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JOANA DE BARROS ALEXANDRE

ENCAPSULAÇÃO POR GELIFICAÇÃO IÔNICA DE EXTRATO AQUOSO DE BETERRABA E DE PROBIÓTICO *Bifidobacterium animalis* BB-12

FORTALEZA – CEARÁ
2023

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Tese apresentada ao Programa de Pós-Graduação em Ciências Naturais, do Centro de Ciências e Tecnologia, da Universidade Estadual do Ceará, como parte dos requisitos para a obtenção do título de Doutora em Ciências Naturais. Área de concentração: Aproveitamento de Recursos Naturais.

Orientadora: Profa. Dr.ª Roselayne Ferro Furtado.

Coorientadora: Dr.ª Laura Maria Bruno.

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Centro de Ciências e Tecnologia
Programa de Pós-Graduação em Ciências Naturais

ATA – DEFESA DE TESE

Ata de Defesa de Tese da aluna JOANA DE BARROS ALEXANDRE. Ao oitavo dia do mês de maio do ano de dois mil e vinte e três, às 08:30 horas, reuniu-se a Banca de Defesa de Tese composta pelos Professores Doutores Roselayne Ferro Furtado, Embrapa Agroindústria Tropical, Selene Daiha Benevides, Embrapa Agroindústria Tropical, Terezinha Feitosa Machado, Embrapa Agroindústria Tropical, Luciana de Siqueira Oliveira, Universidade Federal do Ceará, e Ingrid Vieira Machado de Moraes, Embrapa Agroindústria Tropical, perante a qual JOANA DE BARROS ALEXANDRE, aluna regularmente matriculada no Curso de Doutorado em Ciências Naturais, defendeu, para preenchimento do requisito de Doutor, sua Tese intitulada "ENCAPSULAÇÃO POR GELIFICAÇÃO IÔNICA DE EXTRATO AQUOSO DE BETERRABA E DE PROBIÓTICO *Bifidobacterium animalis* BB-12". A defesa da referida Tese ocorreu, das 08:30 horas às 12:00 horas, tendo a doutoranda sido submetida à sabatina, dispondo cada Membro da Banca do tempo para tal. Finalmente, a Banca reuniu-se em separado e concluiu por considerar a doutoranda aprovada por sua Tese e sua defesa tendo, por unanimidade, recebido o conceito satisfatório, obtendo nota 9 (nove).

Eu, Roselayne Ferro Furtado, que presidi a Banca de Tese, assino a presente Ata, juntamente com os demais membros e dou fé. Em Fortaleza, 08 de maio de 2023.

Profa. Dra. Roselayne Ferro Furtado
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(Examinadora)

A Nossa Senhora de Fátima, mãe amada.

Aos meus pais, Antonio Alexandre Leitão e Maria Gessina de Barros Alexandre pelo amor incondicional.

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RESUMO

A tecnologia de encapsulação é capaz de proteger compostos ativos, como os probióticos e os pigmentos naturais, em alimentos de condições ambientais adversas, como temperatura, variação de pH, presença de oxigênio ou incidência de luz. Este trabalho teve o objetivo de encapsular o probiótico *Bifidumbacterium animalis* BB-12 e o extrato de beterraba rico em betalaínas por gelificação iônica. No que concerne aos probióticos, estes foram encapsulados por gelificação iônica externa em uma bebida de soja sabor uva, com alginato (0,75% – 1,50% m/v) e goma xantana (0 – 0,50% m/v), por meio de um delineamento de composto central rotacional. Foram avaliadas características tecnológicas como tamanho de partícula, esfericidade, viscosidade, eficiência de encapsulação, resistência ao pH e condições de armazenamento. A condição de 1,125% m/v de alginato e 0,50% m/v de goma xantana foi escolhida para o encapsulamento dos probióticos por gelificação iônica externa. A eficiência de encapsulação variou entre 50% e 87,5%. Os probióticos encapsulados foram resistentes a pH semelhante ao do estômago (pH 2) e mantiveram a viabilidade de 8 log UFC g^{-1} por 30 dias quando armazenados em bebida de soja em condição refrigerada. Quanto ao extrato aquoso de beterraba, foram avaliadas características de encapsulamento usando alginato de sódio 2% (m/v) por gelificação iônica externa e interna, e realizadas análises físicas, morfológicas e colorimétricas. As partículas apresentaram eficiência de encapsulação de 10,72% e 89,90% e capacidade de carreamento de 18,90% e 25,60%, usando as técnicas de gelificação iônica externa e interna, respectivamente. Os obtidos por gelificação interna apresentaram maior capacidade de absorção de água, enquanto os obtidos por gelificação externa apresentaram maior dureza da matriz encapsulante e menor taxa de liberação das betalaínas ao longo de 120 minutos. Assim, a escolha da técnica de gelificação iônica (externa ou interna) deve ser feita em função da matriz alimentar a ser adicionada e do objetivo de entrega das moléculas ativas encapsuladas.

Palavras-chave: Alimentos funcionais; Alginato; Goma Xantana; Extrusão; Macroesferas.

ABSTRACT

The encapsulation technology is able to protect active compounds, such as probiotics and natural pigments, in foods from adverse environmental conditions, such as temperature, pH variation, presence of oxygen or incidence of light. This work aimed to encapsulate the probiotic *Bifidumbacterium animalis* BB-12 and the beetroot extract rich in betalains by ionic gelation. With regard to probiotics, these were encapsulated by external ionic gelation in a grape-flavored soy drink, with alginate (0.75% - 1.50% m/v) and xanthan gum (0 - 0.50% m/v) using a rotational central composite design. Technological characteristics such as particle size, sphericity, efficiency, encapsulation efficiency, resistance to pH and storage conditions were evaluated in this work. The condition of 1.125% m/v of alginate and 0.50% m/v of xanthan gum was chosen for the encapsulation of probiotics by external ionic gelation. The encapsulation efficiency varied between 50% and 87.5%. The encapsulated probiotics were resistant to pH similar to that of the stomach (pH 2) and maintained a viability of 8 log CFU g-1 for 30 days when stored in soy beverage under refrigerated condition. The encapsulation characteristics of the beet aqueous extract using alginate solution 2% (m/v) by external and internal ionic gelation were evaluated, through physical, morphological, and colorimetric analyses. The particles showed encapsulation efficiency between 10.72% and 89.90% and capacity loading between 18.90% and 25.60%, using external and internal gelation, respectively. Those obtained by internal gelation showed greater water absorption capacity, while those obtained by external gelation showed greater hardness of the encapsulating matrix and lower release rate of betalains over 120 minutes. Thus, the choice of the ionic gelling technique (external or internal) must be made depending on the food matrix where will be added the spheres and the objective of delivering the encapsulated active.

Keywords: Functional foods; Alginate; Xanthan Gum; Extrusion; Macrospheres.

LISTA DE FIGURAS

Figura 1 – Representação de uma cápsula mononuclear (A) cápsula polinuclear (B) e esfera (C)..... 15
Figura 2 - Representação esquemática da gelificação iônica..... 17
Figura 3 - Estrutura da goma xantana..... 20
Figura 4 - Bactérias probióticas aderindo e produzindo agentes antimicrobianos na parede intestinal..... 22
Figura 5 - Estrutura molecular das betalaínas..... 30

LISTA DE QUADROS

Quadro 1 - Atividades potenciais recentemente relatadas na literatura de diferentes cepas de *Bifidobacterium*..... 23

Quadro 2 - Viabilidade dos probióticos encapsulados apresentados na literatura 25

Quadro 3 - Trabalho recentes sobre o encapsulamento de pigmentos naturais..... 27

Quadro 4 - Técnicas de encapsulamento de extrato de beterraba..... 28

SUMÁRIO

1	**INTRODUÇÃO**	**12**
2	**OBJETIVOS**	**14**
2.1	**Objetivo geral**	**14**
2.2	**Objetivos específicos**	**14**
3	**REVISÃO DE LITERATURA**	**15**
3.1	**Tecnologia de encapsulação**	**15**
3.1.1	Gelificação iônica	**16**
3.1.2	Materiais de parede	**18**
3.1.2.1	***Alginato***	**18**
3.1.2.2	***Goma xantana***	**19**
3.2	**Alimentos funcionais**	**21**
3.2.1	Probióticos	**21**
3.2.1.1	***Bifidobactérias***	**23**
3.2.1.2	***Alimentos probióticos***	**24**
3.2.1.3	***Viabilidade dos probióticos encapsulados***	**25**
3.3	**Pigmentos naturais**	**27**
3.3.1	Beterraba (*Beta vulgaris* L.)	**28**
3.3.1.1	***Betalaínas***	**29**
4	**ARTIGOS**	**31**
4.1	**Artigo 1**	**31**
4.2	**Artigo 2**	**50**
5	**CONSIDERAÇÕES GERAIS**	**75**
	REFERÊNCIAS	**76**
	APÊNDICES	**89**
	APÊNDICE A – SUPPLEMENTARY MATERIAL	**89**

1 INTRODUÇÃO

Os alimentos funcionais são aqueles que contêm compostos biologicamente ativos e que proporcionam impactos benéficos à saúde, a exemplo, compostos antioxidantes e probióticos (REQUE; BRANDELLI, 2021). Todavia, esses suplementos devem permanecer ativos durante o processamento e armazenamento, sendo necessárias alternativas para preservar e prolongar sua funcionalidade (MISRA; PANDEY; MISHRA, 2021).

