (inulina, maltodextrina e amido modificado) nas propriedades físico-químicas das micropartículas e posteriormente, as micropartículas foram aplicadas diretamente em matriz alimentícia como corante natural e indicador colorimétrico comestível a fim de monitorar a qualidade do leite.

O fluxograma abaixo (Figura 1) sumariza as etapas metodológicas adotadas neste projeto, onde a cor roxa se refere a etapa 1, a cor verde a etapa 2 e a cor azul a etapa 3.

Figura 1 - Fluxograma das etapas do projeto



Fonte: Da autora (2022).

3 REFERENCIAL TEÓRICO

3.1 Uva

A uva é uma das principais frutas cultivadas e consumidas mundialmente sendo utilizada tanto para o consumo *in natura* quanto como matéria-prima para o processamento de produtos como vinhos, geleias e sucos (DHEKNEY *et al.*, 2019). No Brasil, a viticultura foi introduzida no ano de 1535 pelos colonizadores portugueses, na então Capitania de São Vicente, hoje conhecido como o estado de São Paulo. Eram uvas europeias (*Vitis vinifera*) e seu cultivo e produção eram baseados principalmente nos conhecimentos e experiências pessoais dos viticultores europeus (CAMARGO, U. A.; PEREIRA; GUERRA, 2011).

No entanto, a viticultura brasileira consolidou-se somente em meados do século XIX, com a chegada dos imigrantes italianos, quando ocorreu a introdução das cultivares americanas *Vitis labrusca* e *Vitis bourquina*, culminando na rápida substituição dos vinhedos de uvas europeias. No século XX, as uvas europeias voltam a ganhar destaque para a produção principalmente de vinhos finos (Cabernet Sauvignon, Cabernet Franc, Malbec, Merlot, Pinotage, Pinot Noir, Syrah, Chardonnay, Riesling Itálico, dentre outras) tornando-se a base para o desenvolvimento da viticultura comercial nos Estados do Rio Grande do Sul e de São Paulo (CAMARGO, U. A.; PEREIRA; GUERRA, 2011).

Segundo a FAO-OIV (2019), a produção mundial de uvas em 2018 alcançou 77.8 milhões de toneladas em todo o mundo, sendo 57% destinada a produção de vinhos, 36% para o consumo *in natura* e 7% de passas. No Brasil, a produção da safra de uvas em 2019, foi de 1.445.705 toneladas, sendo a região sul a maior produtora, representando cerca de 59% da produção nacional, seguida pela região nordeste que contribuiu com 30%. A região sudeste foi a terceira maior produtora de uvas, representando 11% (MELLO, 2019).

Dentre as uvas americanas (*Vitis labrusca*) cultivadas no Brasil, a cultivar ‘Bordô’ tem-se destacado por apresentar excelente fertilidade e alto conteúdo de matéria corante, originando sucos e vinhos de mesa intensamente coloridos (CAMARGO; MAIA, 2005).

3.2 Uva Bordô

A uva Bordô (Figura 2) originalmente chamada de *Ives Seedling* ou simplesmente *Ives*, tem origem na costa leste americana (Cincinnati), e foi obtida a partir da seleção realizada por Henry Ives, proveniente de sementes de *Hartford Prolific*, no ano de 1840 (CAMARGO, UMBERTO ALMEIDA; RITSCHEL; MAIA, 2010).

Figura 2 - Cacho de uva da variedade Bordô

Fonte: Safari Garden (2020).

Em 1872, Tower Fogg, trouxe a uva Bordô para o Brasil, inicialmente para o bairro paulistano do Morumbi. Por obra de viveiristas da época, logo seguiu para o Rio Grande do Sul, sendo depois levada para os estados de Santa Catarina e Paraná, onde é conhecida por 'Terci' (SOUZA; MARTINS, 2002). Nas regiões mineiras, foi introduzida em 1904 nas cidades de Caldas e Andradas, nomeada por eles como 'Folha de Figo' (CAMARGO, UMBERTO ALMEIDA; RITSCHEL; MAIA, 2010).

Devido à grande dificuldade de desenvolvimento em climas tropicais, o cultivo da uva Bordô concentra-se em regiões com inverno definido. Assim sendo, a recomendação de cultivo está no sul de Minas Gerais, norte do Paraná, além dos Estados de Santa Catarina e do Rio Grande do Sul (CAMARGO, UMBERTO ALMEIDA; TONIETTO; HOFFMANN, 2011). Destaca-se como uma das cultivares *Vitis labrusca* mais importantes no Brasil e, em 2018, no estado do Rio Grande do Sul, foram produzidas cerca de 158.405 toneladas (MELLO, 2019).

É uma cultivar bastante rústica e resistente às principais pragas e doenças fúngicas. Apresenta conteúdo de açúcar em torno de 15°Brix e acidez total de 70 meq.L^{-1} (KUCK; NOREÑA, 2016). Possui coloração vermelho-púrpura intensa e destaca-se pela elevada

concentração de matéria corante, resultado de seu alto teor de polifenóis, principalmente das antocianinas. Os compostos fenólicos também são responsáveis pela atividade antioxidante da fruta e, tem como principais funções o impedimento e a neutralização da formação de radicais livres (RICE-EVANS; MILLER; PAGANGA, 1997).

Diversos estudos relatam a presença de compostos fenólicos em grandes quantidades na uva Bordô (CARMONA-GÓMEZ et al., 2018; DA SILVA HAAS et al., 2019; DE CASTILHOS et al., 2016; DOS SANTOS LACERDA et al., 2018; KUCK; NOREÑA, 2016; PERTUZATTI et al., 2020). Nas uvas, as áreas de maior acúmulo de polifenóis são a película, sementes, polpa e engaço. Sendo assim, a utilização de subprodutos do processamento de uvas torna-se uma alternativa promissora devido a presença de compostos bioativos de fontes naturais, resultando em benefícios para a saúde, dentre eles, capacidades anti-inflamatória, antimicrobianas, antidiabética e cardioprotetora (AMORIM et al., 2019; KUCK; NOREÑA, 2016; RODRÍGUEZ-ROQUE et al., 2013).

3.3 Subprodutos do processamento de uvas

Em todo o mundo, são gerados milhões de toneladas de resíduos provenientes de atividades agroindustriais. Estima-se que anualmente são gerados aproximadamente 13 milhões de toneladas de resíduos provenientes de vinícolas de todo o mundo (DÁVILA et al., 2017). No Brasil, são gerados aproximadamente 60 milhões de quilos. Esses subprodutos, como o bagaço (composto por casca e sementes) e o engaço, juntos podem representar até 30% do peso total da uva processada (ROCKENBACH *et al.*, 2011).

Muitas vezes esses resíduos são descartados de forma inadequada, acarretando diversos impactos ambientais. De acordo com Dávila et al. (2017), apenas 3% dos resíduos da vitivinicultura são destinados ao reaproveitamento, pois são considerados subprodutos de baixo valor agregado destinados principalmente para o complemento de ração animal, disposição no campo, adubação do solo ou até mesmo, incinerado.

A forma como esses resíduos são destinados causa um déficit econômico na cadeia produtiva, uma vez que muitos deles são ricos em compostos bioativos, que permanecem nas

cascas e sementes em razão da sua incompleta extração durante o processo de vinificação, podendo ser reaproveitados (BRAZINHA; CADIMA; CRESPO, 2014; CALDAS et al., 2018; DROSOU et al., 2015; FAVRE *et al.*, 2019; KAMMERER *et al.*, 2014; KY; TEISSEDRE, 2015; ROCKENBACH et al., 2011; TRIKAS et al., 2016).

De acordo com Kammerer et al. (2014), apesar da grande variabilidade de compostos bioativos dentre as cultivares de uvas, tanto as cascas como as sementes constituem uma fonte promissora de compostos fenólicos. As cascas de uvas representam, em média, até 82% do peso seco do bagaço de uva, contendo uma grande quantidade de compostos fenólicos, incluindo ácidos hidroxicinâmicos, flavonóis, catequinas e antocianinas (ROCKENBACH et al., 2011). Além disso, apresentam alto valor nutritivo, devido aos teores significativos de fibra alimentar (65-80%) (LLOBERA; CAÑELLAS, 2008).

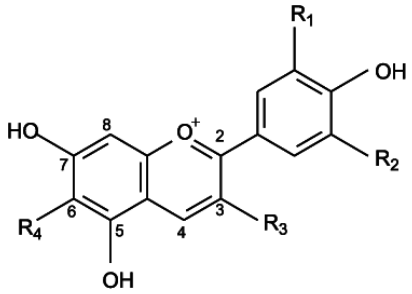
Diversos estudos comprovaram o potencial antioxidante de bagaço de uva introduzido em diversos produtos alimentícios como, pães (AGHAMIRZAEI et al., 2015), biscoitos (PIOVESANA; BUENO; KLAJN, 2013), iogurtes (MARCHIANI et al., 2016; TSENG; ZHAO, 2013), cookies (KARNOPP et al., 2015), muffins (BENDER, ANA B.B. et al., 2017; MILDNER-SZKUDLARZ et al., 2013), snacks (BENDER, ANA BETINE BEUTINGER et al., 2016), cereais matinais (OLIVEIRA et al., 2013), macarrão (MARINELLI et al., 2015), queijo (FELIX DA SILVA et al., 2015; HAN et al., 2011), produtos cárneos (JUNG et al., 2012; ÖZVURAL; VURAL, 2011), leite fermentado (ALIAKBARIAN et al., 2015; FRUMENTO et al., 2013) e frutos do mar (RIBEIRO, BERNARDO et al., 2013). Também pôde ser comprovado a atividade antimicrobiana de extrato de bagaço de uva contra diferentes patógenos transmitidos por alimentos (XU *et al.*, 2016).

Assim sendo, o resíduo de uva constitui um subproduto de grande interesse para a indústria de alimentos, visto que apresenta grande potencial de aplicação em diversos produtos/ingredientes. Isto deve-se ao fato de seu conteúdo ser rico em diversos compostos fenólicos, como as antocianinas.

3.4 Antocianinas

As antocianinas (do grego *anthos* = flor e *kianos* = azul) pertencem à ampla classe de compostos fenólicos e são coletivamente chamadas de flavonóides. São compostos solúveis em água e responsáveis pela coloração azul, violeta, rosa, vermelho e até mesmo laranja brilhantede frutos, flores, grãos e vegetais (SINELA et al., 2017). A estrutura básica das antocianinas é 2-fenilbenzopirona, conhecido como sal *flavylium* ou cátion f*lavylium* (Figura 3).

Figura 3 - Cátion flavylium. R1 e R2 = -H, -OH ou -OCH3, R3 = -glicosil, R4 = -H ou glicosil

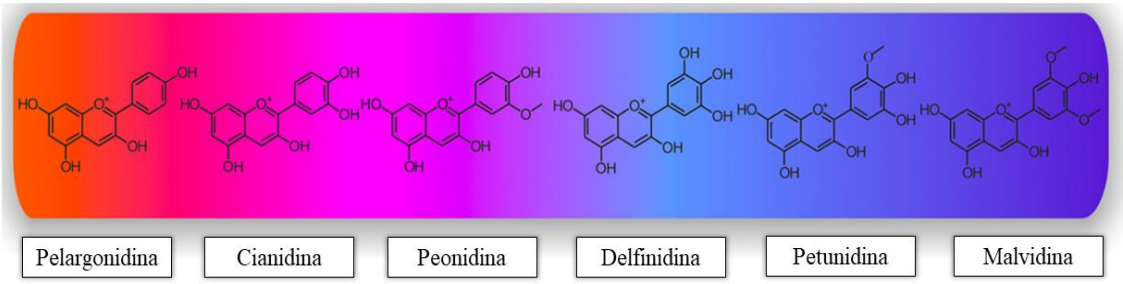


Fonte: Adaptado de Damodaran; Parkin; Fennema (2010).

Cada molécula de antocianina é constituída por uma aglicona, um ou mais grupos de açúcares e, frequentemente, um grupo de ácidos orgânicos. A diferença entre as antocianinas está relacionada ao número de hidroxilas, natureza, posição e número de açúcares associados à molécula e à quantidade de ácidos alifáticos ou aromáticos ligados a esses açúcares (KONG et al., 2003).

Quando o açúcar das antocianinas é hidrolisado, a aglicona é chamada de antocianidina. Dentre esses açúcares tem-se a glicose, xilose, galactose, arabinose, frutose e ramnose. Aproximadamente 27 agliconas são conhecidas, no entanto,6 são as mais frequentemente encontradas na natureza: pelargonidina, cianidina, delfinidina, malvidina, peonidina e petunidina (KONG et al., 2003). As seis principais formas de antocianidinas encontradas na natureza estão mostradas na Figura 4.

Figura 4 - Faixa de cores visível de antocianidinas mais comuns



Fonte: Adaptado de Ananga et al. (2013).

De acordo com Hogervorst, Miljić e Puškaš (2017), a antocianidina mais valiosa encontrada principalmente nas cascas das uvas são as catequinas (flavan-3-ols) e a malvidina-3-O-glicósido, seguida da peonidina-3-O-glicósido. Os teores de antocianinas totais em extratos de cascas de uva Bordô podem variar entre 20 e 53 mg de malvidina-3,5-diglicosídeo/g de extrato em base seca (KUCK; NOREÑA, 2016; KUCK; WESOLOWSKI; NOREÑA, 2017).

Além do potencial corante, diversos estudos têm relacionado as antocianinas, com efeitos benéficos para a saúde, como anticancerígena (CHEN et al., 2018), antinflamatória (LI et al., 2017) e antioxidante (SMERIGLIO et al., 2017). Em particular, diversos extratos obtidos a partir de resíduos da vitinicultura têm sido associados as atividades contra o estresse oxidativo (GONÇALVES et al., 2017; HOGAN et al., 2010), atividades antibacterianas e antifúngicas (KATALINIĆ et al., 2010; SAGDIC et al., 2012) e neuroprotetoras (GRANZOTTO; ZATTA, 2014; WU et al., 2018).

Ainda, as antocianinas podem fornecer informações imediatas sobre a qualidade de alimentos, por meio de mudanças colorimétricas visuais provocadas por alterações estruturais do pigmento e, consequentemente, indicar o frescor ou o estágio de deterioração do alimento, apresentando-se como um método conveniente, rápido e não destrutivo (ALMASI; FORGHANI; MORADI, 2022).

3.5 Utilização de antocianinas como sensores indicadores inteligentes para monitorar a qualidade de alimentos

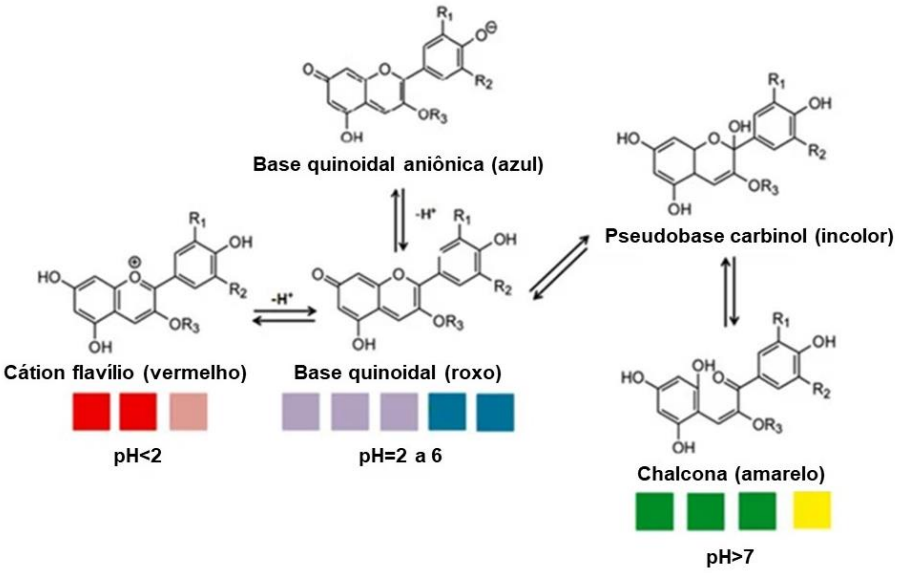
No processo de crescimento e reprodução, os microrganismos liberam vários metabólitos (sulfeto de hidrogênio, aminas, dióxido de carbono, água, etc.), resultando em

alterações na acidez ou alcalinidade do alimento e do ambiente ao redor do alimento. Por esta razão, corantes sensíveis ao pH e outros metabólitos foram introduzidos na preparação de sensores indicadores de frescor. Durante as mudanças no frescor dos alimentos, o sensor indicador responsivo reage com os metabólitos dos microrganismos no alimento e mostra uma mudança de coloração (ALMASI; FORGHANI; MORADI, 2022; DA SILVA FILIPINI; ROMANI; GUIMARÃES MARTINS, 2020).

Assim, sensores indicadores de frescor inteligentes convertem a detecção de alterações qualitativas ou quantitativas na concentração de uma ou mais substâncias associadas à deterioração de alimentos em um sinal perceptível que pode ser detectado visualmente pelo consumidor. As mudanças de cor nos indicadores são geralmente atribuídas à protonação ou desprotonação de grupos funcionais de ácido carboxílico ou amidocianogênio. Recentemente, pesquisadores têm focado sua atenção em corantes indicadores como as antocianinas, derivadas de fontes naturais, para a confecção de sensores colorimétricos (ALMASI; FORGHANI; MORADI, 2022; CHHIKARA *et al.*, 2019).