Os probióticos são microrganismos vivos que, quando administrados em concentrações adequadas (10^6 – 10^7 UFC/ g) devem ser capazes de aderir e colonizar o trato gastrointestinal, proporcionando os benefícios específicos da cepa (GU et al., 2022). Os atributos essenciais para bactérias probióticas são sobrevivência, atividade em condições ácidas e biliares do trato gastrointestinal e capacidade de resistir ao processamento industrial de alimentos, como congelamento ou desidratação/reidratação (MOGHADAM et al., 2022).

Além dos probióticos, compostos naturais podem ser utilizados na composição de alimentos funcionais. A beterraba é uma hortaliça rica em propriedades nutricionais, antioxidantes e funcionais, sendo consumida, principalmente, na forma *in natura*, cozida ou minimamente processada. Sua coloração é decorrente do alto teor de seu principal composto funcional, as betalaínas, que são pigmentos hidrossolúveis presentes em todos os tecidos da beterraba e usados na indústria como corante de alimentos (BANGAR et al., 2022). São também compostos funcionais para a saúde humana, atuando, principalmente, na inibição da peroxidase lipídica. Todavia, as betalaínas são suscetíveis à temperatura, variações de pH, presença de oxigênio e luminosidade (RODRÍGUEZ-MENA et al., 2023).

A encapsulação é uma tecnologia que visa proteger substâncias de efeitos deletérios que ocasionam a perda de sua função. Baseia-se na proteção de um agente ativo através do condicionamento deste em um invólucro produzido por polímeros (RISEH et al., 2023). Além disso, a tecnologia de encapsulação permite mascarar sabor/ odor e promover a liberação controlada do ativo ao longo do tempo (LAGES; NICOLAS, 2023). Entre as técnicas de encapsulação, a gelificação iônica tem sido aplicada por ser simples, de baixo custo, sendo o alginato a matriz encapsulante mais amplamente utilizada, devido ao baixo custo, simplicidade e alta resistência térmica.

O mecanismo de gelificação iônica é baseado na interação eletrostática entre pelo menos um polímero e um íon ou polieletrólito. A gelificação pode ser realizada utilizando dois mecanismos diferentes: gelificação externa (GE) e interna (GI). Na GE, a solução polimérica é gotejada em uma solução rica em íons bivalentes, que interagem com os grupos

gluconato do alginato (CARVALHO et al., 2019; RAJMOHAN; BELLMER, 2019). A GI, por sua vez, ocorre quando uma solução contendo a matriz polimérica, o composto bioativo e um sal de cálcio insolúvel é gotejada em um meio composto por óleo acidificado (NARANJO-DURÁN et al., 2021). É sabido que o tipo de gelificação iônica adotado pode influenciar as características finais das partículas resultantes.

Este trabalho está dividido em dois artigos acerca da encapsulação de probiótico e do extrato aquoso de beterraba. O primeiro envolve a encapsulação do *Bifidobacterium animalis* BB-12 em bebida de soja, com alginato e goma xantana, por gelificação iônica externa, avaliando as características das macroesferas obtidas. No segundo artigo, é realizado um estudo comparativo de dois mecanismos de gelificação iônica, interna e externa, usando alginato de sódio na encapsulação do extrato aquoso da beterraba.

2 OBJETIVOS

2.1 Objetivo geral

Determinar condições para a formação de macroesferas probióticas de *Bifidumbacterium animalis* BB-12 e de extrato aquoso de beterraba por gelificação iônica, a partir do uso de alginato de sódio.

2.2 Objetivos específicos

a) Encapsular bactérias probióticas por gelificação iônica externa em bebidas de soja, utilizando alginato/goma xantana como material de parede;

b) Otimizar as proporções dos materiais poliméricos na encapsulação de probióticos e por meio de um delineamento composto central rotacional (DCCR);

c) Avaliar a viabilidade probiótica em diferentes condições de pH, ao longo do tempo e em relação ao processo de encapsulação;

d) Caracterizar as macroesferas probióticas quanto aos aspectos tecnológicos de tamanho de partícula e esfericidade;

e) Encapsular o extrato aquoso de beterraba por gelificação iônica externa e interna utilizando alginato como material de parede;

f) Caracterizar as macroesferas quanto às análises físico-químicas e de cinética de liberação das betalaínas encapsuladas.

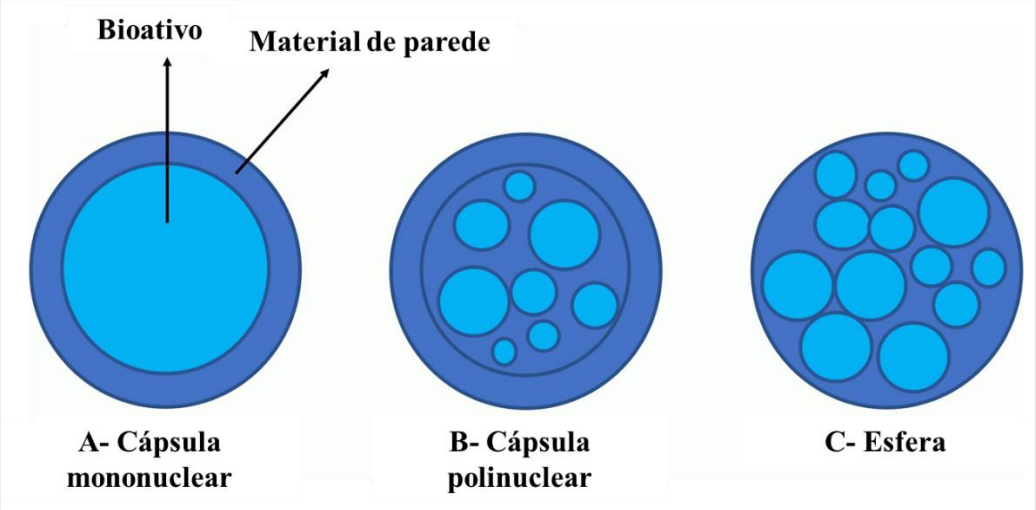
3 REVISÃO DE LITERATURA

3.1 Tecnologia de encapsulação

A encapsulação é definida como uma tecnologia de empacotamento de materiais sólidos, líquidos e gasosos em pequenas partículas, que liberam seus conteúdos durante um período controlado ou sob condições específicas (ALU'DATT et al., 2022). Assim, os componentes bioativos são revestidos por uma matriz polimérica, os quais são protegidos de fatores ambientais externos, como luz, oxigênio, umidade e calor. Além disso, a encapsulação pode mascarar sabor desagradável e coloração, preservando a vida útil dos compostos bioativos (SULTANA et al., 2022; VIVEK et al., 2023).

As partículas produzidas pelas técnicas de encapsulamento são conhecidas como cápsulas ou esferas (Figura 1). As cápsulas são do tipo reservatório, em que o núcleo é envolvido por uma membrana, podendo ter um ou vários núcleos. As esferas, por sua vez, são do tipo matriz, ou seja, o composto bioativo não está separado por um núcleo, mas espalhado por todo o volume da partícula (COMUNIAN; FAVARO-TRINDADE, 2016). Com base nos tamanhos de partícula obtidos, a tecnologia de encapsulação pode ser classificada em três tipos: nano-(<0,2 μm), micro- (0,2-5000 μm) e macro- (>5000 μm) cápsulas ou esferas (ALU'DATT et al., 2022).

Figura 1 – Representação de uma cápsula mononuclear (A) cápsula polinuclear (B) e esfera (C)



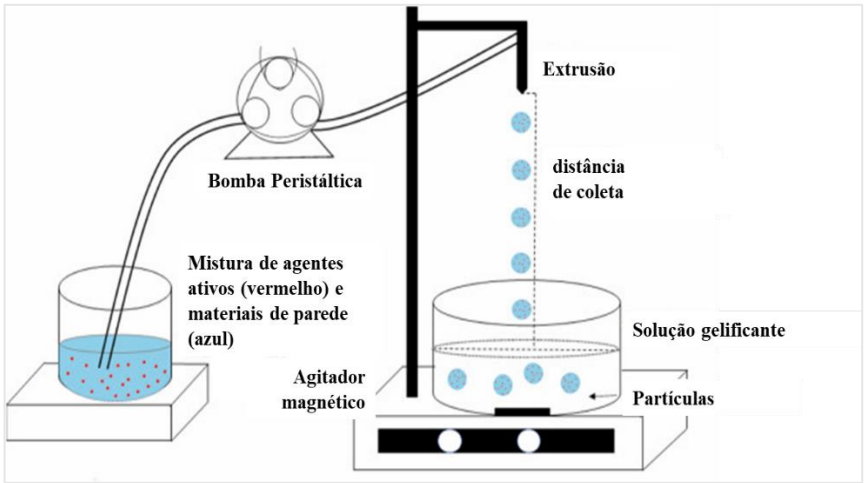
Fonte: Adaptado de Arenas, Negre e Montoya, (2020).

As técnicas empregadas no encapsulamento de compostos bioativos podem ser classificadas em processos físicos (secagem por *spray*, *spray chillin*g, precipitação de fluido supercrítico), químicos (polimerização interfacial, complexação de inclusão molecular) ou, físico-químicos (coacervação simples e complexa, gelificação iônica, lipossomas) (RIBEIRO; VELOSO, 2021; SANTOS et al., 2021). A escolha da técnica é baseada principalmente em função das características da substância de interesse, dos custos e mecanismo de liberação. A gelificação iônica é uma técnica viável, pois é considerada de baixo custo e não requer altas temperaturas e solventes orgânicos (KUROZAWA; HUBINGER, 2017; ETCHEPARE et al., 2020).

3.1.1 Gelificação iônica

A gelificação iônica é baseada na capacidade de hidrocoloides, a exemplo o alginato, tornarem-se géis em meio aquoso na presença de um íon de carga oposta, dando-se por meio da interação desses íons com os grupos químicos das cadeias poliméricas dos polissacarídeos, resultando na formação de géis insolúveis (CASTELO; SILVA; FURTADO, 2020). Quanto ao processo de encapsulação, a técnica está representada pela Figura 2.

Figura 2 – Representação esquemática do processo de encapsulação por gelificação iônica



Fonte: Adaptado de Sultana et al., 2022.

Existem dois principais tipos de gelificação iônica: gelificação externa (GE) e gelificação interna (GI). Na GE, uma solução polimérica contendo o composto ativo é gotejada em uma solução salina contendo íons de cálcio (Ca^{2+}). Neste caso, a partícula é

formada quando os íons se difundem da solução salina em direção ao hidrocoloide, sendo, portanto, considerada uma reação rápida, com a formação de partículas pouco homogêneas (CARVALHO et al., 2019; RAJMOHAN; BELLMER, 2019). A GI, por sua vez, ocorre quando uma solução contendo a matriz polimérica, o composto bioativo e um sal de cálcio insolúvel, é gotejada em um meio composto por óleo acidificado. A reação se inicia quando os íons Ca^{2+} se solubilizam no meio acidificado, reagindo com a solução polimérica e formam hidrogéis, que aprisionam o composto ativo (NARANJO-DURÁN et al., 2021). Este mecanismo é um processo lento, e à formação de uma rede de gel mais homogênea (SHU et al., 2023).

A gelificação iônica tem sido amplamente utilizada na encapsulação de compostos bioativos. Somacal et al. (2022) encapsularam óleo microbiano produzido por *Umbelopsis isabelina* por meio de gelificação iônica externa com alginato de sódio 1,5%. As micropartículas produzidas encontraram eficiência de 80%, se mostrando um método adequado para encapsulação de óleos. O estudo mostrou que a combinação da encapsulação com armazenamento adequado, aumentou a estabilidade oxidativa do óleo. Azevedo e Noreña (2021) utilizaram da mesma técnica como ferramenta para encapsular betacianinas do extrato de brácteas de *Bougainvillea glabra.* Os autores utilizaram alginato de sódio e inulina como materiais de parede, e tiveram eficiências de encapsulamento variando entre 79,28% e 89,01%.

Sánchez et al. (2017) empregaram a gelificação interna para encapsular *Lactobacillus* spp. As micropartículas de alginato protegeram os microrganismos de meios ácidos aumentando a sua viabilidade, além de manter a estabilidade sob diferentes temperaturas por 150 dias. Raddatz et al. (2022) utilizaram o extrato de resíduo da cebola roxa na co-microencapsulação de probióticos. Os autores constataram que o extrato auxilia na sobrevivência de *L. casei* microencapsulado durante a passagem pelo trato gastrointestinal. Holkem et al. (2016) encapsularam *Bifidobacterium* BB-12 em micropartículas de alginato produzidas por gelificação iônica interna, seguida de liofilização. Neste estudo, as bactérias permaneceram viáveis durante o armazenamento refrigerado (7 ºC e -18 ºC) por 120 dias, e à temperatura ambiente (25 ºC) por até 60 dias de armazenamento, com viabilidade de 7,88 log UFC g^{-1}, tendo uma eficiência de encapsulamento de 89,71%. As células bacterianas permaneceram protegidas em pH 4,5. Contudo, em pH 7,5 foram completamente liberadas.

Alguns autores apresentaram estudos comparativos entre as técnicas de gelificação iônica. Lupo et al. (2015) encapsularam o extrato de cacau com alginato de sódio e concluíram que as partículas formadas por gelificação iônica externa são mais duras do que as

preparadas por gelificação iônica interna. Neste caso, a escolha da técnica poderá ser direcionada por meio da aplicação desejada. Foi constatado que o mecanismo da gelificação tem influência no tamanho e na estrutura interna das partículas. Rajmohan e Bellmer (2019) encapsularam espirulina com alginato de sódio e perceberam que as partículas obtidas por GE apresentaram maior dureza, maior teor de proteína bruta e superfície mais homogênea quando comparado com a GI. Independentemente da técnica, o aumento da porcentagem de alginato proporcionou maior firmeza das partículas.

3.1.2 Materiais de parede

A seleção do material de parede é fundamental para a proteção do composto ativo, exercendo influência direta na eficiência de encapsulamento, na estabilidade das partículas e no grau de proteção do núcleo (TIMILSENA et al., 2019). Assim, a seleção do material de parede deve ser cuidadosa, seguindo a avaliação de alguns critérios como, a capacidade de formação de filmes, não apresentar toxicidade, não serem reativos com o material do núcleo, e proteger de fatores que afetam sua viabilidade (RODRIGUES et al., 2020; RIBEIRO; VELOSO, 2021). Além disso, a matriz deve apresentar resistência mecânica e às condições gastrointestinais (PANDEY et al., 2021).

Estudos envolvendo a encapsulação por gelificação iônica relatam com frequência o uso de alginato como material de parede (WU; ZHANG, 2018; BASU et al., 2018; ARRIOLA et al., 2019; QI et al., 2020).

3.1.2.1 Alginato

O alginato é um polissacarídeo aniônico derivado de algas marrons, composto por ácido 1–4 β- D- manurônico e ácido α- L -gulurônico. Na tecnologia de encapsulamento, o alginato é a matriz encapsulante mais amplamente utilizada. Isso é favorecido devido a baixos custos, simplicidade, alta resistência térmica, formação de matrizes suaves com cloreto de cálcio para aprisionar materiais sensíveis, ser biocompatível e biodegradável. Na encapsulação de probióticos, os alginatos apresentam-se adequados devido às suas condições de gelificação e por ser atóxico (IZNAGA et al., 2020; NAMI et al., 2020).

O alginato produz hidrogéis pela interação coordenada com cátions bivalentes. O mecanismo de gelificação é associado como modelo “caixa de ovo”, no qual os cátions,

presentes na solução gelificante, interagem com os grupos carboxílicos levando a formação de um material forte e termoestável (SHARMA et al., 2023; PAIBOON et al., 2023).