A função de mudança de cor das antocianinas como corantes sensíveis ao pH depende das mudanças estruturais causadas pelas propriedades ácido-base do ambiente (Figura 5). Nos valores de pH mais baixos (pH < 2), a coloração vermelha causada pelo cátion flavílio é a cor predominante. Com um ligeiro aumento no pH (pH = 2-4), a cor muda para uma base quinoidal roxa/azul. Então, a pseudobase de carbinol incolor será a cor dominante aumentando o pH de condições ácidas para ligeiramente ácidas/quase neutras. Um aumento adicional no valor do pH (pH > 7) causa uma diminuição gradual na estabilidade das antocianinas e gera uma cor verde-amarelada como resultado da formação de chalcona. Assim, a atividade responsiva das antocianinas pode ser útil para promover sistemas inteligentes de monitoramento da qualidade de alimentos. Diversos estudos tem explorado o potencial das antocianinas como corante ph-responsivo (DA SILVA FILIPINI; ROMANI; GUIMARÃES MARTINS, 2020; MENDES *et al.*, 2022; PEREIRA, VALDIR ANICETO; DE ARRUDA; STEFANI, 2015).

Figura 5 - Alterações nas estruturas das antocianinas devido a mudanças de pH



Fonte: Adaptado de Alizadeh-Sani e colaboradores (2020).

No entanto, as antocianinas são pigmentos instáveis, e bastante sensíveis às condições do ambiente ou processos tecnológicos, como pH, temperatura, umidade, luz, presença de oxigênio, degradação enzimática, interações entre os componentes dos alimentos, tais como ácido ascórbico, íons metálicos e açúcares, dificultando sua incorporação direta em produtos alimentícios. Sendo assim, o grande desafio para as indústrias em utilizar pigmentos de origem natural está justamente relacionado a instabilidade apresentada por esses compostos, dessa forma, a microencapsulação torna-se uma alternativa promissora para confecção de sensores colorimétricos afim de melhorar a estabilidade e biodisponibilidade de indicadores naturais, durante o armazenamento ou processamento (ALMASI; FORGHANI; MORADI, 2022; DIAS *et al.*, 2020; ROCKENBACH *et al.*, 2011).

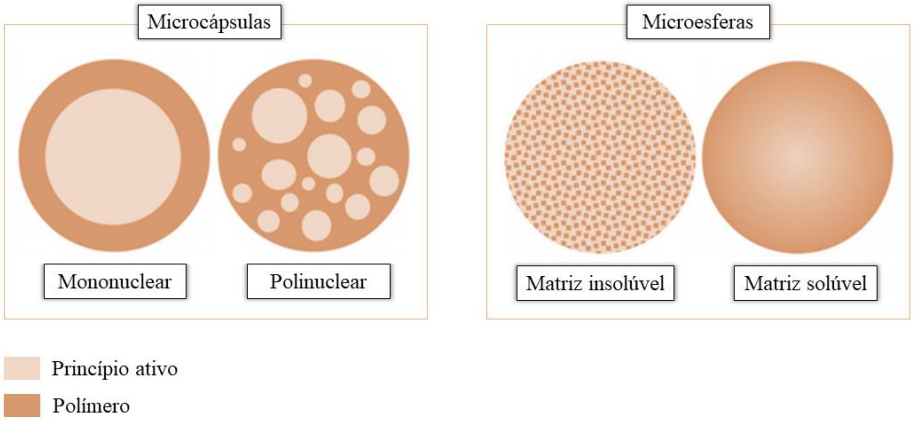
3.6 Microencapsulação

A microencapsulação consiste basicamente em um processo de aprisionamento de um ingrediente ativo (sólido, líquido ou gasoso), no interior de um material de revestimento, a fim de ser protegido contra diversos fatores, tais como a luz, calor, umidade e elevada concentração de oxigênio, evitando a evaporação de compostos voláteis, mascarando sabores e odores desagradáveis, garantindo uma liberação controlada, além de permitir o desenvolvimento de

produtos de alto valor agregado (COMUNIAN; FAVARO-TRINDADE, 2016). É considerada uma técnica versátil, permitindo a aplicação em diversas áreas, tais como farmacêutica, alimentícia, agrícola, cosmética, sistemas de fixação de tintas automotivas e fotocopiadoras, dentre outras (LEE; KIM, 2011).

A escolha do material de revestimento é estabelecida principalmente pela melhoria que este composto pode trazer para um ingrediente e pelo papel funcional da partícula. Ainda, é dependente das características desejadas das micropartículas e do tipo de material ativo. Diferentes tipos de materiais podem ser utilizados, como carboidratos (pectinas, amidos, dextrinas, gomas, quitosana, inulina, maltodextrina), proteínas (isolado proteico de soja, isolado proteico de soro de leite, gelatina, albumina, caseína) e lipídeos (cera, ácido esteárico, monoglicerídeo, óleos, gorduras hidrogenadas, parafina).A escolha vai depender da técnica de microencapsulação a ser utilizada e o composto a ser encapsulado (BOTREL et al., 2012). A retenção do material encapsulado pela micropartícula pode ser conduzida por sua polaridade, funcionalidade química, volatilidade e solubilidade (DESAI; PARK, 2005; GHARSALLAOUI et al., 2007; JYOTHI et al., 2010). Os produtos obtidos do processo de encapsulação se diferem em relação à estrutura física interna e morfologia e são chamados de microcápsulas, quando o núcleo interno é bem definido e microesferas, quando o ingrediente ativo encontra-se disperso na matriz (Figura 6) (DESAI; PARK, 2005; GHARSALLAOUI et al., 2007; JYOTHI et al., 2010).

Figura 6 - Principais morfologias de micropartículas



Fonte: Adaptado de Nesterenko et al. (2013).

Atualmente, existem diversos métodos para preparação ou obtenção de micropartículas, os quais podem ser classificados em físicos: *spray chilling*, *spray drying*, *spray cooling*, leito

fluidizado, co-cristalização e processos utilizando fluidos supercríticos; químicos: inclusão molecular e polimerização interfacial; e físico-químicos: coacervação, formação de lipossomas e gelificação iônica (GOUIN, 2004; MUNIN; EDWARDS-LÉVY, 2011).

Portanto, para a microencapsulação de extratos hidrofílicos, como o extrato de casca de uva, o ideal é que a técnica utilize baixas temperaturas de processo, garantindo assim uma melhor estabilidade dos compostos bioativos. Sendo assim, a utilização da técnica de gelificação iônica é uma alternativa muito interessante, devido as condições brandas de processamento.

3.7 Gelificação iônica

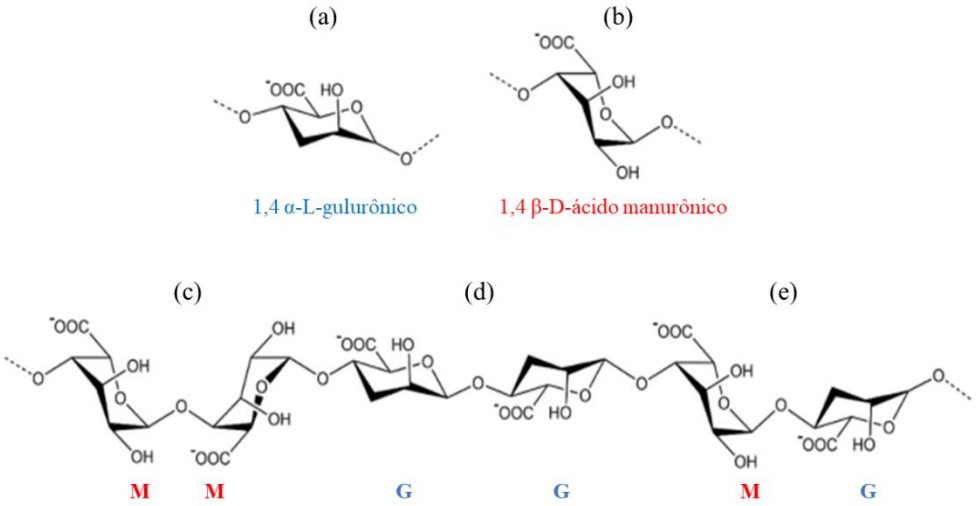
A técnica de gelificação iônica consiste na habilidade de vários hidrocolóides (goma gelana, carragena, alginato, pectinas, dentre outros) tornarem-se gel na presença de íons, normalmente cátions bivalentes (BUREY et al., 2008; ZAM et al., 2014). Resumidamente, uma solução polimérica ou hidrocolóide contendo o material polimérico encapsulante e o composto ativo é gotejada ou atomizada sob uma solução contendo íons divalentes (geralmente o Ca^{2+}). Após o contato, ocorre a formação instantânea de estruturas tridimensionais com elevado teor de água que são responsáveis pela proteção do composto ativo (OTÁLORA et al., 2016).

A pectina e o alginato de sódio são os polissacarídeos mais utilizados no processo de gelificação iônica, uma vez que são capazes de formar gel em presença de cátions bivalentes e não apresentam riscos à saúde humana, desta forma são bastante utilizados pela indústria de alimentos e farmacêutica (COVIELLO et al., 2007).

O alginato é um polissacarídeo hidrossolúvel de ocorrência natural, proveniente de algumas bactérias e encontrado nas paredes celulares e intracelulares de algas marrons, principalmente da *Laminaria hyperborea*, *Ascophyllum nodosum* e *Macrocystis pyrifera* (GARCIA-CRUZ; FOGGETTI; DA SILVA, 2008). Se apresenta na forma de um pó insípido e inodoro e possui coloração branca pálida ou marrom amarelado (SILVA; ANDRADE, 2012). São polímeros lineares não ramificados, formados por ligações β-(1 → 4) ligada ao D-ácido manurônico (estrutura M) e por ligações α-(1 → 4) ligada ao ácido L-gulurônico (estrutura G) (Figura 7).

De acordo com Zia et al. (2015), as propriedades físico-químicas dos alginatos são influenciadas pela proporção e distribuição destes monômeros M e G ao longo da molécula, que são unidos formando “blocos” homopoliméricos (contendo somente monômeros M ou G) e heteropoliméricos (alternando unidades M e G) (Figura 7). A distribuição dos copolímeros, composição e pesos moleculares variam de acordo com a fonte biológica, o estado de maturação da planta e espécies que formam estes monômeros (COMUNIAN; FAVARO-TRINDADE, 2016; HAMBLETON et al., 2009; ZIA et al., 2015).

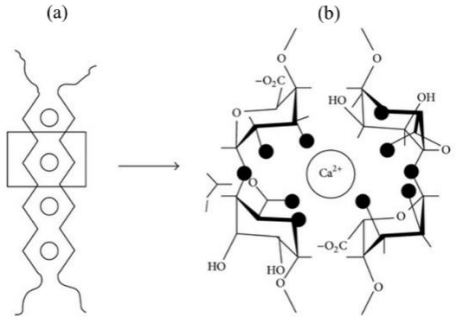
Figura 7 - Estruturas do 1-4 α-L-gulurônico (G) (a), 1-4 β-D-ácido manurônico (M) (b), blocos homopoliméricos M-M (c), G-G (d) e blocos heteropoliméricos M-G (e)



Fonte: Adaptado de Burey, Bhandari e Howes (2012).

Estruturas formadas somente por blocos G são eficazes na formação de géis mais resistentes e estáveis, pois as interações iônicas ocorrem entre os resíduos de ácido gulurônico e Ca^{2+}. Os íons Ca^{2+} se acomodam nas cavidades entre duas ou mais cadeias do bloco G mediante a ligação dos cátions divalentes (Ca^{2+}) com os grupos carboxilas dos resíduos gulurônicos, resultando assim em um arranjo de rede tridimensional comumente denominado de “caixa de ovos” (Figura 8) (GRANT et al., 1973; ROOPA; BHATTACHARYA, 2010; ZIA et al., 2015).

Figura 8 - Ligações do Ca2+ com o resíduo gulurônico (a) e Formação do gel de alginato de cálcio em solução (b)

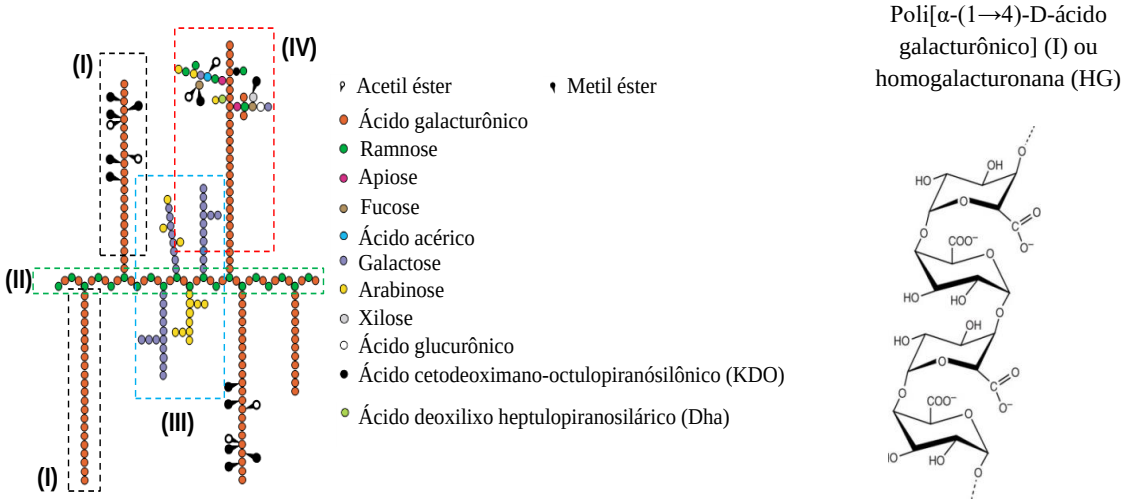


Fonte: Adaptado de Clarck, Chen e Lin (1987).

Além das características citadas anteriormente, o alginato é um polímero natural de custo relativamente baixo, de fácil obtenção, biocompatível, atóxico, biodegradável e que não necessita de solventes orgânicos e nem condições rigorosas de temperatura para a formação das partículas (FUJIWARA et al., 2013). Sendo assim, o uso do alginato como material encapsulante é bastante relevante.

A pectina, é um termo utilizado para designar um grupo de polissacarídeos encontrados na parede celular e em regiões intercelulares de plantas e frutos. São polissacarídeos aniônicos, amorfos, atóxicos e facilmente solubilizados em água. É amplamente utilizada pela indústria de alimentos como agente espessante, gelificante e emulsificante. A estrutura química da pectina é a mais complexa dentre todos os polissacarídeos conhecidos, Figura 9 (WILLATS; KNOX; MIKKELSEN, 2006b)

Figura 9 - Esquema geral para a estrutura molecular da pectina com destaque para o poli(ácido galacturônico), seu maior constituinte



Fonte: Adaptado de Willats et al. (2006).

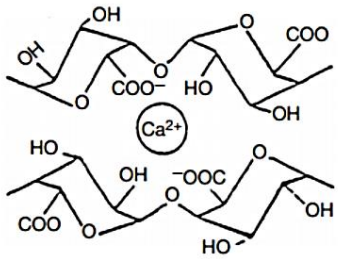
Esta composição química é altamente variável, sendo influenciada por modificações enzimáticas decorrentes do metabolismo dos vegetais, bem como das condições utilizadas para sua extração (SCHOLS; VORAGEN, 2002). As maiores fontes de pectinas são os resíduos do processamento de frutas cítricas, cana-de-açúcar e maçã (MAY, 1990).

A composição química das pectinas é majoritariamente definida por cadeias helicoidais de poli[α-(1→4)-D-ácido galacturônico] (I) ou homogalacturonana (HG). O percentual de HG nas pectinas geralmente supera 75%. Os grupos carboxílicos ocorrem parcialmente na forma de ésteres metílicos, definindo um grau de metoxilação (GM) que varia entre 5 e 95% (SCHOLS; VORAGEN, 2002). A pectina com alto grau de metoxilação contém mais de 50% de grupos carboxílicos esterificados e as com baixo grau de metoxilação possuem menos de 50% de seus grupos na forma esterificada (MAY, 1990; WICKER; KIM, 2015).

A estrutura molecular das pectinas apresenta ainda cadeias de ramnogalacturonanas (II), as quais são formadas por unidades de α-(1→4)-D-ácido galacturônico alternadas por unidades de α-(1→2)-ramnose (RGI). Algumas cadeias de RGI podem estar ligadas a segmentos de cadeias de açúcares neutros como arabinanas, galactanas, arabinogalactanas (III). Outros domínios na estrutura das pectinas envolvem cadeias de HG ligadas a segmentos quimicamente complexos de açúcares (IV) envolvendo xilose, apiose, ácido acérico, Dha e KDO (RGII) (SHOLS; VORAGEN, 2002; WILLATS; KNOX; MIKKELSEN, 2006).

A capacidade de formar gel da pectina está associada ao tamanho da cadeia de poli[α-(1→4)-D-ácido galacturônico]e do grau de metoxilação de seus grupos carboxílicos. Estas características variam de acordo com a fonte da qual o biopolímero é extraído. Pectinas com baixo teor de metoxilação (BTM) formam géis rígidos quando em contato com íons divalentes como o cálcio, que reticula as cadeias do ácido galacturônico. O modelo "caixa de ovos" (Figura 10) também é utilizado para demonstrar a formação da rede de gel com íons cálcio, sendo procedida de maneira semelhante aos géis de alginato (MUKAI-CORREA et al., 2004; DE CINDIO et al., 2016).

Figura 10 - Modelo "caixa de ovos" da interação Pectina-Ca2+



Fonte: Thakur et al. (1997).

Sendo assim, a gelificação iônica é um método que emprega condições simples e brandas, não utiliza altas temperaturas, agitação vigorosa e nem solventes orgânicos, permitindo a encapsulação de substâncias que degradariam sob essas condições (MUKAI-CORRÊA et al., 2005). Além disso, é considerada de baixo custo quando comparada a outras técnicas de encapsulação. Estas vantagens têm sido motivo de sua utilização crescente, principalmente na encapsulação de fármacos e compostos alimentícios de interesse (AGNIHOTRI; MALLIKARJUNA; AMINABHAVI, 2004; PATIL et al., 2010). Diversos estudos tem explorado a utilização de polissacarídeos na produção de géis (CHOJNICKA et al., 2009; LORENZO; ZARITZKY; CALIFANO, 2013; SATO; MORAES; CUNHA, 2014; PARADISO et al., 2015; DEVEZEAUX DE LAVERGNE et al., 2016; FENG et al., 2018).

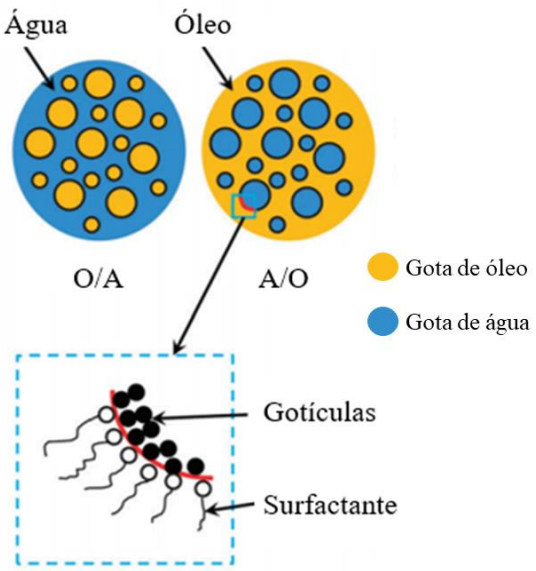
Entretanto, devido a porosidade apresentada pelas micropartículas formadas, a técnica tem como desvantagem o encapsulamento de materiais hidrofílicos ou de baixo peso molecular, pois apresentam fácil difusão e rápida liberação através dos espaçamentos da rede de gel, independentemente do pH (KIM et al., 2014). Para contornar essa problemática, algumas

estratégias podem ser aplicadas para reter os compostos ativos hidrofílicos presentes no extrato de casca de uva, como os sistemas de emulsões duplas.

3.7 Emulsões: definições e estabilização com polissacarídeos

Emulsões são sistemas constituídos de dois ou mais líquidos imiscíveis, sendo, um deles disperso na forma de gotas ou glóbulos (fase dispersa) no outro (fase contínua). As emulsões simples podem ser de dois tipos: óleo em água (O/A) onde gotas de óleo estão dispersas em uma fase contínua aquosa ou o inverso, água em óleo (A/O) onde as gotas de água estão dispersas numa fase contínua de óleo (Figura 11) (SINGH *et al.*, 2017).

Figura 11 - Emulsão concentrada de óleo em água e emulsão concentrada de água em óleo, com destaque para o surfactante



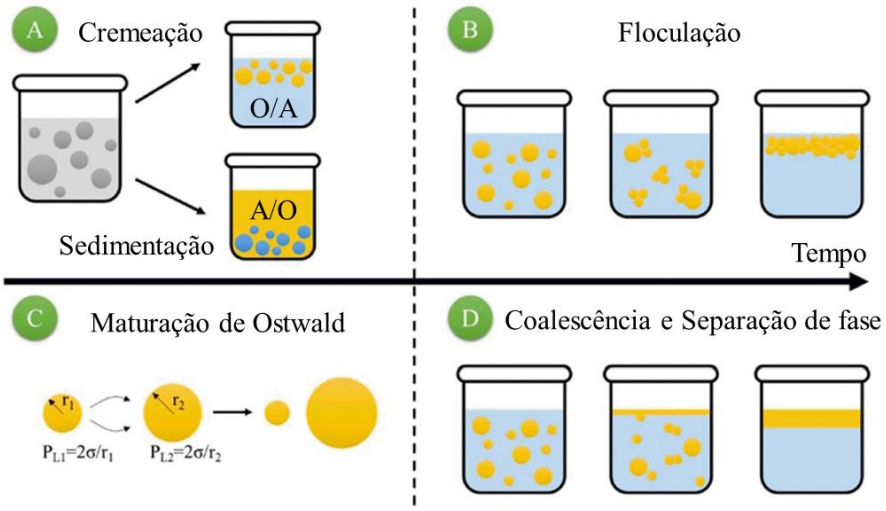
Fonte: Adaptado de Singh et al. (2017).

As emulsões podem ser classificadas conforme o diâmetro das gotas em: macroemulsões (0,5-100 μm), miniemulsões (100-1000 nm) e nanoemulsões (10-100 nm) (WINDHAB et al., 2005). Conforme tem-se a diminuição do tamanho das gotas dispersas, menor será a diferença da densidade entre as fases e maior será a viscosidade da fase contínua,

sendo assim, maior será a estabilidade da emulsão (MCCLEMENTS; RAO, 2011; SAMAVATI et al., 2012).

De acordo com a segunda lei da termodinâmica, os sistemas tendem a retornar ao seu estado inicial de menor energia. No caso das emulsões, ocorre uma tendência natural de diminuição da área de contato interfacial, o que resulta na separação de fase, bem como outras instabilidades como floculação, coalescência, cremeação, sedimentação e maturação de Ostwald (Figura 12). Em emulsões múltiplas, os processos de instabilidade mais observados são a maturação de Ostwald e a coalescência (WALSTRA e VAN VLIET, 2010; BOUYER et al., 2012).

Figura 12 - Principais fenômenos de instabilidades das emulsões



Fonte: Adaptado de Pereira e Garcia-Rojas (2015).

Para melhorar a estabilidade cinética das emulsões, ou seja, aumentar a sua capacidade em resistir aos processos de desestabilização, torna-se necessário o uso de emulsificantes e/ou espessantes. Os emulsificantes são uma espécie química que se adsorve na superfície das gotas produzidas durante a homogeneização, impondo uma barreira estérica ou eletrostática na interface, formando camadas protetoras e com duas funções principais: diminuir a tensão interfacial entre as fases, de forma a facilitar a formação da emulsão e estabilizar a fase dispersa contra os mecanismos de desestabilização após sua formação(MCCLEMENTS, 2012).

Os emulsificantes são classificados em duas grandes classes: de baixa massa molar (monoglicerídeos, polissorbatos, lecitina, dentre outros) e emulsificantes macromoleculares

(usualmente proteínas, principalmente do leite, da soja e do ovo) (DICKINSON, 2003). Diversos estudos utilizam biopolímeros como agentes tensoativos, dentro os mais utilizados pode-se citar os isolados proteicos, concentrado proteicos, proteínas isoladas do leite (beta-lactoglobulina e caseinato de sódio, por exemplo), pectina, galactomananas, goma arábica e carragena (GUZEY; MCCLEMENTS, 2006; OZTURK; MCCLEMENTS, 2016).

Em relação aos polissacarídeos, seu uso para estabilização de gotículas de emulsão pode ser de duas formas: os polissacarídeos capazes de adsorver na superfície do glóbulo da emulsão, atuando como um emulsificante, e os que atuam aumentando a viscosidade da fase externa sem que adsorvam aos glóbulos da emulsão, funcionando como um espessante (BOUYER et al., 2012).

Os polissacarídeos adsorventes apresentam atividade interfacial/superficial e atuam estabilizando as emulsões por adsorção na superfície da gota de óleo. Assim, o glóbulo de óleo é praticamente recoberto por um filme polimérico, em seguida eles atuam impedindo a floculação dos glóbulos e coalescência por meio de forças de repulsão estérica e eletrostática. Alguns exemplos de polissacarídeos com esse mecanismo de atuação incluem a goma arábica, pectina, goma de fibra de milho, quitosana, amidos e celuloses modificados, mucilagem de mostarda amarela e goma de tragacanto (MCCLEMENTS; BAI; CHUNG, 2017).

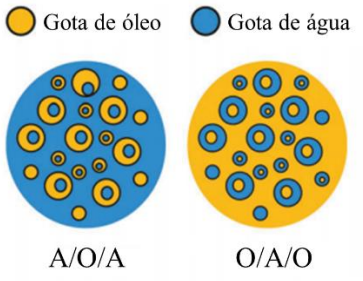
Os espessantes por outro lado, apresentam a propriedade de aumentar a viscosidade ou gelificar a fase contínua das emulsões, fazendo com que a velocidade de movimento da fase dispersa, devido à gravidade ou ao movimento browniano, seja diminuída reduzindo, assim, a probabilidade de choques entre as gotas, evitando a fenômenos de desestabilização (MCCLEMENTS, 2012). Podem-se citar como principais espessantes utilizados os polissacarídeos alginato, gomas carragena, goma xantana e goma gelana (GUZEY; MCCLEMENTS, 2006; MCCLEMENTS, 2010).

3.7.1 Emulsões múltiplas

Emulsões múltiplas, também chamadas de emulsões duplas, são sistemas mais complexos, formados por processos de emulsificação seguidos, onde os dois tipos de emulsões (A/O e O/A) existem conjuntamente, constituindo emulsões do tipo A/O/A ou O/A/O (Figura 13). As emulsões A/O/A, por exemplo, são compostas de pequenas gotas de água (fase aquosa

interna) dispersas em gotas maiores de óleo, dispersas ainda em outra fase aquosa (fase aquosa externa) (BOUYER et al., 2012).

Figura 13 - Esquema simplificado de emulsões múltiplas



Fonte: Adaptado de Singh et al. (2017).

Para a formação de uma emulsão múltipla, primeiramente é formada uma emulsão chamada primária, podendo ser do tipo A/O ou O/A. Essa emulsão normalmente é formada a partir de métodos que envolvem uma homogeneização mais intensa (alta pressão ou alta velocidade de agitação/mistura). Posteriormente, a emulsão primária é homogeneizada com outra fase aquosa ou oleosa, a partir de uma homogeneização com menor intensidade de agitação. Por fim, tem-se a formação de uma emulsão múltipla do tipo A/O/A ou O/A/O. Vale ressaltar que na segunda etapa são aplicados processos de homogeneização mais suaves a fim de evitar a ruptura das gotas presentes na emulsão primária e com isso diminuir a eficiência da encapsulação (GARTI, 1997). Da mesma forma que as emulsões simples, as emulsões múltiplas são sistemas termodinamicamente instáveis e requerem o uso de agentes tensoativos e/ou espessantes para estabilizar e delimitar as interfaces (MCCLEMENTS, 2012).

Diante disso, o uso de emulsões duplas apresenta vantagens em relação às emulsões convencionais, principalmente, no que se refere ao encapsulamento, proteção e liberação controlada de bioativos. Tal fato se deve à presença de duas fases imiscíveis que limitam a difusão do ativo da fase interna para a externa. Assim, a fase interna funciona como um reservatório confinado, protegendo compostos sensíveis à luz, à degradação enzimática, à oxidação e a outros fatores externos (PEREIRA, LUCIANO JOSÉ BARRETO; GARCIA-ROJAS, 2014; TATAR; SUMNU; SAHIN, 2017; VRIGNAUD; BENOIT; SAULNIER, 2011). Por isso, a técnica de emulsão dupla juntamente com a gelificação iônica, pode ser o método

mais adequado para incorporar e administrar compostos hidrofílicos (GÈZE et al., 1999; YE; KIM; PARK, 2010). Ainda, podem ser utilizados materiais encapsulantes complementares como a inulina, maltodextrina e o amido modificado, visando a melhora da retenção e maior proteção dos compostos bioativos presentes no extrato de casca de uva.

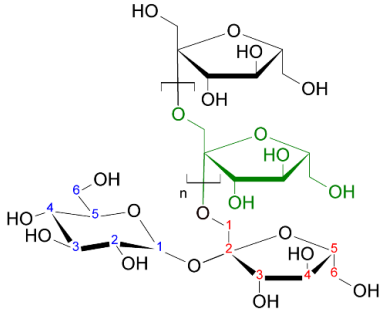
3.8 Materiais encapsulantes complementares

3.8.1 Inulina

A inulina é um carboidrato de reserva naturalmente presente nas plantas da família *Asteracea*e (RONKART et al., 2009). No entanto, a maior parte da extração industrial é obtida a partir de raízes da espécie *Cichoriumintybus* (chicória) (ROBERFROID, 2018). Outras fontes como alho, alho-poró, cebola, alcachofra, batata yacon, banana e aspargo também podem ser utilizadas para a sua extração (FERNANDES et al., 2016; KARIMI et al., 2015; KAUR; GUPTA, 2002; MEYER et al., 2011).

Pertence ao grupo de polissacarídeos denominados frutanos e é constituída de uma mistura de polímeros e oligômeros superiores lineares de frutose. Sua estrutura química consiste em unidades de β-D-frutosil unidas por ligações glicosídicas do tipo β(2→1) e uma molécula de glicose na porção inicial de cada cadeia linear de frutose, a qual é unida por uma ligação tipo (α1- β2), como na molécula de sacarose (Figura 14). O comprimento das cadeias de frutose pode variar de 2 a 60 monômeros e, a partir da hidrólise enzimática parcial da inulina pode-se obter frações de frutooligossacarídeos (FOS), denominados também como oligofrutoses, os quais apresentam no máximo 10 unidades de frutose (MENSINK et al., 2015).

Figura 14 - Estrutura química da inulina



Fonte: Adaptado de Mensink et al. (2015).

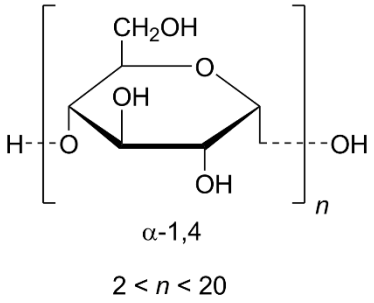
A inulina tem atraído grande atenção das indústrias alimentícia devido a apresentar diversos benefícios, tais como adoçante de baixo teor calórico, agente formador de gel e espessante, substância que proporciona uma dispersão sólida para aumentar a velocidade de dissolução e também, como fibra não digerível (FERNANDES et al., 2016). Além disso, de acordo com Mesink et al. (2015), apresenta a capacidade de alterar a composição da flora intestinal após um curto período de alimentação, funcionando como um prebiótico, estimulando principalmente o crescimento de bifidobactérias e lactobacilos.

Diversos estudos tem explorado a utilização da inulina como material de parede para proteção de compostos bioativos e liberação controlada (BEIRÃO-DA-COSTA et al., 2013; FERNANDES et al., 2016; KARIMI et al., 2015; MEHRAN; MASOUM; MEMARZADEH, 2020; SHAHIDI NOGHABI; MOLAVEISI, 2020).

3.8.2 Maltodextrina

A maltodextrina é um polímero baseado em sacarídeo contendo D unidades ligadas por ligações glicosídicas de glicose α-l, 4 ou α-l, 6 (Figura 15), é produzida pela hidrólise ácida parcial ou enzimática do amido (QIU et al., 2017). São classificadas de acordo com a sua equivalência de dextrose (DE), que está relacionada com o grau de polimerização (DP) através da relação: DE=100/DP. As que apresentam menores valores de DE não são higroscópicas, enquanto as com maiores DE tendem a absorver umidade (BEMILLER; HUBER, 2010). É um carboidrato que apresenta baixa viscosidade e baixo custo e é dita como um pó de coloração branca e inodoro (LACERDA et al., 2016).

Figura 15 - Estrutura química da maltodextrina



Fonte: Adaptado de Gupta et al. (2015).

A maltodextrina tem a capacidade de formar uma cobertura para os agentes de núcleo que encapsulam sabores, aromas e compostos bioativos, minimizando efeitos de degradação (LACERDA et al., 2016). Diversos estudos tem explorado sua utilização como material de parede para proteção de compostos bioativos (BÖGER; GEORGETTI; KUROZAWA, 2018; CARNEIRO et al., 2020; ETZBACH et al., 2020; LACERDA et al., 2016).

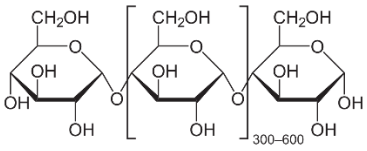
3.8.3 Amido modificado

Amido e ingredientes à base de amido (β-ciclodextrinas, amidos modificados, etc.) são amplamente utilizados pelas indústrias alimentícias com diversas funcionalidades, tais como a de reter e proteger compostos voláteis, substituintes de gorduras e também como estabilizantes de emulsões (MADENE et al., 2006).