Vega-Carranza et al. (2021) estudaram o encapsulamento de *Bacillus licheniformis* em micropartículas de alginato obtidas por gelificação iônica para avaliar sua liberação controlada e direcionada em trato digestivo simulado do camarão, onde foram comprovados que, as micropartículas de alginato podem proteger as bactérias e fornecer maior concentração de probióticos no intestino dos camarões. A sobrevivência bacteriana e eficiência de encapsulamento apresentaram alta viabilidade celular e rendimento de 99%. Além disso, a avaliação da estabilidade indicou que em temperatura de armazenamento de 4 ºC as bactérias permaneceram viáveis por 15 dias, tendo sua viabilidade reduzida de 100% para 55% de sobrevivência após 30 dias de armazenamento sob a mesma temperatura. Quanto à liberação e sobrevivência, a encapsulação apresentou efeito protetor sobre as bactérias, mantendo 51,29% de probióticos viáveis no intestino do camarão, quando comparados as bactérias livres, que alcançaram 27% de viabilidade.

Apesar das diversas vantagens de aplicação, o alginato também apresenta algumas limitações, como estabilidade fraca, baixa barreira e instabilidade em relação a tratamentos térmicos (SANI et al., 2023). Tais fatores podem ser melhorados pela sua combinação do alginato com outros biopolímeros, como relatado no estudo de Carvalho et al. (2019). Os autores encapsularam extrato de antocianinas de juçara, carreadas com alginato de cálcio por gelificação iônica, combinando com o processo de complexação. usando quitosana, concentrado de proteína de soro de leite ou gelatina. Os polímeros usados como matrizes encapsulantes foram eficazes na proteção dos pigmentos durante a estabilidade de armazenamento, sendo o produto revestido com quitosana, o que apresentou maior retenção de antocianinas.

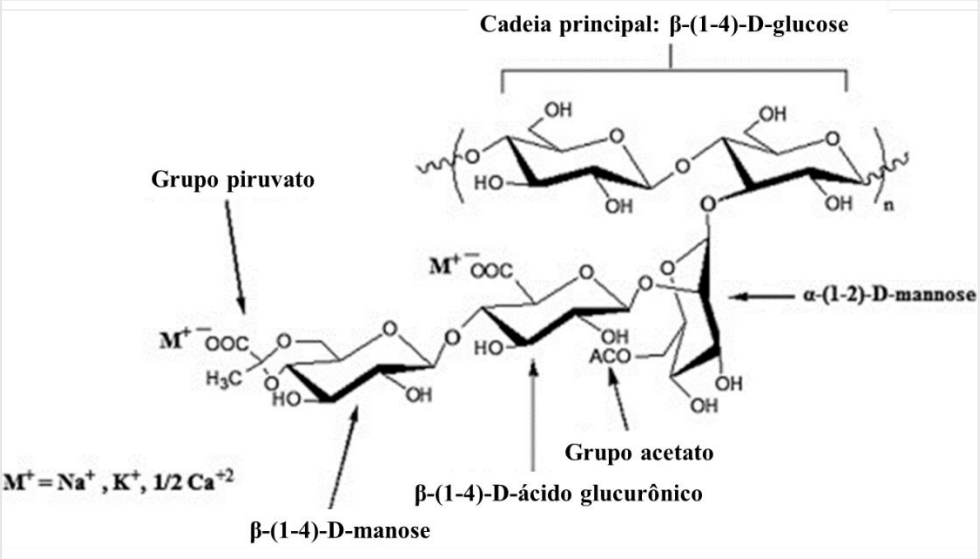
3.1.2.2 Goma xantana

Produzida pelo processo de fermentação do substrato pelo microrganismo *Xanthomonas campestris* a goma xantana (GX) é um polissacarídeo de alto peso molecular muito utilizado como espessante, emulsificante e estabilizante, além de apresentar propriedades de biocompatibilidade, biodegradabilidade e atoxicidade (BHAT et al., 2022).

A GX é um polieletrólito aniônico devido à presença de grupos acetato e piruvato em sua estrutura (Figura 3). O composto é constituído de glucano ligado a β -1,4 com cadeias laterais trissacarídicas carregadas (β-d-manopiranosil-(1,4)-α-d-glucopiranosil-(1,2)-β-d-

manopiranosil-6-O-acetato) em resíduos de esqueleto alternados (ELELLA et al., 2021; NSENGIYUMVA; ALEXANDRIDIS, 2022).

Figura 3 - Estrutura da goma xantana



Fonte: Adaptado de Elella et al., (2021).

A literatura apresenta trabalhos utilizando a goma xantana na tecnologia de encapsulação, principalmente, na estabilização de emulsões e em combinação a outros polissacarídeos. Pongjanyakul e Puttipipatkhachorn (2007) utilizaram a goma xantana para reforçar esferas de alginato de cálcio. Os autores encapsularam o fármaco diclofenaco de sódio por gelificação iônica, e perceberam que a sua eficiência de encapsulação aumentou significativamente com o aumento do percentual de goma xantana, indicando que a interação desses biopolímeros proporcionou aumento na barreira que reduz a perda do fármaco das partículas. Vega-Sagardía et al. (2018) encapsularam, por gelificação iônica, *Lactobacillus fermentum* em goma xantana e alginato. Os probióticos foram encapsulados a fim de avaliar a liberação e o comportamento frente à atividade inibitória de *Helicobacter pylori*. Os autores observaram que os probióticos encapsulados submetidos a pH 3,0 mantiveram sua atividade anti- *H. pylori* e o meio refrigerado proporcionou maiores contagens bacterianas quando comparadas à temperatura ambiente.

De Farias e Noreña (2019) utilizaram a gelificação reversa no encapsulamento de molho de soja, produto grande interesse na gastronomia molecular. A goma xantana foi utilizada como espessante e controlador difusivo no suco de soja, tendo como material de parede o alginato de sódio, e como reticulante, o lactato de cálcio. Foi constatado que, a goma

xantana teve influência no diâmetro e no peso das partículas. Apesar da goma não ter sido utilizada como material de parede, os autores sugerem que a mesmo pode ter se misturado com o alginato realizando ligações de hidrogênio, influenciando nos aspectos morfológicos das partículas.

Outros trabalhos envolvendo goma xantana combinada a outros biopolímeros relataram ainda a encapsulação de carotenoides (FU et al., 2019; BOONLAO et al., 2020); de polifenóis (TIAN; XIANG; LI, 2021); de antocianinas (JANG; KOH, 2023), e fármacos como piroxicam e cetoconazol (DIMOFTE et al., 2022).

3.2 Alimentos funcionais

A boa alimentação é cada vez mais reconhecida como fundamental para os cuidados da saúde, tendo uma relação direta na prevenção de doenças (SHARMA; RAO; SINGH, 2021). A conscientização dos consumidores ampliou o interesse em alimentos funcionais que ofereça benefícios à saúde, além do valor nutricional. Dessa forma, produtos alimentícios funcionais com adição de compostos bioativos são constantemente alvos de estudo, incluindo, especialmente, polifenóis, vitaminas, lipídios, probióticos e pigmentos (XIE et al., 2022).

3.2.1 Probióticos

As bactérias benéficas que estão presentes no sistema intestinal ajudam na qualidade de vida dos indivíduos. O sistema microbiano intestinal pode ficar desregulado devido às mudanças na ingestão de alimentos e para restaurar a homeostase microbiana intestinal, o consumo de probióticos é essencial (AREPALLY; REDDY; GODWAMI, 2020). Probióticos são definidos como microrganismos vivos que quando consumidos em quantidade adequada, conferem benefícios à saúde do hospedeiro (FAO/WHO, 2002).

Dentre os benefícios proporcionados pelas bactérias probióticas, estudos relataram: a inibição do crescimento de bactérias patogênicas, a regulação do trânsito intestinal (SILVA et al., 2020), e a redução dos níveis de colesterol (NICULESCU et al., 2019; KUERMAN et al., 2020; JITPAKDEE et al., 2021). Além disso, os probióticos apresentaram benefícios também relacionados a atividades antiobesidade (CHEN et al., 2018; WANG et al., 2020), antidiabética (CAI et al., 2019; ZHONG et al., 2020), anticancerígenas

(SABER et al., 2017; YUE et al., 2020), e de recomposição da microbiota bucal (BOSCH et al., 2012; LIM et al., 2020).

A microbiota intestinal desempenha um papel importante na defesa contra infecções. Os principais mecanismos de ação dos probióticos incluem: aumento da barreira epitelial, aumento da adesão à mucosa intestinal e inibição da adesão de patógenos, exclusão competitiva de microrganismos patogênicos, produção de substâncias antimicrobianas (Figura 4) e modulação do sistema imunológico (BERMUDEZ-BRITO et al., 2012). A literatura recomenda a porção de $10^6 – 10^7$ UFC.g^{-1} ao dia (SANI et al, 2023). Como forma de veículo, diversos estudos aplicaram probióticos em laticínios, como iogurte e leite, e produtos não lácteos, como sucos (MARINI et al., 2022; ROSA et al., 2022; USAGA et al., 2022). De acordo com Vivek et al. (2023), as propriedades gerais das bactérias probióticas incluem:

- Não patogenicidade;
- Resistência à antibióticos;
- Eficaz na produção de benefícios à saúde;
- Parte natural do sistema gastrointestinal humano;
- Seguro para consumo;
- Aderência à parede intestinal;
- Produz agentes antimicrobianos para proteção da parede intestinal.

Figura 4 - Bactérias probióticas aderindo e produzindo agentes antimicrobianos na parede intestinal

Fonte: Adaptado de Vivek et al., 2023.

3.2.1.1 Bifidobactérias

O gênero *Bifidobacterium*, pertencente ao filo Actinobacteria, é da família *Bifidobacteriaceae*. São caracterizadas como microrganismos gram-positivos, anaeróbicos, não formadoras de esporos e não móveis (LI et al., 2023a). Podem sintetizar vitaminas como riboflavina, tiamina, vitamina B6 e vitamina K, além de moléculas bioativas como, ácido fólico, niacina e piridoxina (SHARMA; WASAN; SHARMA, 2021).

Consideradas como seguras e comprovando seus potenciais em atividades ao organismo, algumas espécies e cepas de bifidobactérias usadas comercialmente foram estudadas (quadro 1).

Quadro 1 - Atividades potenciais recentemente relatadas na literatura de diferentes cepas de *Bifidobacterium*

Cepa bacteriana	Atributo funcional	Modelo	Referência
Bifidobacterium longum CECT7894	Alívio da alergia alimentar.	Camundongos BALB/c fêmeas.	CUI et al., 2023
Bifidobacterium longum Bif10 e *Bifidobacterium breve* Bif11	Melhora inflamações do cólon e ulcerações.	Camundongos balb/c machos.	SHARMA et al., 2023b
Bifidobacterium longum NSP001	Potencial no tratamento clínico da colite.	Camundongos machos C57BL/6.	CHEN et al., 2023
Bifidobacterium animalis subsp. Lactis NFBAL23	Potencial como agente anti-Salmonella neonatal.	Ratos Wistar.	LIN et al., 2023
Bifidobacterium longum R0175	Potencial em tratamento de lesão hepática.	Camundongos machos C57BL/6J.	LI et al., 2023b
Bifidobacterium animalis subsp. lactis	Retarda o movimento dentário ortodôntico.	Camundongos machos C57BL6/J.	DUFFLES et al., 2022
Bifidobacterium longum 5 1A	Potencial em atenuar lesão durante a mucosite.	Camundongos BALB/c fêmeas.	QUINTANILHA et al., 2022
Bifidobacterium longum	Regular o desenvolvimento intestinal.	Lesão celular induzida por lipopolissacarídeo (LPS).	GUAN et al., 2021
B. longum -C-CPE-PE23	Potencial para o tratamento do câncer de mama.	Camundongos BALB/c fêmeas e camundongos KSN fêmea.	SHIMIZU et al., 2020
Bifidobacterium breve CCFM1025	Efeitos antidepressivos consideráveis e reguladores da microbiota.	Camundongos machos C57BL/6J	TIAN et al., 2020

Fonte: Elaborado pela autora.

3.2.1.2 Alimentos probióticos

Os benefícios dos alimentos probióticos são alcançados quando os microrganismos chegam vivos e em quantidades adequadas ao intestino, e então, são capazes de colonizá-lo. Os produtos probióticos são costumeiramente vinculados a produtos lácteos. Entretanto, há um interesse crescente na busca de matrizes não lácteas como carreadoras de probióticos, em virtude ao aumento mundial de populações vegetarianas e veganas, bem como de pessoas com intolerância à lactose, ou alergia à proteína do leite (KUMAR et al., 2022; CASES; FRUTOS; LLAMAS, 2023).

A adição de probióticos em alimentos depende da cepa, tipo de alimento, pH e possíveis interações com outras bactérias. Torna-se necessário avaliar a segurança de produtos alimentícios contra contaminação cruzada; compatibilidade do produto com a cepa probiótica, além da viabilidade e estabilidade durante armazenamento, e as condições de fermentação (VIVEK et al., 2023).

Recentemente, diversos pesquisadores têm estudado a elaboração de novos produtos que visam alternativas de veiculação dos probióticos, acompanhando a tendência do mercado global. Cases, Frutos e Lhamas (2023) desenvolveram uma bebida simbiótica - um suco misto de cenoura e laranja, enriquecido com *Lactiplantibacillus plantarum* e inulina, o que apresentou influência no crescimento bacteriano. D' Alessandro et al. (2023), desenvolveram bebidas fermentadas de soja contendo probióticos encapsulados e não encapsulados. As cepas encapsuladas, *Lactobacillus crispatus* BC4 e *Lactobacillus gasseri*, BC9, apresentaram viabilidade estável em 7 log UFC.mL^{-1} de produto, durante os 28 dias de armazenamento e maior capacidade de retenção de água.

Usaga et al. (2022) avaliaram a sobrevivência de probióticos e a estabilidade de betalaínas em suco de pitaya roxa, uma bebida promissora com apelo funcional, e apropriada para veiculação probiótica. Lopes et al. (2020) incorporaram *Lactobacillus acidophilus* La-05 microencapsulados em leites veganos (arroz e soja). Os autores observaram que ambos são transportadores adequados de probióticos. Além disso, as culturas probióticas encapsuladas sobreviveram em contagens adequadas (> 6 log CFU/mL) quando armazenadas sob refrigeração (7 °C) por 120 dias, além de ter sua sobrevivência aumentada durante a digestão *in vitro*. Além de sucos e leites veganos, a literatura apresenta potenciais produtos de veiculação probiótica, como café fermentado (CHAN; TOH; LIU, 2021) massas (KONURAY; ERGINKAYA, 2020), maçãs desidratatas com revestimento probiótico

(AKMAN et al., 2019), banana em pó (SORNSENEE et al., 2022), e produtos cárneos (SIRINI et al., 2023).

3.2.1.3 Viabilidade dos probióticos encapsulados

É sabido que para promover os efeitos benéficos, os microrganismos probióticos devem ser capazes de aderir e colonizar o trato gastrointestinal (RODRIGUES et al., 2020). Entretanto, alguns desafios podem afetar a sobrevivência dessas bactérias, como a suscetibilidade aos fatores ambientais, como oxigênio, pH ácido, enzimas digestivas, sais biliares e a sobrevivência às operações de processamento e armazenamento após serem veiculados em alimentos (RIBEIRO et al., 2014; LOPES et al., 2020).

A tecnologia de encapsulação mostra-se eficiente e promissora na proteção de probióticos devido a formação de barreira em torno das bactérias, evitando, a exposição aos fatores deletérios, permitindo que um maior número de células viáveis chegue ao intestino (BEVILACQUA et al., 2019; POLETTO et al., 2019; LOPES et al., 2020). O quadro 2 apresenta alguns trabalhos em que a microencapsulação dos probióticos proporcionou maior viabilidade, seja em relação à estocagem ou a aplicação em alimentos.