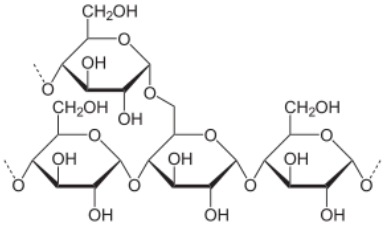
O amido é o principal carboidrato armazenado em tubérculos, raízes e cereais, estando depositado em grânulos insolúveis constituídos por estruturas semicristalinas. É composto por dois polímeros de D-glicose. O primeiro, denominado amilose (Figura, é um glucano constituído principalmente de ligações α[1-4]. O segundo é a amilopectina, a qual tem cadeias de glicose ligadas nas posições α[1-4], arranjadas em uma estrutura ramificada com ligações α[1-6]. Esses dois glucanos representam de 98 a 99 % do material seco do grânulo, dependendo da origem botânica do amido (MADENE et al., 2006). As estruturas químicas da amilose e amilopectina podem ser visualizadas na Figura 16.

Figura 16 - Estrutura química da amilose (a) e amilopectina (b)

(a)



(b)



Fonte: Adaptado de Tester et al. (2004).

O mercado de amidos vem crescendo e se aperfeiçoando nos últimos anos, visando produtos com características especificas que atendam às exigências da indústria. A produção de amidos modificados tem como principal objetivo superar as limitações dos amidos e, assim, aumentar sua faixa de aplicação industrial. Essas modificações podem, por exemplo, modificar as características de gelatinização e a tendência em formarem géis, melhorar a textura das pastas ou géis, adicionar grupamentos específicos e introdução de poder emulsificante (RIBEIRO et al., 2020).

Sendo assim, o amido modificado é um agente encapsulante muito utilizado devido a sua excelente retenção de voláteis e pela estabilização de emulsões. Diversos estudos tem explorado sua utilização como material de parede para proteção de compostos bioativos (CHARLES et al., 2021; DE CÁSSIA SOUSA MENDES et al., 2021; ITURRI; CALADO; PRENTICE, 2021; RIBEIRO, A. MARISA et al., 2020; ZHANG et al., 2021).

Portanto, para a microencapsulação de extratos hidrofílicos, como o extrato de uva, é importante escolher materiais encapsulantes adequados e também elucidar novas abordagens, como por exemplo, à aplicação como sensor colorimétrico comestível a fim de desenvolver novos sistemas para monitorar a qualidade de alimentos em tempo real, como forma de inovação no setor alimentício.

3.9 Aplicação de corantes naturais para o monitoramento da qualidade do leite

O leite é consumido por grande parte da população, destacando-se por suas propriedades sensoriais, excelente valor nutricional e também poder terapêutico, visto que seu consumo tem sido associado com efeitos benéficos à saúde (NOORI *et al.*, 2017). Em circunstâncias de práticas de fabricação e condições de armazenamento não adequadas, a deterioração desses produtos ocorre dentro de um tempo relativamente curto. Sua deterioração é o resultado de mudanças em suas características físicas, químicas e organolépticas/sensoriais, tornando-o inaceitável para consumo humano. A deterioração do leite, deve-se principalmente ao desenvolvimento de leveduras e bolores, percebida pelo aparecimento e formação de agregados na superfície do produto, descoloração e odores desagradáveis, tornando o alimento mais ácido e com isso, mudanças significativas no seu pH são observadas (MATARAGAS *et al.*, 2011).

Desse modo, a utilização de sensores comestíveis funcionalizados com corantes naturais, como as antocianinas, pode ser utilizada para o monitoramento da qualidade de produtos lácteos, e fornecer como resultado uma resposta imediata (mudança de cor, por exemplo) que se correlaciona com as propriedades físico-químicas e biológicas dos alimentos. Weston e colaboradores (WESTON; GENG; CHANDRAWATI, 2021), desenvolveram um sistema sensível ao pH para monitorar a qualidade do leite, usando antocianinas de rabanete, e o leite exibiu uma mudança de coloração de laranja-vermelho para verde, demonstrando o potencial deste sensor em predizer a vida útil do alimento.

No entanto, a utilização de corantes sensíveis ao pH aplicados diretamente em alimentos na forma de micropartículas para monitorar a qualidade e colorir alimentos é pouco explorada. Portanto, o desenvolvimento de sensores comestíveis inovadores utilizando corantes alimentícios naturais, atóxicos e sensíveis a mudanças de pH, que possam ser aplicados diretamente no leite para o monitoramento da sua qualidade, torna-se extremamente relevante, permitindo ao consumidor detectar facilmente a olho nu se o alimento está apto ou não para o consumo, além de ser um ingrediente colorante.

4 CONSIDERAÇÕES FINAIS

A busca por potenciais fontes de baixo custo para extração de compostos bioativos corantes naturais, como os resíduos agroindustriais da uva, vem ganhando destaque nos últimos anos, podendo ser utilizados para confecção de micropartículas multifuncionais de interesse para indústria alimentícia. Como forma de proporcionar melhor estabilização e retenção dos compostos bioativos (antocianinas) presentes no extrato de uva, o método combinado de emulsificação e gelificação iônica, bem como a utilização de materiais de parede complementares, se mostrou uma estratégia bastante promissora, fornecendo propriedades funcionais adicionais às micropartículas. Ainda, abordagens inovadoras, utilizando corantes alimentícios naturais, como as antocianinas, que possam ser aplicados diretamente no alimento para o monitoramento da sua qualidade, são necessárias, permitindo confeccionar novos sistemas colorantes e inteligentes.

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SEGUNDA PARTE – ARTIGOS

ARTICLE 1 - Development of alginate/pectin microcapsules by a dual process combining emulsification and ultrasonic gelation for encapsulation and controlled release of anthocyanins from grapes (*Vitis labrusca* L.)

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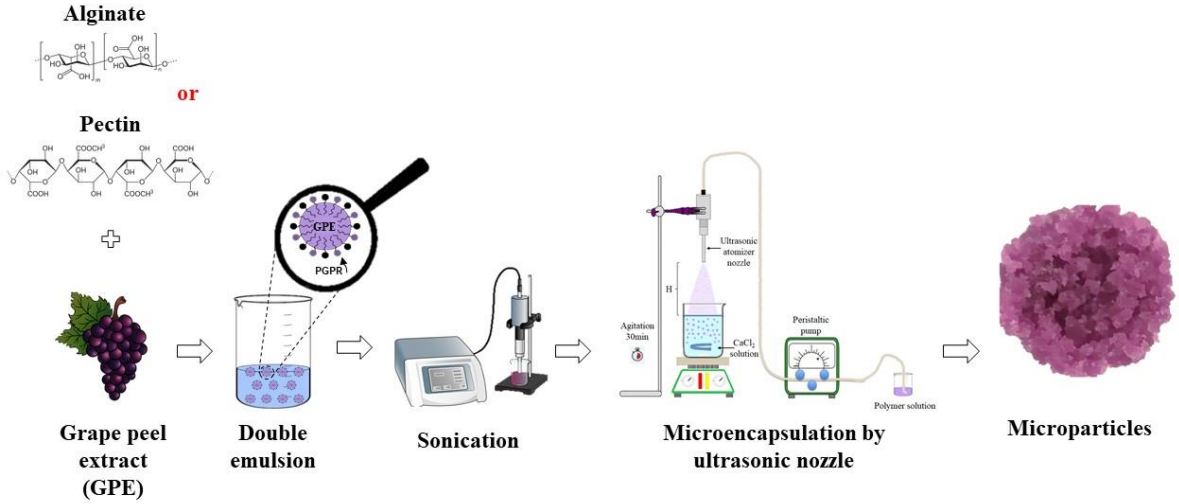

Development of alginate/pectin microcapsules by a dual process combining emulsification and ultrasonic gelation for encapsulation and controlled release of anthocyanins from grapes (*Vitis labrusca* L.)

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Abstract

The aim of this study was to investigate the physicochemical, morphological, and gastrointestinal release properties of an anthocyanin-rich extract of grapes in alginate and pectin beads as carriers; the effects of ultrasonic gelation combined with emulsification were also investigated. In general, the alginate beads showed smaller size and more regular shape compared to pectin. The effect of emulsification combined with ionic gelation was more pronounced in the alginate beads and resulted in higher retention of anthocyanins, higher antioxidant capacity, and also allowed the best release profile during intestinal digestion. Thus, the simultaneous strategy could be an interesting delivery system and enhance the release of anthocyanins, providing an opportunity for the development of ingredients with different bioactive properties.

Keywords: Ultrasonic gelation; Anthocyanins; Sodium alginate; Pectin; Grape peel extract.

1. Introduction

In recent decades, consumer demand for antioxidants from natural sources has increased in importance due to the trend toward healthier and more balanced diets. They are seeking out additional benefits such as health promotion and disease prevention. On this feel, grapes occupy an outstanding location as a supply of phenolic compounds associated with the advertising of human health, acting, for instance, as a natural antioxidant and minimizing the outcomes of premature aging (Asioli et al., 2017).

Grape production occupies a prominent position in world cultivation and is considered an important fruit for the agro-industrial sector. It is consumed both in fresh form, *in natura*, and for the preparation of various foods such as jellies, kinds of vinegar, oils, juices, and wines. However, tons of agro-industrial by-products such as seeds, stems, and hulls are generated during processing. It is estimated that only 3% of these byproducts are reused, as they are considered low value-added wastes and are often used as soil fertilizer, to supplement animal feed, or even incinerated. Therefore, it is mandatory that there is economic and environmental responsibility for a more appropriate destination for these byproducts (Dávila et al., 2017).

Grape by-products, especially skins, represent a promising and cost-effective source of natural phenolic compounds for the food industries. They are responsible for biological activities such as: antioxidant, antidiabetic, antimutagenic, anti-inflammatory and prevention of coronary diseases (Dávila et al., 2017). Among these interesting phenolic compounds, anthocyanins stand out.

Anthocyanins are hydrophilic non-toxic natural pigments and are found in flowers, vegetables and fruits. Several studies have shown the biological effects related to anthocyanins, such as: antioxidant and anti-inflammatory properties, suggesting that they may have the

potential to prevent obesity, colon cancer and diabetes. Thereby, it is suggested that the ingestion of anthocyanins could be attributed to the improvement of several parameters related to the intestinal health (Han et al., 2021; Lin, Gong, Song, & Cui, 2017). However, they have low stability under unfavorable conditions and are affected by the environment (temperature, pH, oxygen and light), interaction with other components and gastrointestinal conditions (pH and enzymes) (de Moura, Berling, Germer, Alvim, & Hubinger, 2018). Therefore, the main challenge is to find technologies to preserve and incorporate these phenolic compounds into food products. These limitations can be overcome by microencapsulation.

Among the various microencapsulation techniques used for the protection of phenolic compounds, ionic gelation is very promising. It is considered a simple technique that requires mild preparation conditions and does not require temperature or toxic solvents to the environment (McClements, 2017). Various atomization nozzles are available for ionic gelation and the selection must take into account relevant factors such as the desired size and size distribution of the microparticles and also their final application. Recently, ultrasonic nozzle has emerged as an interesting alternative for the preparation of microparticles with smaller, uniform and spherical size, facilitating their dispersion in various products, such as foods, and also leads to better sensory acceptance by consumers due to the properties of the microparticles obtained by ultrasonic gelation (Barba, Dalmoro, d'Amore, & Lamberti, 2015; Turan et al., 2016). The anionic polymers sodium alginate and low amidated pectin are the most commonly polymers used on the development of microparticles by ionic gelation (McClements, 2017).

Alginates are nature-based water-soluble polysaccharides found in some bacteria and brown algae. Their chemical structure consists of residues of a linear 1,4-linked copolymer of L-guluronic and D-mannuronic acids. It is biodegradable and biocompatible, and the interaction

of Ca^{2+} are responsible for the 3D-network gel formation (Comunian & Favaro-Trindade, 2016; Hambleton, Debeaufort, Bonnotte, & Voilley, 2009; McClements, 2017; Zia, Zia, Zuber, Rehman, & Ahmad, 2015).

On the other hand, pectin consists of a polysaccharide fiber with a very complex structure. Its chemical structure is formed by linear units of galacturonic acid linked by partially methoxylated glycosidic bonds. Its outstanding properties include biocompatibility, biodegradability and gelling capacity. In addition, pectins are consider safe for consumption (GRAS) and low cost (Wicker & Kim, 2015).

However, the hydrogel beads produced with these polymers are porous, which may compromise the release and protection of the microencapsulated hydrophilic anthocyanins. An alternative to overcome this technical obstacle is to combine microencapsulation and emulsion systems. When dispersed in emulsion gels, bioactive ingredients are protected and immobilized by the interaction between the emulsion and the network formed, and therefore show an improvement in physicochemical properties from adverse conditions, control their release, and increase their release during digestion (Beldarrain-Iznaga, Villalobos-Carvajal, Leiva-Vega, & Sevillano Armesto, 2020; Geremias-Andrade, Souki, Moraes, & Pinho, 2016). Recently, the efficacy of gel microparticles for encapsulating bioactive compounds has been showed several times (Geremias-Andrade et al., 2016; Feng et al., 2018).

In the present work, the potential use of microencapsulation by ion gelation in combination with an emulsification process was investigated to improve the stability of hydrophilic natural anthocyanins extracted from the grape skin. It is worth noting that this is the first study in which a combined approach of emulsification and ionic ultrasonic gelation with alginate/pectin as gelling agent was used. The microcapsules were evaluated for their

moisture content, water activity, mean size, morphological properties, anthocyanin content, total phenolic compounds, antioxidant capacity, encapsulation efficiency (EE), vibrational spectroscopy and gastrointestinal release. The results of this study could allow the development of active microparticles to meet the current market trends in the food sector and also provide an alternative for the preservation of the physicochemical properties of anthocyanins.

2. Materials and Methods

2.1 Materials

The grapes of the Bordô variety (*Vitis labrusca* L.) were kindly donated by producers from a farm located in Campos Gerais (Minas Gerais, Brazil). Hydrogel beads were prepared using sodium alginate (Sigma-Aldrich, Darmstadt, Germany); low methoxyl amidated pectin LM-104 AS-Z Genu® (CP Kelco, Limeira, SP, Brazil); calcium chloride (Anidrol, Diadema, SP, Brazil); soybean oil (Bunge Alimentos, Brazil) and polyglycerol polyricinoleate (PGPR) emulsifier (Concepta Ingredients, SP, Brazil).

2.2 Preparation of the grape peel extract (GPE)

The grapes were washed and disinfected. To obtain the grape peel extract, the seeds, peel, and pulp were removed manually. Anthocyanins from the peel were extracted with distilled water and citric acid (1% wt. of water) in a 1:1 (w/v) ratio (peel: acidified water). The mixture was kept overnight in the dark at a refrigeration temperature of 5 ± 1 °C and covered with aluminum foil to improve the extraction of anthocyanins. Then, the extract was filtered through organza and centrifuged at 5,000rpm to remove suspended solids. Finally, the grape

peel extract (GPE) was stored in amber bottles to avoid degradation of anthocyanins at a refrigeration temperature of 5 ± 1 °C.

2.3 Encapsulation by ultrasonic ionic gelation method

For the preparation of control microspheres, gelation of alginate (ALG) and pectin (PEC) was performed according to the adapted method of Belščak-Cvitanović et al. (2015). The alginate or pectin solutions (2%, w/w) were prepared by dissolving the polymer in the previously prepared grape skin extract (GPE) and homogenized for 20 min at 10,000 rpm in an Ultra-Turrax. The particles were atomizer on the cross-linking solution ($CaCl_2$ - 1.5% w/w) using an ultrasonic nozzle (0.5 mm diameter) and with a flow rate of 0.565 mL/min. The beads were stirred at 150 rpm for 30 minutes. Then, they were filtered with a vacuum pump, dried at 30 °C in a vacuum oven until completely dry, and stored in aluminum pouches.

The combined emulsification and ionic gelation for microencapsulation was performed following the methodology proposed by Moura et al. (2018). The primary w_1/o emulsion was prepared as follows. First, a measured amount of PGPR (4% w/w) was dissolved in soybean oil to prepare the oil phase. Then, grape skin extract was added at a ratio of 35:65 w/w and mixed for 15 min and 7,000 rpm in an Ultra-Turrax. The double emulsion ($w_1/O/w_2$) was prepared with the polysaccharide (pectin or alginate) solution (2% w/w) in an Ultra-Turrax IKA operating at 7,000 rpm for 10 min at a ratio of 20:80 w/w. A ultrasonifier (30°C, 200 W) (Model S-450D, Branson Ultrasonic Corporation, Danbury, USA) was also used for emulsification for 7 minutes. Samples contain pectin (PEC - DE) and alginate (ALG - DE) were prepared according to the aforementioned gelation steps of the control microbeads.

2.4. Microparticles characterization

2.4.1 Moisture content and water activity

The moisture content of the dried samples was determined using the infrared radiation in a thermobalance (Moisture Balance MOC-120H; Shimadzu Corporation, Tokyo, Japan), in triplicate, at 70 °C until constant weight. The water activity (a_w) of samples (1 g of each) was measured in triplicate by using a hygrometer HygroPalm AW1 (Rotronic Instruments, Huntington, NY, USA) at 25 °C.