Quadro 2 - Viabilidade dos probióticos encapsulados apresentados na literatura

Técnica	Material de Parede	Probiótico	Resultado	Referência
Coacervação Complexa	Gelatina e goma arábica	*Lactobacillus plantarum*	A viabilidade das células encapsuladas foi de 80,4% enquanto as células livres eram de 25,0%. A viabilidade probiótica foi mantida durante o armazenamento a 8 ºC e 18 ºC por 45 dias.	PAULA *et al*., 2019
Gelificação iônica e coacervação complexa	Pectina e proteína de soro de leite	*Lactobacillus acidophilus* LA -5	O iogurte probiótico com LA-5 encapsulado apresentou menor acidificação. A microencapsulação protegeu durante 35 dias de armazenamento refrigerado.	RIBEIRO *et al*., 2014
Gelificação iônica externa	Alginato e quitosana	*Lactobacillus acidophilus* LA -05	A microencapsulação aumentou a sobrevivência do probiótico durante o armazenamento refrigerado; Aumento da resistência do	LOPES *et al*., 2020

			probiótico à temperatura, pH e NaCl; Aumento da sobrevivência do probiótico durante a digestão *in vitro* de leites veganos.	
Gelificação iônica interna	Pectina + prebióticos	*Lactobacillus acidophilus* LA -05	As micropartículas adicionadas dos diferentes prebióticos apresentaram melhor proteção ao microrganismo.	RADDATZ *et al.*, 2020
Gelificação iônica	Alginato	*Lactobacillus casei*	A microencapsulação melhorou a estabilidade térmica e a sobrevivência celular na digestão e armazenamento.	IZNAGA *et al.*, 2020
Gelificação iônica	Proteína de soro de leite + alginato	*Lactobacillus bulgaricus*	Proteção aos probióticos sob condições gastrointestinais simuladas. Melhora da sobrevivência durante o armazenamento.	CHEN *et al*., 2017
Gelificação iônica	Alginato + goma persa + prebióticos	*Lactococcus lactis*	Viabilidade em condições digestivas simuladas foi maior ou igual a 61% em comparação com as células livres. Alta estabilidade de armazenamento em suco de laranja durante 6 semanas.	NAMI *et al*., 2020
Gelificação iônica	Alginato + quitosana + prebióticos	*Lactobacillus acidophilus TISTR 1338*	Proteção aos probióticos quando submetidos aos processos de liofilização e aquecimento.	JANTARATHIN, BOROMPICHAICHARTKUL, SANGUANDEEKUL; 2017
Gelificação iônica	Alginato	*Lactobacillus plantarum NCDC201 e L. casei NCDC297*	Melhora significativa na capacidade de sobrevivência ao suco gástrico simulado e a tratamentos térmicos.	RATHER *et al*., 2017
Electrospray	Alginato + quitosana + prebióticos	*Lactobacillus plantarum*	As bactérias permaneceram viáveis após 90 dias incorporadas em sorvete.	ZAEIM *et al*., 2020

Fonte: Elaborada pela autora.

3.3 Pigmentos naturais

Os pigmentos são compostos químicos visíveis ao olho humano em cores variadas, e que proporcionam benefícios à saúde, apresentando altos níveis de atividade antioxidante, além de serem necessários para a proteção (OLIVEIRA FILHO et al., 2022; ROCHA et al., 2023). Diante disso, os pigmentos são constantemente utilizados como aditivos alimentares para proporcionar coloração ou melhorar a aparência dos produtos alimentícios, manter a qualidade sensorial, e aumentar a aceitabilidade do produto (NABI et al., 2023).

Clorofilas, carotenoides, antocianinas e betalaínas são classes de corantes naturais que contribuem com tonalidades de cores nos alimentos. Apesar de vantajosos em aplicações de alimentos em substituição aos sintéticos, os pigmentos apresentam alguns desafios, como a baixa estabilidade em pH, temperatura, luz, mas que podem ser melhorados com a tecnologia de encapsulamento, podendo ser protegidos contra condições ambientais, preservando seus compostos bioativos (GHOSH et al., 2022). O quadro 3 apresenta trabalhos recentes acerca da encapsulação de pigmentos.

Quadro 3 - Trabalhos recentes sobre o encapsulamento de alguns pigmentos naturais

Técnica	Material de Parede	Pigmento	Referência
Coacervação complexa	Isolado de proteína de soja e quitosana	Clorofila de espinafre	AGARRY et al., 2022
Coacervação complexa	Maltodextrina combinada com carboximetilcelulose, goma arábica, goma xantana	Antocianinas de *Aronia melanocarpa*	JANG; KOH, 2023
Liofilização	Leveduras	Antocianinas de *Aronia melanocarpa*	KUREK et al., 2023
Gelificação iônica	Alginato e pectina	Antocianinas de uva	NORCINO et al., 2022
Liofilização	Maltodextrina	Antocianinas e ácidos fenólicos de *Prunus spinosa* L.	BLAGOJEVIC et al., 2022
Spray drying	Galactomananas	Bixina	PASCOAL et al., 2021
Liofilização e *spray drying*	Maltodextrina combinada com pectina e cera	Óleo de linhaça enriquecido com carotenoides	ELIK; YANIK; GOGUS, 2021
Coacervação complexa	Proteína de soro de leite e goma arábica	Suco de cenoura	PARENTE et al., 2021
Coacervação complexa	Gelatina e goma arábica	Casca de guaraná e probiótico	SILVA et al., 2022

Fonte: Elaborado pela autora.

3.3.1 Beterraba (*Beta vulgaris* L.)

A beterraba, originária da Ásia e da Europa, pertencente à família *Chenopodiaceae (*TUTUNCHI et al., 2019), é considerada uma das raízes mais populares para consumo humano, podendo ser ingerida cozida, *in natura*, em saladas ou sucos, e possui elevado aspecto nutricional. Apresenta excelentes atividades antioxidante e anti-inflamatória, por possuir compostos bioativos, como polifenóis, carotenoides, betalaínas, betaínas e nitratos. Além disso, apresenta vitaminas (A, K, C, E, B2, B3, B6) e minerais (sódio, potássio, fósforo, cálcio, ferro, zinco) (CHHIKARA et al., 2019), que a caracterizam como alimento funcional.

Diversos estudos relataram outras potenciais atividades do extrato aquoso da beterraba. Nade et al. (2015) avaliaram o potencial neuroprotetor na doença de Parkinson, sendo o mecanismo de proteção, relacionado à presença de antioxidantes. Rehman et al. (2021) evidenciaram também a atividade terapêutica da beterraba em doenças neurodegenerativas. Albrahim e Alonazi (2020) estudaram a influência do suco de beterraba na hepatotoxicidade induzida em ratos machos, confirmando seu potencial em atividade hepatoprotetora. Gheith e El- Mahmoudy (2018) forneceram resultados que evidenciaram o efeito hematopoiético do extrato aquoso da folha de beterraba no modelo de anemia induzida em ratos albinos.

Visando preservar os compostos bioativos da beterraba, que são susceptíveis à diversas condições ambientais, estudos apresentam técnicas de encapsulação como alternativa viável de proteção (quadro 4).

Quadro 4 – Técnicas de encapsulamento de extrato de beterraba

Técnica	Composto ativo	Resultados	Referência
Spray drying	Extrato de beterraba	A estabilidade das betalaínas depende da atividade de água em que são armazenadas. O pó armazenado em menores atividades de água apresentou a maior estabilidade.	PITALUA et al., 2010.
Spray drying	Extrato de beterraba	As microcápsulas de goma arábica e pigmentos de beterraba foram estáveis por maior período, devido à menor higroscopicidade em comparação com as microcápsulas de maltodextrina.	JANISZEWSKA, 2014
Gelificação iônica externa	Extrato das folhas de beterraba	As cápsulas de alginato permaneceram intactas na simulação gástrica, preservando o composto bioativo. O inchaço da rede de alginato ocorreu na simulação intestinal, o que permitiu a liberação de compostos bioativos dos extratos de folhas de beterraba.	GORBUNOVA et al., 2018.

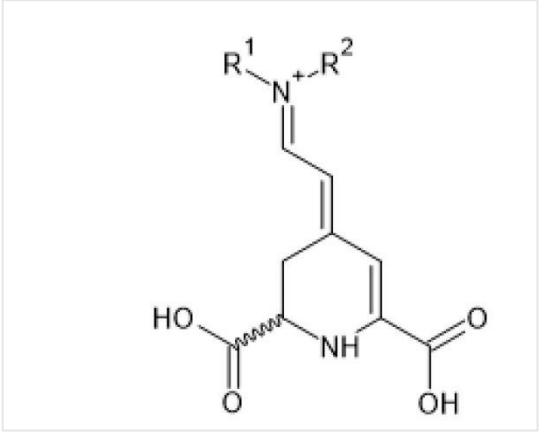
Spray drying	Extrato de beterraba	Amostras preparadas com mucilagem de chia e maltodextrina apresentaram maior carga de betacianina após *spray drying* do que amostras preparadas apenas com maltodextrina ou maltodextrina e goma arábica. As microcápsulas dispersas em soluções aquosas com diferentes temperaturas e pHs. apresentaram boa capacidade de proteção do corante.	ANTIGO et al., 2020
Coacervação complexa	Extrato da beterraba vermelha	A beterraba encapsulada pelo complexo goma persa e quitosana apresentou alta eficiência de encapsulação (90%) estabilidade térmica.	NAMAZZADEH; EHSANI; GHASEMPOUR (2022)
Gelificação iônica	Extrato de caule de beterraba	O encapsulamento das esferas reduziu a perda da capacidade antioxidante ao longo da digestão.	AGUIRRE-CALVO et al., 2022
Spray drying	Probiótico (*L. Plantarum*) com extrato aquoso do caule da beterraba vermelha.	O extrato de caule de beterraba não afeta a sobrevivência de *L. plantarum* LP01 sob condições de refrigeração e congelamento. A estabilidade das microcápsulas por três meses é assegurada a baixas temperaturas.	DE DEUS et al., 2023

Fonte: Elaborado pela autora.

3.3.1.1 Betalaínas

As betalaínas (Figura 6) são pigmentos nitrogenados, hidrossolúveis, encontrados em frutas, flores, raízes e folhas de algumas plantas, sendo a beterraba uma importante fonte dessas substâncias. São derivadas do ácido betalâmico, a qual são divididas em dois grupos: betaxantinas de coloração amarelo alaranjada (absorbância em torno de 480 nm) e betacianinas vermelho-púrpura (absorbância em torno de 530 nm) (NABI et al., 2023).

Figura 5 - Estrutura molecular das betalaínas



Fonte: Adaptado de Oliveira Filho et al., 2022.

Diversos fatores afetam a estabilidade das betalaínas, sejam eles intrínsecos ou extrínsecos. Os fatores intrínsecos incluem a estrutura química e a presença de enzimas dos tecidos vegetais. Quanto aos extrínsecos, incluem a temperatura acima de 50 ºC quando as betalaínas degradam-se rapidamente. A variação de pH pode degradar as betalaínas em valores maiores que 8 e menores que 3. A presença de oxigênio causa reações de oxidação, e a incidência de luz, que favorece a reatividade dos compostos (CARREÓN-HIDALGO et al., 2022).

No entanto, quando todos esses fatores são controlados, as betalaínas apresentam potencial antioxidante, agregando valor aos produtos aplicados (RODRÍGUEZ-MENA et al., 2023). A literatura apresenta a encapsulação como uma tecnologia interessante para melhorar a estabilidade das betalaínas (OTÁLORA et al., 2016; OTÁLORA et al., 2018; OTÁLORA et al., 2019; FATHORDOOBABY et al., 2021).

4 ARTIGOS

4.1 Artigo 1

Application of probiotic (*Bifidobacterium animalis* BB-12®) macrospheres in soy beverage: functional and technological evaluation

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Highlights

- Higher concentrations of xanthan gum or alginate caused higher encapsulation efficiency and viscosity.
- Macrospheres stored in soy drink juice showed a viability of 8 log CFU g^{-1} for 30 days.
- Encapsulated probiotics showed resistance at different pH values.

Abstract

Some conditions may affect probiotic survival, and encapsulation technology is an alternative for their protection and delivery. This work had the objective of encapsulating the probiotic *Bifidobacterium animalis* BB-12 in soy-based beverages and evaluating the viability of microorganisms under different conditions. Macrospheres were obtained by dissolving sodium alginate and xanthan gum in a grape-flavored soy drink and cross-linked with calcium lactate by external ionic gelation. The alginate (0.75% - 1.50%, m v^{-1}) and xanthan gum (0% - 0.50%, m v^{-1}) concentrations were evaluated in this work for probiotic viability, encapsulation efficiency, particle size, sphericity, and dynamic viscosity. The condition of alginate 1.125% m v^{-1} and xanthan gum 0.50% m v^{-1} was selected for formation of the probiotic spheres by external ionic gelation. The encapsulated probiotics showed resistance at different pH values (2 to 5). The storage conditions of the macrospheres in soy beverage showed a viability of 8 log CFU g^{-1} for 30 days.

Keywords: Encapsulation; Xanthan Gum, Alginate, Bifidobacterium BB-12, soy drink.

1. Introduction

Studies show that a diet rich in probiotics can boost the immunity of the human body (immunomodulation) and effectively inhibit the growth of pathogenic bacteria to ensure the normal functioning of organisms (Marchesin et al., 2018). Moreover, are several show the benefits of prophylactic use of probiotics in different types of cancer and cancer-associated side effects (Vinceković et al., 2017; Juan et al., 2022; Liu et al., 2023). Recently, significant advances have been observed in the field of probiotics area, with the application of these microorganisms in the area of functional foods and nutraceuticals (Campos et al., 2019; Morais et al., 2019; Nguyen et al., 2019). Probiotics are classified as live microorganisms that, when administered in adequate amounts, 10^6-10^7 CFU g^{-1}, cause benefits to the host. They are from the genera *Lactobacillus, Bifidobacterium, Lactococcus, Streptococcus* and *Enterococcus* (Vinceković et al., 2017; Paludo et al., 2021). However, probiotics must survive gastrointestinal transit, which is a challenge for the food industry since temperature and oxygen variations can influence cell survival.

Soy-based foods have a stimulating effect on the growth of some bacterial strains, such as those of the *Bifidobacterium* genus, because these foods contain oligosaccharides that are used as a nutritional source of probiotics (Rasika et al., 2021). The fermentation process

with bacterial cells increases protein content, improving protein solubility, amino acid composition and availability, and optimizing probiotic viability over time (Bastida et al., 2023). Moreover, soybean drinks can be a good source of plant proteins and are rich in isoflavones, which promote health benefits such as the prevention of osteoporosis and cardiovascular diseases (Xu et al., 2022).

Since probiotics must survive gastrointestinal transit and adhere to and colonize the intestinal mucosa, encapsulation technology may be an alternative to protect probiotics (Vivek et al., 2023). External ionic gelling is an encapsulation technique in which an aqueous polymeric solution interacts with oppositely charged ions, reacting and forming an insoluble gel (Kurozawa & Hubinger, 2017). It is an attractive technique because it is low cost and not use organic solvents or high temperatures. Among the available biopolymers, studies report that alginate is one of the most commonly used in the encapsulation of probiotics (Tan et al., 2022; Yuan et al., 2022; Li et al., 2023) due to its biocompatibility and not toxicity. Alginate is a natural polymer extracted from brown seaweed and is capable of gelling when divalent cation ions (for example, Ca^{2+}) bind to the gluconate blocks of alginate chains. The gluconate blocks of a polymer then form junctions with the gluconate blocks adjacent to the polymer chains called the egg-box crosslinking model, resulting in a stable gel structure. However, this material can be improved by combining with other polymers, increasing the stability of the particles (Ta et al., 2021).

Xanthan gum is an extracellular polysaccharide produced by Xanthomonas spp. during aerobic fermentation. It is used as a stabilizing and emulsifying agent in the food and pharmaceutical industries (Cofelice et al., 2023) This polymer can be used to reinforce calcium alginate granules (Pongjanyakul & Puttipipatkhachorn, 2007). The application of two polymers as a wall material in adequate proportions provides greater resistance to the medium and can delay the release of substances.

This study proposed encapsulating *Bifidobacterium* BB-12 with alginate (AG) and xanthan gum (XG) and adding it to grape-flavored soy beverages to provide better condition of probiotic viability.

2. Materials and Methods

2.1 Materials

Bifidobacterium animalis BB-12 strains are from Chr. Hansen. Sodium alginate (MW: 405.21 g mol^{-1}) and calcium lactate were obtained from Scientific Exodus. Xanthan

gum (MW: 933.75 g mol^{-1}), sodium citrate and hydrochloric acid were purchased from Chemical Dynamics. Grape-flavored soy drink was purchased in local supermarkets.

2.2 Preparation of probiotic inoculum for encapsulation

The probiotic culture of *Bifidobacterium animalis* BB-12 was grown in three steps in MRS broth (from Man, Rogosa and Sharpe) with 0.1% (v v^{-1}) cysteine 10% (m v^{-1}) at 37 °C for a total of 56 hours. Each step was performed under anaerobic conditions. Then, the cells were centrifuged at 5.509 x g for 15 min at 5 °C and washed with a phosphate buffer solution (1.0 M, pH 7.4). Cells were resuspended in 10% maltodextrin solution (m v^{-1}) and 10% sucrose (m v^{-1}) to obtain a 10 log CFU mL^{-1} solution. Finally, the suspension was aliquoted (1 mL) in Eppendorf tubes, frozen at -80 °C, and used directly in the production of macrospheres.