2.4.2 Mean diameter and size distribution

The beads were homogenized in isopropanol as dispersing medium using a sonifier (Model S-450D, Branson Ultrasonics Corporation, Danbury, USA) for 1 min at a power of 200 W. De Brouckere mean diameter ($d_{4,3}$) and diameter distribution (indicated by polydispersity index, PdI) were determined using a Mastersizer (Model 3000E, Malvern Instruments Inc., Worcestershire, U.K.).

2.4.3 Morphology

Particle morphology was observed using an optical inverted light microscope (Carl Zeiss Sports Optics, model Axio Scope.A1, Zeiss, Germany), with 40X magnification. The acquisition of the images was carried out through the software AxioVision Rel. 4.8.

2.4.4 Particles dissolution to bioactive quantifications

The samples with extract were placed in sodium citrate solution in order to promote the disorganization of the gel structure and release the phenolic compounds present in the beads. So, 1.5 g of wet particles were dissolved in sodium citrate 3% (w/v) and kept in an ultrasonic bath for 2 hours. The samples were filtered, transferred into a flask covered with aluminum foil and the subsequent analysis were performed: anthocyanin content, content of total phenolic compounds and antioxidant assay.

2.4.5 Anthocyanin content

The pH differential method (AOAC, 2006) was used to estimate the total anthocyanin content. Aliquots of the samples were placed at pH 1 and 4.5 and incubated at room temperature for 15 minutes. Then, the absorbance was read using a UV-Vis- spectrophotometer against a blank at 520 and 700 nm.

2.4.6 Content of total phenolic compounds

About 1.5 ± 0.01 g of sample was used to determine the total phenolic compounds according to the Folin-Ciocalteu method (Turfan, Türkyilmaz, Yemi, & Özkan, 2011). The absorbance of the samples was made in a UV-Vis- spectrophotometer (model UV 2600i, Shimadzu, Kyoto, Japan) at 760 nm.

2.4.7 Antioxidant assay

About 1.5 ± 0.01 g of sample was used to evaluation of antioxidant activity by DPPH method performed according Brand-Williams, Cuvelier, & Berset (1995). The absorbance

readings were made in a UV-Vis- spectrophotometer (model UV 2600i, Shimadzu, Kyoto, Japan). The results were expressed in mg of Trolox equivalent/g of dry beads.

2.4.8 Encapsulation efficiency (EE)

The encapsulation efficiency was calculated on dry basis according to De Moura et al. (2018) and was determined by Eq. (1).

$$EE\ (\%) = \frac{\text{mg of anthocyanin in microparticle}}{\text{mg of anthocyanin in the mixture}} \times 100 \qquad (1)$$

For the control groups (ALG and PEC) the mixture was only composed of: grape peel extract + alginate/pectin solution. For ALG-DE and PEC-DE the mixture was composed of: emulsion (soybean oil + grape peel extract) + pectin or alginate solution.

2.5 FTIR vibrational spectroscopy

Fourier transform infrared spectroscopy (FTIR) of the microcapsules were obtained using a spectrophotometer (model Vertex 70v, Bruker, Massachusetts, United States). Measurements were done at 25 °C and with the recording from 4000 to 400 cm^{-1} at a scan rate of 40 scans with a 4 cm^{-1} spectral resolution.

2.6 Anthocyanin release profile under gastrointestinal conditions

The release profile of anthocyanins was carried out using an *in vitro* model according to Belscak-Cvitanovic et al. (2015). About 2.5 g of microencapsulated anthocyanins or free extract were dispersed in 50 mL of simulated gastric fluid (pH 1.2) and incubated at 37°C in thermostatic bath with constant shaking for 120 min to mimic stomach conditions. Afterward, the microparticles were dispersed in 50 mL of simulated intestinal fluid (pH 7.4) and incubated at 37°C for an additional 120 minutes. At defined time intervals (5-30 min), an aliquot of 5 mL of the fluids were taken for subsequent quantification of total monomeric anthocyanins (differential pH method).

2.7 Statistical analyses

For data analysis was applied a one-way analysis of variance (ANOVA), using the software OriginPro v9.9 (Origin Lab, Northampton, United States). The means differences were performed employing the Tukey test, with a significance level of $\alpha = 0.05\%$.

3. Results and discussion

3.1 Moisture content and water activity

Moisture content and water activity of grape powder beads are important parameters for predicting storage stability as they are related to microbial degradation rate and growth reactions (Geremias-Andrade et al., 2016). Table 1 shows the A_w and moisture content of dried pectin and alginate beads influenced by emulsification process.

Table 1. Water activity and moisture content of GPE-loaded beads influenced by emulsification process.

Parameter	Sample			
	ALG	**PEC**	**ALG - DE**	**PEC - DE**
A_w at 25°C	0.40 ± 0.01^{b}	0.42 ± 0.01^{a}	0.37 ± 0.02^{c}	0.43 ± 0.01^{a}
Moisture (%)	6.7 ± 0.9^{b}	9.1 ± 1.1^{a}	2.9 ± 1.3^{d}	4.7 ± 1.5^{c}

[a-d] Different letters in the same line indicate a significant difference ($p < 0.05$) using Turkey test.

According to the obtained results shown in Table 1, the moisture content of the beads varied between 2.9 and 9.1%. The higher moisture content was observed in the pectin-based beads when compared to the alginate ones. This tendency can be explained by the different packing density arrangement in the polysaccharides network, which affects the water affinity to the polymer components (Beldarrain-Iznaga, Villalobos-Carvajal, Leiva-Vega, & Sevillano Armesto, 2020).

ALG -DE and PEC- DE exhibited lower moisture content than ALG and PEC. This effect can be attributed to a less porous polymeric structure capable of adsorbing low-water content in their 3D-networks, because the oil (lipophilic) filling the space between the macromolecules (Belščak-Cvitanovic et al., 2016). Similar results were obtained by other authors who used alginate and pectin as gelling materials by ionic/ionotropic gelation (Beldarrain-Iznaga et al., 2020; Belščak-Cvitanovic et al., 2016). In relation to a_w, the values

ranged from 0.37 to 0.43, values considered interesting since a_w < 0.60 can guarantee microbiological stability for food.

3.2 Mean diameter and size distribution

Particle size is a fundamental parameter in industrial applications of microencapsulation as it can be a limiting factor in food products (Belščak-Cvitanovic et al., 2016). The polydispersity index and mean droplet diameter of the beads generated by ultrasonic gelation technique are shown in Table 2.

Table 2. Average diameter (µm) and polydispersity index (PdI) of GPE-loaded beads.

Sample	Diameter (µm)	PdI
ALG	17.1 ± 1.2^{b}	0.27 ± 0.02^{b}
PEC	21.9 ± 1.7^{a}	0.30 ± 0.03^{a}
ALG – DE	11.1 ± 1.5^{d}	0.31 ± 0.03^{a}
PEC – DE	15.3 ± 1.1^{c}	0.33 ± 0.02^{a}

[a-d] Different letters in the same column indicate a significant difference ($p < 0.05$) using Turkey test.

All the beads showed a narrow distribution range, confirmed by the low PdI values, below 0.6, indicating a unimodal behavior with respect to size. ALG beads diameters were smaller than those of PEC. This difference could be attributed to the more reticulated structure

of alginate, which promotes higher shrinkage of the polymer gel, resulting in lower diameters, when compared to pectin (Sandoval-Castilla et al., 2010). Moreover, there was a significant decrease in droplet size of the beads that combined the emulsification system and ionic gelation strategy. This reduction in size was also observed by Beldarrain-Iznaga et al. (2020) and Lu et al. (2019), who attributed this effect of size reduction to the formation of a more cohesive surface structure due to the interaction between the emulsion, calcium ions, and polymer chains, which could compress the hydrogel bead structure. In addition, the presence of phenolic acids in GPE could lead to the formation of smaller droplets as they reduce the interfacial tension between the aqueous phase and the flexible interfacial formation, thus acting as co-surfactants and co-solvent (Fasolin, Santana, & Cunha, 2014).

3.3 Morphology

Fig. 1 shows the morphology of the GPE-loaded beads.

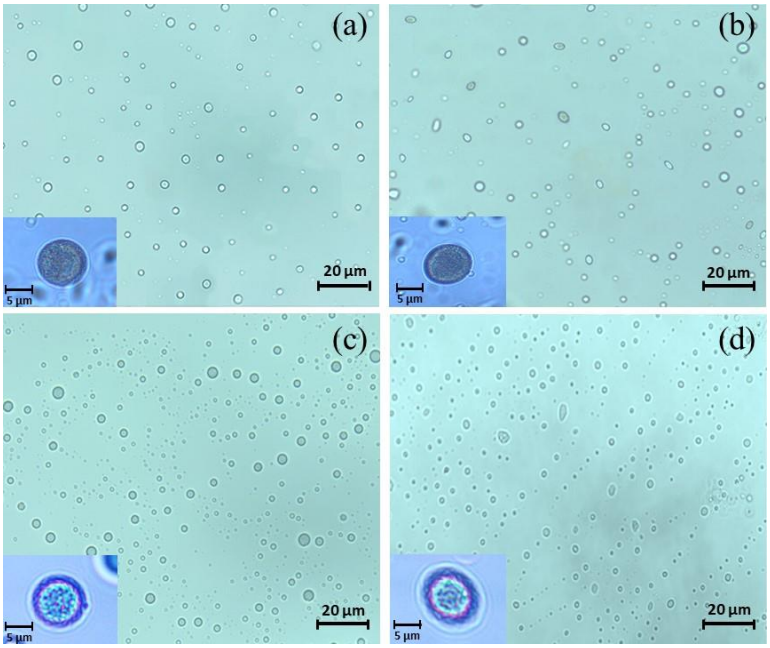

Fig. 1. Morphology of: (a) ALG, (b) PEC, (c) ALG- DE and (d) PEC- DE.

Optical microscopy photographs revealed that the alginate beads prepared by ionic gelation exhibit a regular, round and spherical shape (Fig. 1a and Fig. 1c), whereas the pectin beads have a slightly irregular shape (Fig. 1b and Fig. 1d). One possible explanation is that this irregularity is due to the weaker mechanical stability of the Ca-crosslinked pectin-bead network compared to alginate (Fasolin et al., 2014). Similar trends were reported by Belščak-Cvitanovic et al. (2016) with pectin beads of dandelion polyphenols and ß-carotene generated by ionotropic gelation. These authors showed a lower regularity in spherical form for particles using pectin than particles with alginate.

The results also showed that the emulsion droplets were multinucleated and had defined walls, which should ensure better protection to the grape peel bioactive compounds. Furthermore, the integrity and tightness of the coating wall which can be observed by perfectly

enclosing the core material, leading to a gradual diffusion of the core material from the hydrogel beads.

3.4 Anthocyanin content, total phenolic compound content, and antioxidant capacity of GPE-loaded beads

The encapsulation technique using ionic gelation is an effective way to prevent the degradation of bioactive compounds that can occur due to their interaction with temperature/pH variations, and light/oxygen exposure (Fasolin et al., 2014). Anthocyanin content, total phenolic compound content, and antioxidant capacity are shown in Table 3.

Table 3. Anthocyanin content, total phenolic compound content, and antioxidant capacity of GPE-loaded beads.

Sample	Total monomeric anthocyanin (mg 100 g^{-1})	Total phenolic content (mg GAE 100g^{-1})	Antioxidant activity (mg TEAC 100g^{-1})
GPE	1048.5 ± 0.10[a]	978.2 ± 0.10[a]	2016.9 ± 0.80[a]
ALG	671.0 ± 0.66[e]	143.6 ± 0.01[e]	306.4 ± 1.30 [e]
PEC	482.1 ± 0.92[f]	63.2 ± 0.01[f]	168.2 ± 0.40[f]
GPE - EM	987.2 ± 0.44[b]	831.7 ± 0.07[b]	1787.4 ± 0.94[b]
ALG - DE	878.6 ± 0.84[c]	768.3 ± 0.02[c]	1353.9 ± 0.60[c]
PEC - DE	750.3 ± 0.70[d]	456.2 ± 0.04[d]	653.1 ± 0.70[d]

[a-e] Different letters in the same column indicate a significant difference ($p < 0.05$) using the Turkey test.

Table 3 shows that the parameters of total anthocyanins, total phenolic compound content, and antioxidant activity decreased significantly in the emulsion (GPE-EM) compared with GPE. This effect could be related to the preparation conditions of the emulsions, such as contact with oxygen and the use of an ultrasonic sonicator, which generates cavitation bubbles that collapse and produce high local heat that could degrade some of the phenolic compounds present in the GPE due to their instability under these conditions (Wang et al., 2017). This effect was also observed by Moura et al. (2018).

According to Table 3, PEC and ALG showed higher losses of monomeric anthocyanins during the encapsulation process than PEC-DE and ALG-DE beads. This loss of anthocyanins may be attributed to the high porosity of the beads and the easy diffusion of these bioactive compounds under hydrophilic conditions, especially because of their polar nature (Fasolin et al., 2014).

The emulsion/ionic gelation approach provided higher levels of monomeric anthocyanins content. One possible explanation is that the combined techniques may have reduced the loss of anthocyanins by reducing the effective diffusivity through the microstructures of the hydrogels. In this way, the emulsification process acted as an additional barrier compared to the free anthocyanin extract and affected the polarity of the anthocyanins, hindering diffusion transport from the interior to the surface of the beads. These results are in accordance with da Silva et al. (2019).

Antioxidant capacity analysis and total phenolic content followed the same trend as monomeric anthocyanin content, as displayed in Table 3. The beads containing only grape peel extract showed the lowest antioxidant capacity and total phenolic content, followed by the samples ALG-DE and PEC-DE. In this way, the antioxidant capacity and phenolic content of the beads were directly related to the emulsification process. Pectin and alginate hydrogel beads with combined techniques were more efficient in incorporating and protecting the anthocyanins, resulting in higher antioxidant capacity and phenolic content values in relation to samples do not use the emulsification process. These results are in agreement with the previous studies that showed that ionic gelation/double emulsion of jussara extract (Carvalho et al., 2019) and *Lactobacillus casei* (Beldarrain-Iznaga et al., 2020) exhibited higher antioxidant activity.

The encapsulation efficiency (EE) of GPE was investigated by comparing the ability of encapsulate or hold the grape peel anthocyanins inside the microcapsules using or not the emulsification process. EE was around 46% (PEC), 64% (ALG), 76% (PEC-DE) and 89% (ALG-DE). Therefore, a possible explanation for the increase in the encapsulation efficiency of PEC-DE and ALG-DE microparticles is that soybean oil interacted with the polymeric network of polysaccharides, strengthening the existing chemical bonds and thus acting as an additional physicochemical barrier that limited the diffusion rate of anthocyanins and consequently protected them (Liu et al., 2019). This effect was more pronounced at ALG-DE. Previous researches have suggested that alginate with divalent cations forms stronger egg-box structures than pectin (Fang et al., 2008). Similar results were also reported by Carvalho et al. (2019), Liu et al. (2019) and Beldarrain-Iznagaab et al. (2020).

3.5 FTIR spectra GPE-loaded beads

FT-IR analysis was performed to distinguish possible chemical interactions among GPE and also the respective beads constituents. The FTIR-spectra of the samples were displayed in Figure 2.

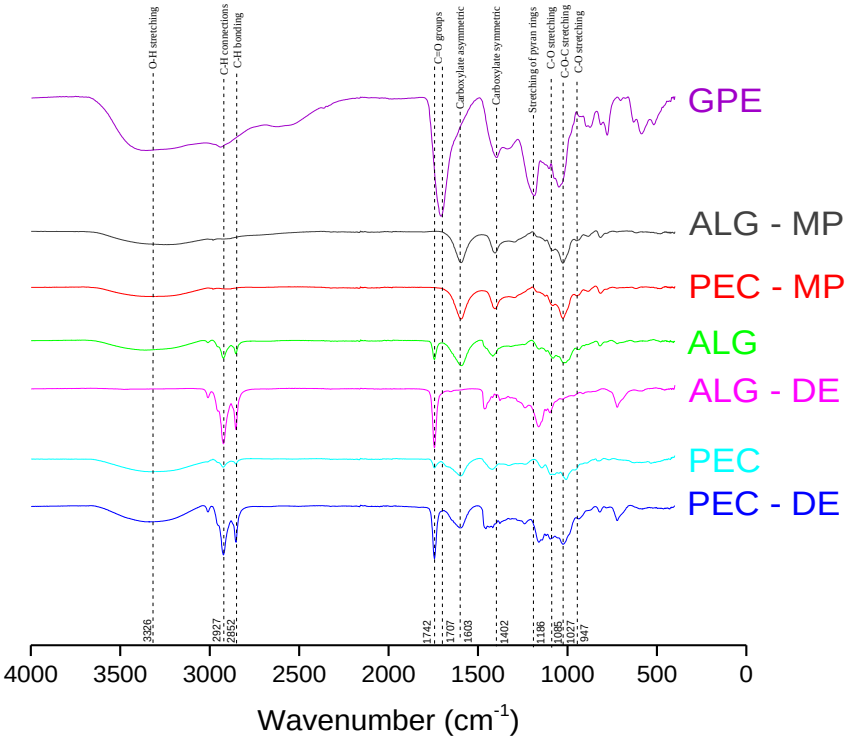

Fig. 2. FTIR spectra of grape peel extract (GPE), neat biopolymers, and GPE-loaded beads. Y-axis: transmittance/a.u.

The spectrum of pure pectin (PEC-MP) showed vibrational bands at 3627-2910 cm^{-1} (O-H and C-H stretching). Two bands at 1603 cm^{-1} and 1405 cm^{-1} (C-O-O weaker asymmetric and stronger symmetric), at 1200-1000 cm^{-1} (C-O and C-C of glycosidic bonds and pyranoid rings) and the very complex region below 1000 cm^{-1} so-called "fingerprint" of polysaccharides (Synytsya, Čopíková, Matějka, & Machovič, 2003).

The infrared spectra of neat alginate (ALG-MP) showed characteristic bands of its composition, highlighting the absorption bands at 3650-3018 cm^{-1} (hydroxyl group), 1603 and 1402 cm^{-1}, which were assigned to asymmetric and symmetric stretching of carboxylate salt groups. In addition, the bands centered at approximately 2927 cm^{-1} was attributed to the elongation of C–H bonds of pyranoid-ring carbons, 1085 cm^{-1} (C–O stretching), 1027 cm^{-1} (C–

O–C stretching), and 947 cm^{-1} (C–O stretching) were attributed to its polysaccharide structure (Synytsya et al., 2003).

For grape peel extract (GPE) the observed bands, as the O-H stretching at 3354 cm^{-1}, 2852 cm^{-1} attributed to the C–H-bonding axial deformations in aliphatic hydrocarbons, 1402 cm^{-1} corresponds to the C-O deformation of phenols and 1742 - 1707 cm^{-1} attributed to the C=O groups for aromatic rings. The absorbance at 1047 cm^{-1} was due to the stretching vibration of the C-O-C esters whereas absorbance at 1186 cm^{-1} was ascribed to the stretching of the pyran rings, which are typical of flavonoid compounds (Ahmed, 2015).

GPE beads peaks were typical of grape peel extract components. Specifically, the addition of GPE caused the appearance of characteristic bands (2852 cm^{-1}, and 1186 cm^{-1}) as well as the increase in the intensity of these bands when combined emulsification and ultrasonic ionic gelation strategy, also suggesting that ALG-DE retained the bioactive compounds more efficiently. In addition, changes in the wavelength number of the bands related to C=O groups, 1707 to 1742 cm^{-1}, may indicate an interaction between phenols present in GPE. These results are in accordance with Beldarrain-Iznaga (2020) and Silva et al. (2018). The FTIR data strongly support the findings obtained by conducted analyses on bioactive encapsulation parameters, such as anthocyanin content and encapsulation efficiency.

3.6 GPE-loaded beads release profile under simulated gastric and intestinal conditions

Anthocyanins (ATC) are characterized by very low bioavailability and enter the bloodstream rapidly after consumption of a meal rich in these compounds, generally due to their low solubility, low stability, low permeability, an active efflux process, and also gastrointestinal tract metabolism (Pedrali, Barbarito, & Lavelli, 2020).

In this way, encapsulation of bioactive components can improve bioavailability by increasing their water solubility and release in a given environment, leading to better absorption by the human body (Pedrali, Barbarito, & Lavelli, 2020). Therefore, anthocyanin release (Fig. 3) from the microcapsules was determined in simulated intestinal fluid to clarify whether the double emulsification approach can be considered a suitable matrix for stabilizing anthocyanins under detrimental intestinal conditions.

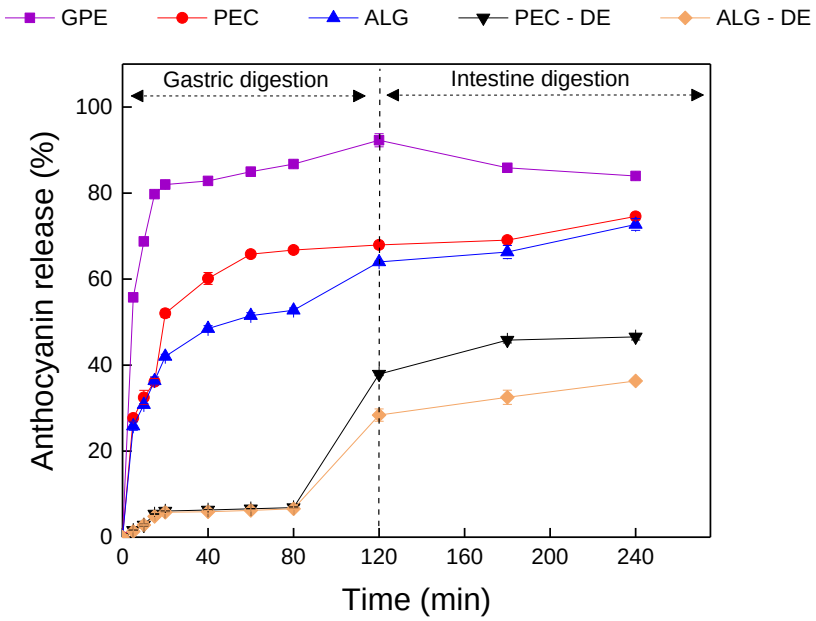

Fig. 3. Release profile of ATC from GPE and GPE-loaded beads in simulated gastric conditions.

In GPE, 70% of anthocyanins were already detected within the first 10 minutes. It was also observed that the total anthocyanins content in the gastric fluid decreased from 120 min, probably due to the degradation of anthocyanins caused by exposure to heat (37 °C) for a longer

period of time (Pedrali, Barbarito, & Lavelli, 2020). When transferred to the simulated intestinal fluid, anthocyanin release was kept almost constant, until the end of 4h incubation.

PEC and ALG had a low protective effect on anthocyanins during gastric digestion, as more than 45% of this pigment was released in the first 20 minutes. This could be because bioactive compounds are generally small molecules that can easily migrate into the porous structure of hydrogels, resulting in low protection and facilitating release in the stomach (Yao et al., 2018).

On the other hand, microparticles prepared using a double emulsion and ultrasonic ionic gelation showed lower release in simulated gastric fluid than particles prepared with a non-combined strategy. The use of soybean oil to elaborate the double emulsion with grape skin extract before the ultrasonic gelation step contributed to a lower release of anthocyanins because the enzyme lipase is not present in gastric fluid. Thus, the soybean oil protected the GPE by acting as a barrier against the gastric fluid and not fully releasing the bioactive compounds during the gastric digestion (Yao et al., 2018).

In ALG - DE, the anthocyanin content lost in the gastric phase was slightly lower than in PEC-DE. This ALG-DE behavior can be attributed to the stronger physicochemical interaction of the hydroxyl groups in the adjacent chains of the alginate with the polar compounds in the grape peel extract (anthocyanins) and with the associated soybean oil. This resulted in the formation of more complex polymer entanglement and lower porosity due to the addition of soybean oil, which reduced the diffusivity of anthocyanins across the polysaccharide chain interface (Rather et al., 2017). Similar results were pointed out by Beldarrain-Iznaga et al. (2020), Carvalho et al. (2019) and Zhang et al. (2015).

In the simulated intestinal phase (SIF), anthocyanins convert to quinonoid, hemiketal, and chalcone forms and are degraded due to higher pH. Conversion and degradation under intestinal conditions could be a possible reason for the low bioavailability of anthocyanins. Therefore, it would be beneficial for anthocyanins to enter the intestine in their flavylium form (more stable cation). For this reason, the release of anthocyanins in simulated intestinal fluid provides information on whether microparticles are suitable matrices for the stabilization of anthocyanins under intestinal conditions (basic pH) (Betz & Kulozik, 2011).

The release of anthocyanins continued to increase significantly in the beads ($p \leq 0.05$), which is due to the fact that the rate of degradation of alginate is due to an increase in the rate of ß-elimination, which is also the case for pectin, facilitating the lipolysis of soybean oil through the action of pancreatic lipases. This process led to the demulsification of the microcapsules, eventually exposing the anthocyanins to the action of bile salts (He et al., 2017).

However, the ALG-DE microparticles containing soybean oil showed a lower release of anthocyanins during the final stages of digestion, indicating the protective effect caused by the combination of microencapsulation techniques. Similar results were shown by He et al. (2017), where chitosan nanoparticles caused slower release of blueberry anthocyanins than non-encapsulated anthocyanins, indicating that the slow release could reduce the degradation of anthocyanins, leading to bioavailability of these bioactive compounds that are more disponible for absorption in the gastrointestinal tract.

4. Conclusion

The combination of techniques for microencapsulation of grape peel extract influenced the physicochemical, morphological, and biological properties during the digestion process. In

particular, ALG-DE showed greater retention of anthocyanins, total phenolic compounds, and antioxidant activity. These results are related to the formation of more complex three-dimensional structures and greater chemical interactions of the alginate/grape skin extract and the oil, resulting in less diffusion of anthocyanins. In addition, smaller particle size, more spherical particles, and greater protection/ bioavailability of anthocyanins during gastrointestinal digestion were evident.

In summary, the combined method for microencapsulation of anthocyanins from grape extract provided additional functional properties to the microparticles and proves to be an interesting strategy for incorporating phenolic compounds into polymeric matrices and directly affects the viability of these compounds during processing, storage, and application, with the advantage of using mild conditions. The proposed technology also highlights the possibility of using agroindustrial by-products of viticulture for the production of extracts rich in anthocyanins, which strengthens the possible application of the combined approach of ionic gelation and emulsification processes

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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ARTICLE 2 - Exploring the potential of anthocyanin-based alginate microparticles for application as a food coloring and edible colorimetric indicator for milk

Artigo elaborado de acordo com as normas da revista Food Hydrocolloids – Versão preliminar

Exploring the potential of anthocyanin-based alginate microparticles for application as a food coloring and edible colorimetric indicator for milk

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Abstract

The objective of this study is to develop natural colorimetric indicator microparticles based on anthocyanins, produced through the ultrasonic-assisted ionic gelation technique, for application as a dye and to monitor the quality of milk. Different wall materials were combined with alginate in the microencapsulation process and microparticles were evaluated for their physicochemical, morphological, thermal, spectroscopic properties, in addition to stability and encapsulation efficiency. The color change during milk storage, for the immediate visual recognition of its freshness status was also evaluated. In general, the microparticles with modified starch resulted in higher retention of anthocyanins, higher antioxidant capacity, and also allowed the best stability. All the microparticles were able to monitor the freshness status of milk. Thus, the use of anthocyanin-based microparticles could be an interesting system for the development of edible colorimetric indicators, opening space for new approaches in the food industry

Keywords: Ultrassonic-assisted ionic gelation; Anthocyanins; Sodium alginate; Grape peel extract; edible colorimetric indicator.

1. Introduction

The food industry is increasingly interested in developing sustainable functional ingredients to replace synthetic or animal-based ingredients. This shift in focus has been driven by growing consumer demand for healthier, more environmentally friendly foods to feed the ever-growing global population. As a result, the last two decades have seen increased interest in obtaining bioactive compounds from low-cost sources, such as agro-industrial residues, which has spurred innovation and created space for the natural additives market (Carocho, Barreiro, Morales, & Ferreira, 2014).

Globally, an estimated 1.3 billion tons of agroindustrial waste is generated annually, with one-third of food potentially destined for human consumption going to waste. This waste, generated by various human activities, poses a major challenge as it is often improperly disposed of, causing public health problems and various environmental impacts (Carocho et al., 2014; Soni et al., 2022).

Therefore, concepts of minimization, recycling, use of by-products and bioconversion of wastes are increasingly widespread and necessary for agro-industrial chains to achieve better use and destination of these materials (Brazinha, Cadima, & Crespo, 2014; Caldas et al., 2018; Drosou, Kyriakopoulou, Bimpilas, Tsimogiannis, & Krokida, 2015; Favre, Hermosín-Gutiérrez, Piccardo, Gómez-Alonso, & González-Neves, 2019; Kammerer, Jaramillo, García, Calderin, & Valbuena, 2014; Ky & Teissedre, 2015; Rockenbach et al., 2011; Trikas, Melidou, Papi, Zachariadis, & Kyriakidis, 2016). In this sense, fruit residues, such as grapes, one of the most cultivated fruits in the world and of great economic importance in Brazil, are considered a potential source for the extraction of natural colorants, known as anthocyanins, which can be

used in an accessible and sustainable way for the production of food colors and edible indicator sensors of interest to the food industry (Al Azad, Jie, & Lal, 2018).

As microorganisms grow and multiply, they release various metabolites that lead to changes in the acidity or alkalinity of the food and the food environment. For this reason, pH-sensitive dyes have been introduced for the production of freshness indicators. When the degree of freshness of food changes, the sensitive indicator sensor reacts with the metabolic products of microorganisms in the food and indicates a color change in the form of a perceptible signal that can be visually recognized by the consumer (Almasi, Forghani, & Moradi, 2022; da Silva Filipini, Romani, & Guimarães Martins, 2020). Recently, researchers have focused their attention on indicator dyes such as anthocyanins, which are derived from natural sources, for the production of colorimetric indicators (Almasi et al., 2022; Chhikara, Kushwaha, Sharma, Gat, & Panghal, 2019).

The color-changing function of anthocyanins as pH-sensitive dyes depends on structural changes caused by the acid-base properties of the environment. At low pH values (pH < 2), the red color caused by the flavylium cation is predominant. At a slight increase in pH (pH = 5-6), the color changes to a purple/blue quinoid base. Then the colorless carbinol pseudobase is the predominant color as the pH increases from acidic to slightly acidic/almost neutral conditions. A further increase in pH (pH > 7) leads to a gradual decrease in anthocyanin stability and produces a yellowish-green color as a result of chalcone formation (Filipini et al., 2020; Mendes et al., 2022; Pereira, de Arruda, & Stefani, 2015). The reactive activity of anthocyanins may therefore be useful in promoting intelligent food quality monitoring systems.

However, anthocyanins are unstable pigments that are very sensitive to environmental conditions or technological processes, making their direct incorporation into food products difficult. Therefore, microencapsulation represents a promising alternative for the production

of colorimetric indicatprs to improve the stability and bioavailability of natural dyes during storage or processing (Almasi et al., 2022; Dias, Castanheira, Gil Fortes, Pereira, & Sameiro, 2020; Rockenbach et al., 2011). The ionic gelation technique is characterized by the fact that it is considered an environmentally friendly technology, since no solvents are used and no environmentally harmful substances are generated. Moreover, mild preparation conditions are used, ensuring better stability of these natural compounds (Sampaio et al., 2019a).

In recent years, various hydrogels, especially alginate, have been used as wall materials for ionic gelation. However, alginate has a porous gel structure and is not suitable for encapsulation of hydrophilic compounds such as anthocyanins (Deladino, Anbinder, Navarro, & Martino, 2008). Therefore, the use of non-toxic complementary wall materials such as maltodextrin, inulin and modified starch can help to reduce the porosity of the alginate and delay the release of the entrapped hydrophilic compounds.

Maltodextrin is a material commonly used in encapsulation processes and is a good stabilizer of components by reducing their mobility in the matrix, resulting in protection against external adverse conditions (Iturri, Calado, & Prentice, 2021). Inulin is increasingly used as a carrier material in recent years because it has good technological properties and is classified as a prebiotic, an interesting property that contributes to the innovation and development of functional foods (Almasi, Azizi, & Amjadi, 2020). Modified starch, such as Capsul®, with both hydrophilic and hydrophobic properties, is often also used by the food industry as an encapsulating agent with excellent retention capacity for bioactive substances and to stabilize emulsions (Zhu, 2017).

In this way, the development of innovative edible microparticles anthocyanins-based smart indicators using natural, non-toxic and pH-sensitive food colorings, which can be applied directly to milk to monitor its quality in real time, becomes extremely relevant, allowing the

consumer to easily detect the naked eye if the food is fit for consumption or not, in addition to being a coloring ingredient, as a form of innovation in the food sector.

In the present work, the potential use of anthocyanin-based microparticles produced by ionic gelation combined with complementary wall materials was investigated to improve the stability of hydrophilic natural compounds extracted from the grape peel and potential use as a colorimetric edible indicator. The intelligent microcapsules were evaluated for their mean size, anthocyanin content, total phenolic compounds, antioxidant capacity, encapsulation efficiency, vibrational spectroscopy, accelerated stability and also, were applied directly in milk. The results of this study could allow the development of intelligent microparticles it provides a fast and reliable inline assessment of food quality and safety, and also provide an alternative for the preservation of the physicochemical properties of anthocyanins.

2. Materials and Methods

2.1 Materials

Sodium alginate (ALG_w) was supplied by Sigma-Aldrich (Darmstadt, Germany); inulin (IN) with a polymerization degree > 10 was obtained by Orafttit® GR (Tienen, Belgium), modified starch (MS) Capsul® and maltodextrin (MD) with dextrose equivalent (DE) 20 from Ingredion (Campinas, Brazil). Calcium chloride (Sigma-Aldrich, Darmstadt, Germany); soybean oil (Bunge Alimentos, Brazil) and polyglycerol polyricinoleate (PGPR) emulsifier (DuPont™ Danisco®, Brazil). The grapes (*Vitis labrusca* L.) were kindly donated by a producer in Minas Gerais (Campos Gerais, Brazil) and milk were purchased from a local supermarket (São Carlos, Brazil).

2.2 Grape peel extract preparation (GPE)

Grape peel extract that was rich in anthocyanins was prepared according to Norcino et al. (2022). Using a blender, the peels were homogenized with distilled water acidified with citric acid (1%, w/w) at a ratio of 1:1 (peel:acidified water). The liquid extract was kept overnight in the dark at a refrigeration temperature (5-7°C) for extraction of anthocyanins. Then, was filtered through organza and centrifuged at 5,000rpm to remove suspended solids. Finally, GPE was stored in amber bottles to avoid degradation of anthocyanins at a refrigeration temperature (5-7°C) until use.

2.3 Microencapsulation of grape anthocyanin-rich extract by ultrassonic-assisted ionic gelation

The preparation of the intelligent microparticles was performed following the methodology proposed by Norcino et al. (2022) and Belscak-Cvitanovic et al. (2015), with slight modifications. Four different sustained release carrier systems based on double emulsions were formulated; sodium alginate (ALG), alginate blended with inulin, maltodextrin and modified starch (A_{INU}, A_{MALTO}, A_{MS}). First, the simple emulsion (w_1) was prepared as follows: PGPR (4% w/w) was dissolved in soybean oil (SO) to prepare the oil phase. Then, GPE was added (ratio of 35:65 w/w) and mixed in an Ultra-Turrax IKA operating at 7,000 rpm for 15 min. The double emulsion ($w_1/O/w_2$) was prepared with the alginate solution (2% w/w) in an Ultra-Turrax IKA operating at 7,000 rpm for 10 min at a ratio of 20:80 w/w. The A_{INU}, A_{MALTO}, A_{MS} blended solutions were obtained by adding (2% w/w) of the respective polysaccharide into the GPE-alginate solution described previously. A ultrasonifier (30°C, 200 W) (Model S-450D, Branson Ultrasonic Corporation, Danbury, USA) was also used for emulsification for 10

minutes. The particles were atomizer into a calcium chloride solution (1.5% w/w) using an ultrasonic nozzle (0.5 mm diameter) (Vibra-Cell model VCX 130, Sonics & Materials INC, Newtown, USA) and with a flow rate of 0.565 mL/min. The beads were stirred at 150 rpm for 30 minutes until complete gelation process. Then, they were filtered with a vacuum pump, dried at 30 °C in a vacuum oven until completely dry, and stored in aluminum pouches before use.

2.4. Microparticles characterization

2.4.1 Particle size and size distribution

The polydispersity index (PDI) and De Brouckere mean diameter ($d_{4,3}$) of the microparticles were obtained using a 1:3 (sample: isopropanol) dilution factor. Then, were homogenized using a sonifier (Model S-450D, Branson Ultrasonics Corporation, Danbury, USA) for 1 min at a power of 200 W. PDI and ($d_{4,3}$) were determined using a Mastersizer (Model 3000E, Malvern Instruments Inc., Worcestershire, U.K.).

2.4.2 Total anthocyanin content (TAC), total phenolic compounds (TPC), antioxidant assay (AA) and encapsulation efficiency (EE)

To evaluate the total anthocyanin content, total phenolic compounds and antioxidant assay of the beads, they were placed in sodium citrate solution 3% (w/v) and kept in an ultrasonic bath for 2 hours, in order to promote the disorganization of the gel structure and release the phenolic compounds present in the beads. After, the samples were filtered, transferred into a flask covered with aluminum foil and the analysis were performed.

The pH differential method described by AOAC (2006) and Mendes et al. (2021) was used to estimate TAC. TPC was determined according to the Folin-Ciocalteu method (Turfan, Türkyilmaz, Yemi, & Özkan, 2011). Antioxidant assay evaluation was performed according to DPPH method by Brand-Williams, Cuvelier, & Berset (1995). The encapsulation efficiency was calculated on according to de Moura et al. (2019) and was determined by Eq. (1).

$$EE\ (\%) = \frac{\text{mg of anthocyanin in microparticle}}{\text{mg of anthocyanin in the mixture}} \times 100 \qquad (1)$$

The mixture was composed of: emulsion (SO + GPE) + alginate solution + complementary polysaccharide (inulin, maltodextrin or modified starch).

2.4.3 FTIR vibrational spectroscopy

Fourier transform infrared spectroscopy (FTIR) of the microcapsules were obtained using a spectrophotometer (model Vertex 70v, Bruker, Massachusetts, United States). Measurements were done at 25 °C and with the recording from 4000 to 400 cm-1 at a scan rate of 40 scans with a 4 cm-1 spectral resolution.

2.5 Stability

Two procedures were used to evaluate the stability of the GPE-microparticles: thermogravimetric analysis and accelerated storage stability. In addition, grape peel extract without the addition of carrier agents was dried using a lyophilizer model L6000 from Terroni®

(São Carlos, Brazil). The lyophilized grape peel extract (LGE) was used as a control treatment to evaluate how the presence of carrier agents influences the stability of the GPE-beads.

2.5.1 Thermogravimetry

The thermogravimetric curves (TG/DTG) were obtained by a thermal TA Q500 (TA Instruments, Inc., New Castle, USA) under the following conditions: atmosphere comprising synthetic air (21% O_2) flowing at 40 mL min^{-1}, heating rate of 10°C min^{-1}, and temperature range from 25 to 600°C. Approximately 5 mg of sample was used for the analysis and at least 2 replicates were performed for each sample.

2.5.2 Accelerated storage stability

Accelerated storage stability was carried out according to Carmo et al. (2018), with some modifications. The vaccum oven-dried samples (2.5 g) were placed in a sterile high transparency polystyrene (PS) Petri dish with dimensions of 49 × 13 mm. The samples were properly sealed and incubated at 60 ± 1°C and relative humidity (UR) of 30%. Samples were characterized (in triplicate) in relation to anthocyanins content and microparticles color every week for 28 days of storage.

The color parameters were determined by using a portable colorimeter (Minolta Camera Co., ltd, Osaka, Japan). A white standard color plate (L* = 91.76, a* = -0.25, b* = -0.40) was used for calibration. L* (lightness), a* (redness-greenness), b* (yellowness-blueness) were measured to evaluate the color change of samples. Three measurements were taken on each sample. The total color difference (ΔE) was calculated according to Eq. (2):

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

The half-life $t_{1/2}$ of anthocyanins for each treatment was calculated according to Tonon, Brabet, & Hubinger (2010), considering that the degradation of these substances follows a first-order kinetics reaction, using Equations (3) and (4):

$$kt = -\ln\left(\frac{C_t}{C_0}\right) \qquad (3)$$

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

where C_0 and C_t correspond to the initial and final anthocyanin concentration (mg/100g d.b.) at the time t (days), and k ($days^{-1}$) is the first-order reaction rate constant, respectively.

2.6. Colorimetric response in milk samples

The colorimetric response in milk samples was performed according to the adapted methodology of Weston, Phan, Arcot, & Chandrawati (2020). The dried microparticles (10% w/w) were mixed with raw milk using an Ultra-Turrax IKA operating at 7,000 rpm for 5 min, to demonstrate the ability of anthocyanin-rich microparticles to discriminate between fresh (ph=6.8), spoiling (ph=5.0) and spoiled milk (ph=2.0). A digital pH meter (digital pH meter, Taiwan) was applied to monitor the milk pH for 12 days under refrigerated conditions (5°C). The samples were photographed using a digital camera and the visual color change was evaluated.

2.7 Statistical analyses

For data analysis was applied a one-way analysis of variance (ANOVA), using the software OriginPro v9.9 (Origin Lab, Northampton, United States). The means differences were performed employing the Tukey test, with a significance level of $\alpha = 0.05\%$.

3. Results and discussion

3.1 Particle size and size distribution

In the case of application of particles in food products, particle size is an extremely important factor because it is directly related to the sensory aspects of food: taste, texture and appearance. (Nabil, Ouaabou, Ouhammou, Saadouni, & Mahrouz, 2020). Table 1 shows the polydispersity index and mean droplet diameter of the anthocyanin-loaded microparticles.

Table 1. Average diameter (μm) and polydispersity index (PdI) of anthocyanin-loaded beads.

Sample	Diameter (μm)	PdI
ALG	17.1 ± 1.2^{b}	0.27 ± 0.02^{b}
ALG_{INU}	15.3 ± 1.1^{c}	0.68 ± 0.17^{a}
ALG_{MALTO}	15.1 ± 0.8^{c}	0.72 ± 0.14^{a}
ALG_{MS}	24.5 ± 1.3^{a}	0.81 ± 0.11^{a}

[a-c] Different letters in the same column indicate a significant difference ($p < 0.05$) using Turkey test.

The particle size of alginate microparticles was significantly affected by the addition of the additional wall materials ($p < 0.05$). The addition of inulin and maltodextrin decreased particle size, although there was no significant difference between treatments. However, ALG_{MS} exhibited the higher particle size, 24.5µm, expressed as $d_{(4,3)}$ (mean De Brouckere diameter). This effect can be explained by the fact that the low concentration of maltodextrin and inulin did not significantly change the viscosity of the system. However, even at low concentration, the modified starch might have increased the viscosity of the feed and increased the amount of soluble solids, forming larger droplets during the atomization process (Etzbach et al., 2020; Rajabi et al., 2015). In terms of the polydispersity index, it was low (0.81-0.27), indicating that the microparticles showed a narrow distribution range.

3.2 Anthocyanin content (TAC), total phenolic contend (TPC), antioxidant assay (AA) and encapsulation efficiency (EE)

Microencapsulation is an effective alternative to incorporate anthocyanins into various products while preserving their bioactive properties. In this context, ionic gelation presents itself as a technique with mild processing conditions, since no temperature and no aggressive solvents are used (Beldarrain-Iznaga, Villalobos-Carvajal, Leiva-Vega, & Sevillano Armesto, 2020; da Silva Carvalho et al., 2019). TAC, TPC, AA and encapsulation efficiency are shown in Table 2.

Table 2. Anthocyanin content (TAC), total phenolic compound content (TPC), antioxidant capacity (AA), and encapsulation efficiency (EE) of anthocyanin-loaded beads.

Sample	TAC (mg/g)	TPC (mg GAE/g)	AA (%)	EE (%)
GPE-emulsion	10.43 ± 0.3^{a}	8.96 ± 0.2^{a}	91.1 ± 0.3^{a}	------
ALG	8.74 ± 0.1^{d}	7.68 ± 0.1^{cd}	83.2 ± 0.3^{e}	84%
ALG_{INU}	8.77 ± 0.2^{d}	7.66 ± 0.1^{d}	84.1 ± 0.2^{d}	84%
ALG_{MALTO}	9.15 ± 0.3^{c}	7.98 ± 0.3^{c}	86.9 ± 0.1^{c}	90%
ALG_{MS}	9.87 ± 0.4^{b}	8.38 ± 0.2^{b}	88.6 ± 0.4^{b}	97%

[a-e] Different letters in the same column indicate a significant difference ($p < 0.05$) using the Turkey test.

Table 2 shows that all parameters (TAC, TPC and AA) decreased significantly in all treatments compared to the GPE emulsion. This decrease could be related to the preparation conditions, which, even if mild, could have caused cavitation of the emulsion bubbles during the sonication phase, generating local heat. In addition, the formulations could have been in contact with the light and oxygen present in the environment during the preparation and ultrasonic atomization, which caused the degradation of the phenolic compounds present in the grape extract and the oxidation of the soybean oil itself. These observations are in accordance with the results reported by other authors that also encapsulated phenolic compounds by ionic gelation using the sonification step (de Moura et al., 2019; Norcino et al., 2022).

The different encapsulation materials had a significant effect on the anthocyanin content and total phenolic compound content parameters ($p < 0.05$). TAC values ranged from 9.87 to 8.74 (mg anthocyanins per gram) and 7.66 to 8.38 (mg gallic acid equivalent per gram) for TPC.

The content of total anthocyanins and phenolic compounds was higher in ALG_{MS}. This behavior may be related to the presence of lipophilic groups along the modified starch polymer chains that interact simultaneously with polar and nonpolar molecules, allowing encapsulation of the grape extract as a whole, including lipids and other lipophilic components present in the soybean oil. In addition, the modified starch can act as an emulsion stabilizer, allowing the formation of emulsions with a close orientation of the polymer around an oil droplet and being an important factor for the microencapsulation of phenolic compounds (Zhu, 2017). Romero-Hernandez et al. (2021) and Lacerda et al. (2016) showed similar observations.

Moreover, the addition of maltodextrin contributed to this high TAC and TPC content, as this material has a good stabilization of the components and reduces their mobility in the matrix (Carmo et al., 2018).The antioxidant capacity content followed the same trend as the content of monomeric anthocyanins and total phenolic compound, as shown in Table 2.

Encapsulation efficiency (EE) was 84% (ALG), 84% (ALG_{INU}), 90% (ALG_{MALTO}), and 97% (ALG_{MS}). One hypothesis for the increase in encapsulation efficiency of ALG_{MS} is the combination of chemical interactions between the modified starch, soybean oil, and phenolic compounds present in the grape peel extract. In general, hydrophobic compounds are not able to show chemical interactions with amylose and amylopectin of starch. However, the hydrophilic compounds in the GPE and the hydrophilic portion of the soybean oil can lead to hydrogen bonds with the hydroxyl groups of the starch molecules and thus act as an additional physicochemical barrier that restricts the diffusion of the anthocyanins and consequently protects them (Hoyos-Leyva, Bello-Pérez, Agama-Acevedo, & Alvarez-Ramirez, 2018; Romero-Hernandez et al., 2021). These results are consistent with previous studies showing that microencapsulation of natural extracts exhibits higher antioxidant activity in the presence

of modified starch as a wall material (Escobar-Puentes, García-Gurrola, Rincón, Zepeda, & Martínez-Bustos, 2020; Hoyos-Leyva et al., 2018; Romero-Hernandez et al., 2021; Zhu, 2017).

Although the encapsulation efficiency of ALG_{MALTO} was higher than those of the other treatments, the encapsulation efficiencies of alginate/maltodextrin and alginate/inulin beads were still high, and the encapsulation efficiency of alginate alone (approximately 84%) can also be considered high, indicating that all carbohydrate-based matrices are efficient in retaining the nuclear material (anthocyanins) in the beads.

3.3 FTIR spectra of anthocyanin-based microparticles

ATR-FTIR spectroscopy was carried out to find possible interactions among the wall materials (alginate, inulin, maltodextrin and modified starch), grape peel extract and others beads constituents. The spectra are shown in Figure 1.

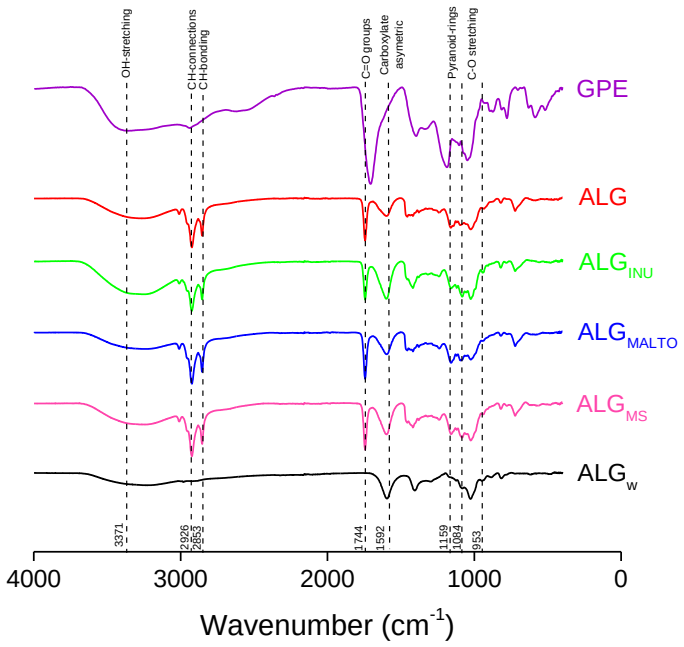

Fig. 1. FTIR spectra GPE, ALG_w – neat polymer, ALG, ALG_{INU}, ALG_{MALTO}, and ALG_{MS} microparticles. Y-axis: transmittance/a.u.

ALG_w showed characteristic bands around 3371, 1592, 2926, and 953 cm− 1, attributed to stretching vibration of hydroxyl group, asymmetric and symmetric stretching vibrations of carboxylate salt ion, stretching vibrations of pyranoid-rings carbons C–H and C–O stretching, respectively (Daemi & Barikani, 2012).

GPE showed bands around 3371 and 2853 cm^{-1} corresponding to O-H stretching and the stretching vibration of C–H bond in aliphatic hydrocarbons. The region at 1790 - 1622 cm^{-1} corresponding to the C=O groups of aromatic rings, and bands at 1159 and 1084 cm^{-1} corresponding to skeletal stretching vibration of the aromatic rings and =C-O-C group of flavonoids (de Cássia Sousa Mendes et al., 2021; Ribeiro et al., 2018).

For ALG, ALG_{INU}, ALG_{MALTO} and ALG_{MS} specific bands (2926 and 1744 cm^{-1}) were revealed, which imply the possible presence of grape peel extract compounds (for example,

anthocyanins) in the microparticles. Changes in the wavelength number 1705 to 1744 cm^{-1} (C=O groups) also indicate an interaction between phenols present in the extract. It is worth noting that the intensity of these bands in ALG_{MALTO} and ALG_{MS} were higher, while ALG and ALG_{INU} were similar, suggesting that modified starch and maltodextrin retained the bioactive compounds more efficiently, supporting the results found for TAC and EE.

3.5 Stability

3.5.1 Thermal properties

Knowledge of the thermal properties of the beads is useful because it can be used to evaluate their ability to resist decomposition at the high temperatures of the process, dehydration and oxidation (Kumorkiewicz-Jamro et al., 2021). The TGs and their first derivatives (DTG) curves obtained for the GPE-loaded beads are shown in Fig. 2, and the temperatures corresponding to the onset (T_{onset}) and offset (T_{offset}) of the thermal degradation are shown in Table 3.

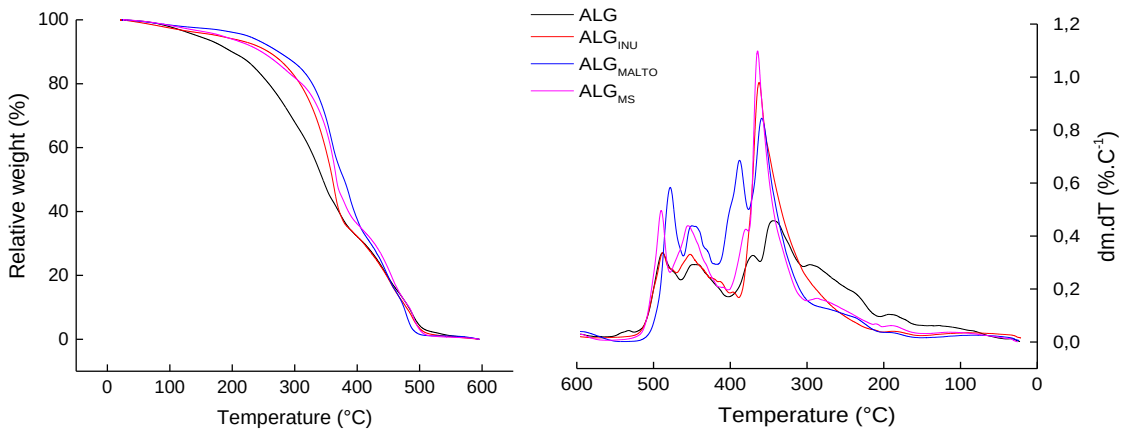

Fig. 2. Thermogravimetric (TG; left) and derivative TG (right) profiles of ALG-beads.

Table 3. Thermal parameters – initial (T_{onset}) and final degradation temperatures (T_{offset}) and residual mass at 600 °C – of ALG-beads as influenced by different wall materials.

Sample	T_{onset} (°C)	T_{offset} (°C)	R_{600} (%)
ALG	**161**	**411**	**0.3**
ALG_{INU}	**209**	**393**	**0.2**
ALG_{MALTO}	**217**	**416**	**0.1**
ALG_{MS}	**198**	**464**	**0.1**

In general, the DTG curve for the microparticles showed three defined mass loss stages. The first occurred between 50 and 150 °C and corresponds to the removal of water, which is physically bound, and the loss of volatile components with low molar mass. The second stage occurred between 180 and 250 °C may be related to the thermal degradation of the carbohydrates resulting from the depolymerization of the polymer chains, the release of CO_2, H_2O, and CO; degradation of the soybeans oil and GPE, and the formation of sodium carbonate (Sampaio et al., 2019b). The third stage (T> 400 °C) was related to the combustion of aromatic carbon residues (Gorrasi, Bugatti, & Vittoria, 2012).

The thermal behavior of the beads was modified after the addition of complementary wall materials. ALG was thermally stable up to 161 °C, while the microparticles were thermally stable up to 198 °C. This increase on T_{onset} may suggest that the addition of the complementary wall materials increased the molecular interactions between adjacent alginate chains, and strengthened the hydrogen bonding between alginate, inulin, starch, maltodextrin and soybean

oil hydroxyl groups and also with the polar compounds (polyphenols) found in the anthocyanins of GPE.

These results demonstrate the importance of using additional wall materials in the ultrasonic gelation of grape peel extract to increase the thermal stability of the microparticles, a relevant fact because many foods are subjected to heat treatments to improve their conservation.

3.5.2 Accelerated storage stability - kinetic parameters

Findings about the stability of anthocyanins provide important information for their use as a natural food coloring. Among the factors that estimate the stability of anthocyanins, temperature and humidity are considered very important during the processing and storage of the product (Furuta & Neoh, 2021). Table 4 show the kinetic parameters under accelerated storage conditions for all samples, respectively.

Table 4. Kinetic parameters data for LGPE, ALG, ALG_{INU}, ALG_{MALTO} and ALG_{MS} under accelerated storage conditions (60°C; UR=30%).

Sample	**k (x 10^{-2}) ($days^{-1}$)**	**$t_{1/2}$ (days)**	**R^2**
LGPE	5.4	13	0.90
ALG	3.2	21	0.89
ALG_{INU}	2.8	24	0.91
ALG_{MALTO}	2.5	26	0.92
ALG_{MS}	1.7	39	0.90

The degradation of anthocyanins followed a first order kinetics ($0.89 < R^2 < 0.92$). Both k and $t_{1/2}$ values were dependent on the complementary wall materials used to make the microparticles. In general, treatments with the addition of complementary wall materials showed longer half-life ($t_{1/2}$) and lower degradation rates (k), when compared to LGPE. This behavior of the grape extract was expected, since the phenolic compounds are fully exposed and are thermally sensitive, favoring the degradation mechanisms (Hoyos-Leyva et al., 2018).

Therefore, it can be seen that the encapsulated samples exhibited a better protective effect under accelerated stability conditions. Among the complementary materials studied, inulin presented the highest k and $t_{1/2.}$ This effect can be explained by its higher degree of polymerization, influencing the greater ability to absorb water on its surface and, thus, promoting an increase in the amount of water molecules and favoring the mechanisms of degradation of phenolic compounds, such as hydrolysis (Etzbach et al., 2020). Maltodextrin is less hygroscopic than inulin and has less free water on its surface, promoting greater stability to degradation reactions. Also, maltodextrin has a higher degree of dextrose equivalent (DE)

and the microparticles tend to be denser, making it difficult for oxygen and moisture to enter, for example (Iturri et al., 2021).

ALG_{MS} was the treatment that showed the lowest k and highest $t_{1/2}$. This can be explained by the presence of the hydrophobic group octenyl succinate in the structure of the modified starch, thus hindering the absorption of water on the surface of the microparticle, preserving more effectively the phenolic compounds (Inada et al., 2015). Similar results were reported by Romero-Hernandez et al. (2021) and Etzbach et al. (2020).

3.5.3 Accelerated storage stability – color

Color is an important parameter to know and can influence the quality and the acceptability of a food product (Tonon et al., 2010). The total color variation ΔE* and visual color of the beads are presented in Fig 3. The colorimetric parameters L*, a*, b*, C* and h* are also shown in Table 5.

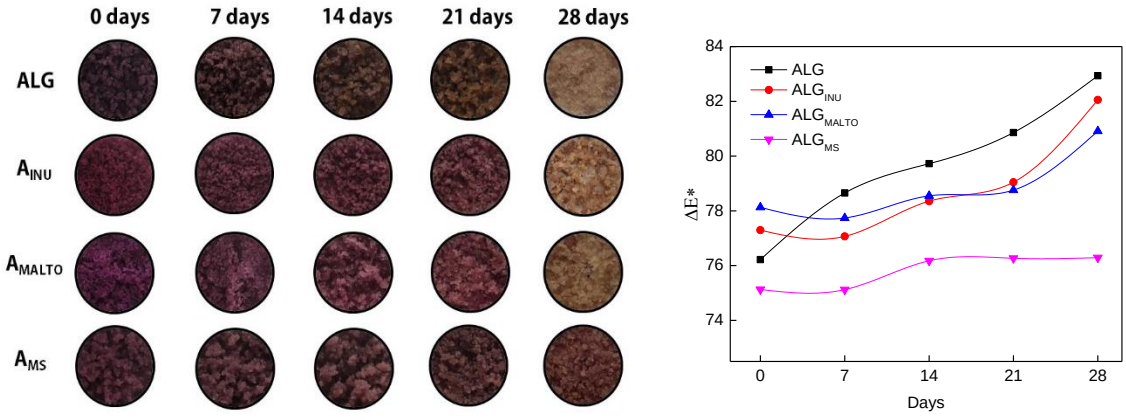

Fig. 3. Visual aspect of the beads and color variation ΔE* under accelerated storage conditions.

Table 5. Colorimetric parameters for ALG, ALG_{INU}, ALG_{MALTO} and ALG_{MS} under accelerated storage conditions (60°C; UR=30%).

		Period			
Sample	**Parameter**	**7 days**	**14 days**	**21 days**	**28 days**
ALG	L*	$8,54 \pm 0.01^{a}$	$4,45 \pm 0.02^{b}$	$4,33 \pm 0.03^{c}$	$2,75 \pm 0.01^{d}$
	a*	$2,76 \pm 0.04^{a}$	$1,72 \pm 0.01^{b}$	$1,53 \pm 0.04^{c}$	$1,24 \pm 0.03^{d}$
	b*	$1,78 \pm 0.03^{d}$	$2,46 \pm 0.01^{c}$	$2,96 \pm 0.01^{b}$	$3,03 \pm 0.02^{a}$
ALG_{INU}	L*	$5,18 \pm 0.03^{b}$	$3,86 \pm 0.03^{c}$	$3,49 \pm 0.01^{d}$	$5,78 \pm 0.04^{a}$
	a*	$3,98 \pm 0.02^{a}$	$3,18 \pm 0.03^{b}$	$2,99 \pm 0.03^{c}$	$2,13 \pm 0.05^{d}$
	b*	$1,77 \pm 0.03^{d}$	$2,08 \pm 0.04^{c}$	$2,31 \pm 0.05^{b}$	$4,59 \pm 0.01^{a}$
ALG_{MALTO}	L*	$3,49 \pm 0.03^{d}$	$3,66 \pm 0.03^{c}$	$3,82 \pm 0.04^{b}$	$6,73 \pm 0.01^{a}$
	a*	$3,88 \pm 0.01^{a}$	$3,16 \pm 0.05^{b}$	$3,09 \pm 0.02^{b}$	$2,33 \pm 0.01^{c}$
	b*	$1,51 \pm 0.01^{d}$	$1,91 \pm 0.03^{c}$	$2,33 \pm 0.05^{b}$	$4,32 \pm 0.01^{a}$
ALG_{MS}	L*	$8,12 \pm 0.05^{a}$	$3,52 \pm 0.03^{b}$	$3,58 \pm 0.02^{c}$	$3,48 \pm 0.04^{b}$
	a*	$3,79 \pm 0.05^{a}$	$2,74 \pm 0.02^{b}$	$2,68 \pm 0.02^{c}$	$2,19 \pm 0.05^{d}$
	b*	$1,78 \pm 0.02^{d}$	$2,13 \pm 0.01^{c}$	$2,19 \pm 0.03^{b}$	$2,63 \pm 0.01^{a}$

$^{a-d}$ Different letters in the same line indicate a significant difference ($p < 0.05$) using the Turkey test.

Under accelerated storage conditions all the treatments had an increase in ΔE* values, indicating that the samples lost its initial characteristic coloration. This lost can be associated to the destruction of their phenolic compounds and the presence of sugars, together with proteins, which can imply in non-enzymatic browning reactions, known as the Maillard

reaction, responsible for color changes in various food products during processes that use temperature or food storage (Oliveira et al., 2021). The presence of sugars or products associated with their degradation can accelerate the anthocyanins degradation, since this reaction rate follows the rate of conversion of sugars to furfural. Furfural condense together with the anthocyanins, leading to the formation of pigments with brown coloration, called melanoidins (Moreira, Nunes, Domingues, & Coimbra, 2012).

This change in coloration of ALG_{MS} was less pronounced and can be explained by the presence of more hydrophobics groups in the chains of modified starch that can minimizes the hydrolysis, and consequently decreasing the degradation of anthocyanins, leading to a less extensive formation of compounds of brown coloration, reducing color change during storage. These results are in accordance with Barretto, Clemente, Santana, & Vasconcelo (2020) and Carmo et al. (2018). Color parameters are also in line with TAC and thermal stability.

3.6. Colorimetric response in milk samples

Milk is a highly perishable food under circumstances of poor manufacturing practices and storage conditions. Its deterioration is the result of physicochemical changes, especially in pH values. In this way, the use of natural dyes, such as anthocyanins, can be used to monitor the quality of dairy products, and provide an immediate response (color change, for example) that correlates with the physicochemical and biological properties of food (Eshaghi, Sadrabad, Jebali, Hekmatimoghaddam, & Mohajeri, 2020). Figure 4 shows the visual color change of samples under refrigerated conditions after 12 days of storage (a) and the visual color of GPE in different pH (b).

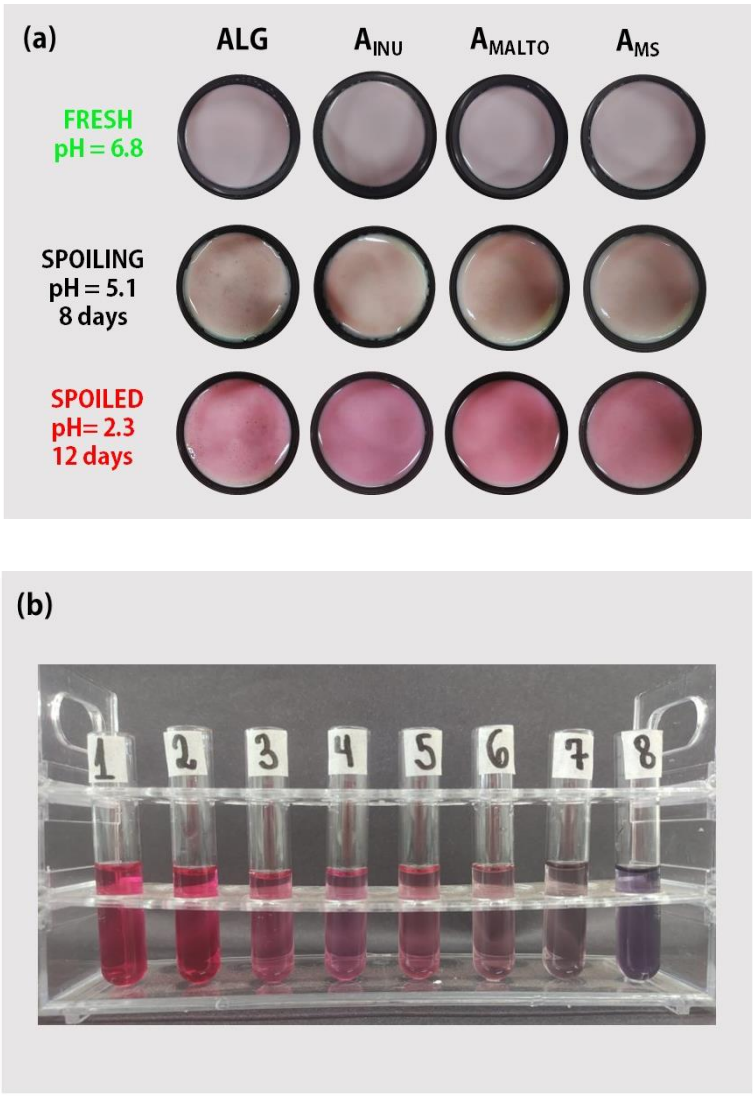

Fig. 4. Visual aspect of milk under refrigerated storage conditions (a) and visual color of GPE in different pH (1-8) (b).

According to Figure 4, it is possible to see changes in the color of all samples with the naked eye. From the eighth day, the purple color slowly started to change to rosy coloring. After twelve days of storage, along with the increase in acidity, the pH decreased from 6.8 to 2.3, due to the bacterial activity that produces lactic acid, promoting color change to intense pink, which indicated milk spoilage. This behavior of color-changing function of anthocyanins is associated

to reaction between the anthocyanins present in the microparticles and acid-base properties of the milk environment, causing structural changes. At lower pH values (pH < 2), the red/pink color caused by the flavylium cation is the predominant color. With an increase in pH (pH = 7), the color changes to a purple/blue quinoidal base (Weston et al., 2020).

Therefore, the observed color change in milk in response to lactic acid suggests that the anthocyanin-based microparticles could be used as an edible indicator, proving to be an efficient dye and also capable of monitoring milk freshness by visually tracking. It is proposed that the microparticles can be incorporated directly into the milk, in a way that is visible to consumers inspection, which enables the detection of milk spoilage without having to open the packaging. This intelligent microparticles could help aid retail and consumer decisions around food management and be a useful tool in minimizing food waste.

4. Conclusion

The incorporation of additional wall materials caused significant changes in the physicochemical properties of the microparticles. In particular, ALG_{MS} achieved high stability under accelerated and thermal conditions and greater TAC, TPC, and AA, this effect can be associated to the presence of more hydrophobics groups in the chains of modified starch compared to inulin and maltodextrin. In addition, the anthocyanin-based microparticles was able to be use to discriminate between fresh, spoiling, and spoiled milk. These findings show the possibility of using these intelligent microparticles as edible colorimetric indicators, opening space for new approaches in the food industry, allowing the consumer to easily detect with the naked eye whether the food is fit for consumption or not, in addition to being a natural coloring ingredient obtained from low-cost sources.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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