2.3 Formation of probiotic macrospheres by external gelation

The process of encapsulation was performed according to Tanganurat (2020), with adaptations. Alginate and xanthan gum were mixed in 100 mL of soy beverage until complete dissolution at approximately 60 °C. After cooling to room temperature (25 °C), 1 mL of the probiotic culture (10 log CFU mL^{-1}) was added to the polymeric solution and homogenized. The mixture was extruded dropwise into a 1% calcium lactate solution (m v^{-1}) and stirred for 2 min on a magnetic stirrer. The macrospheres were washed with distilled water to remove excess salt and stored at 4°C in falcon tubes.

2.4 Experimental Design

A rotational central composite design (RCCD) was used (Table 1) for the formation of probiotic macrospheres, the independent variables were AG and XG (%, m v^{-1}), while the response variables were probiotic viability, encapsulation efficiency, particle size, sphericity, and dynamic viscosity. The design consisted of 11 treatments with four factorial points at two levels (2^2), four axial points and three central points.

Table 1. Coded values and uncoded values (real experimental values) of the independent variables, Alginate and Xanthan Gum, according to the RCCD.

	Coded Values		Decoded Values	
Treatment	**Alginate**	**Xanthan Gum**	**Alginate (%, m.v^{-1})**	**Xanthan Gun (%, m.v^{-1})**
T1	-1	-1	0.86	0.15
T2	+1	-1	1.39	0.15
T3	-1	+1	0.86	0.43
T4	+1	+1	1.39	0.43
T5	-1.41	0	0.75	0.25
T6	+1.41	0	1.50	0.25
T7	0	-1.41	1.125	0
T8	0	+1.41	1.125	0.50
T9	0	0	1.125	0.25
T10	0	0	1.125	0.25
T11	0	0	1.125	0.25

2.5 Characterization of probiotic macrospheres

2.5.1 Viscosity of polymeric solutions

The viscosity of the polymeric solutions was analyzed using a HAAKE MARS III rheometer (Thermo Scientific). The polymeric solution was loaded into the conical plate geometry (C60° Ti L) with a 0.052 mm gap at 25 °C. The samples were conditioned for 1 min at 25 °C to recover the structure, and the readings were performed in 2 min each at a frequency of 0.5000 Hz.

2.5.2 Encapsulation Efficiency

To assess the EE of the probiotic was used a spectrophotometer (model Anthos Zenith 200rt). Inoculum standardization curve was created by relating the absorbance measurements with probiotic counts on MRS - cysteine agar plates. The standardization curve is represented in Equation 1.

$$\text{CFU} = 0.0099 \ln(x) - 0.0861; \quad R^2 = 0.90 \qquad \text{(Equation 1)}$$

The encapsulation efficiency (EE) was calculated from the results obtained in the number of probiotic cells using Equation 2.

$$EE = \frac{N_0}{N} \; x \; 100 \qquad \text{(Equation 2)}$$

Where: N_0 is the number of cells after encapsulation and N is the number of cells before encapsulation.

2.5.3 Particle size distribution and sphericity

Particle size was determined by measuring the transverse and longitudinal diameters of 20 spheres with a digimatic micrometer (Mitutoyo). The average size was calculated using the Ferret diameter (Zanetti et al., 2002), according to Equation 3.

$$TM = \frac{d+D}{2} \qquad \text{(Equation 3)}$$

Where: TM is the average size; d is the smallest diameter of an inscribed circle; and D is the largest diameter of the circumscribed circle, both concerning the most significant cross-section of the particle.

The degree of sphericity of the particles was determined using Riley method (Riley, 1941), according to Equation 4.

$$\Phi_0 = \sqrt{\frac{d}{D}} \qquad \text{(Equation 4)}$$

Where: Φ0 is the sphericity; d is the smallest diameter of an inscribed circle; and D is the largest diameter of the circumscribed circle, both in relation to the largest cross section of the particle.

2.6 Viability of free and encapsulated probiotics

The viable probiotic count was determined by plating on MRS-cysteine agar. One gram of the microparticles was dispersed in 9 mL of 2% sodium citrate solution at 50 ± 1 °C to completely dissolve the macrospheres. 0.1 mL of the material was inoculated plates were incubated at 37 °C ± 1 °C for 48 h. To evaluate the influence of the soy drink, samples of probiotic macrospheres in water as a control were tested under the same conditions.

2.7 Viability of encapsulated probiotics at different pH values

The effect of pH on probiotic survival was evaluated according to Silva et al. (2018), with adaptations. Solutions containing 2% sodium citrate (m v^{-1}) were adjusted to pH 2, 3, 4, and 5 using 2 M hydrochloric acid. Approximately 1 g of probiotic macrosphere was added to 9 mL of each solution. Free and water-encapsulated probiotics were used as controls. Probiotic resistance was evaluated by enumeration for 5 min and after 1 h of exposure to pH. Probiotics were counted according to item 2.6.

2.8 Evaluation of probiotic viability during storage

The survival of encapsulated *Bifidobacterium animalis* BB-12 was evaluated for 0, 2, 5, 10, 15 and 30 days at 8 °C and stored in reverse osmosis water (pH 7.0), in juice (pH 3.9) and without liquid. Probiotics were accounted for in item 2.6.

2.9 Statistical analysis

The response surface and analysis of variance were performed using the Statistica® software (StatSoft version). The regressions were evaluated for their determination coefficients (R^2) and statistical significance ($p < 0.05$).

3. Results and discussion

3.1 Probiotic macrospheres formation

During the formation of spheres by ionic gelation, the sodium alginate interacts with the divalent ions present in the crosslinking solution. These ions bind to the carboxyl groups of mannuronic acid and glucuronic acid present in alginate (Noor et al., 2022). Despite forming a strong gel, alginate spheres can have a porous structure, facilitating the diffusion of some molecules inside or outside them and affecting the viability of the encapsulated material (Li et al., 2023). This problem can be solved with the incorporation of another biopolymer into the polymeric solution, such as xanthan gum.

Alginate is made up of mannuronic and guluronic acid, while xanthan gum contains glucuronic acid. The intermolecular interaction between these acids and the constituent hydroxyls of the polysaccharides influence the increase in intermolecular interactions, making the structure of the macrospheres more compact (Cai et al., 2019). Nsengiyumva & Alexandridis (2022) observed that the performance of xanthan gum is based on its macromolecular conformation. In aqueous media, xanthan gum undergoes

conformational transitions from helix to random spiral in response to some stimuli such as pH, ionic strength and temperature (Nsengiyumya & Alexandridis, 2022).

In Table 1 are found the polymer proportions used for the eleven treatments of the rotational central composite design. The response variables of encapsulation efficiency, viscosity, particle size and sphericity were studied in this work, but according to ANOVA only viscosity and encapsulation efficiency were significant ($p < 0.05$) (supplementary material). Despite the significance found previously, it was not possible to establish a mathematical model for these variables, as the model fit was lower than 90% (supplementary material). Thus, the trends for the best conditions found in all response variables were considered in this work.

3.2 Characterization of probiotic macrospheres

3.2.1 Encapsulation Efficiency

The EE represents the efficiency of the wall materials to hold or encapsulate the materials of interest within the particle (Apiwattanasiri et al., 2022). In this study, the macrospheres presented EE ranging between 50.0% and 87.5% (Figure 1B). A response surface plot for EE has been provided to represent better the trends of the best conditions found in this work. The treatments showed that higher concentrations of xanthan gum can achieve higher EE using a lower amount of alginate. The reverse behavior was also observed; using higher concentrations of alginate, a lower amount of xanthan gum can be used.

Under these conditions, there is a greater availability of active sites in the chains of alginate to bind to Ca^{2+}, resulting in a greater degree of crosslinking. Similar results were found by Farahmand et al. (2022). The authors encapsulated probiotics with alginate by ionic gelation and complexed with chitosan and observed that by increasing the alginate concentration (and, consequently, its viscosity), higher EE values were obtained in the study. According to the authors, when there is a lower viscosity of the polymeric solution, the extrusion dripping movement is facilitated, and a high leakage occurs in the gelation step and plays a role in reducing the EE. Furthermore, alginate and xanthan gum form intermolecular forces through hydrogen bonds (Wen et al., 2022) between the hydroxyl groups of xanthan gum and the carboxyl groups of sodium alginate.

(A) **(B)**

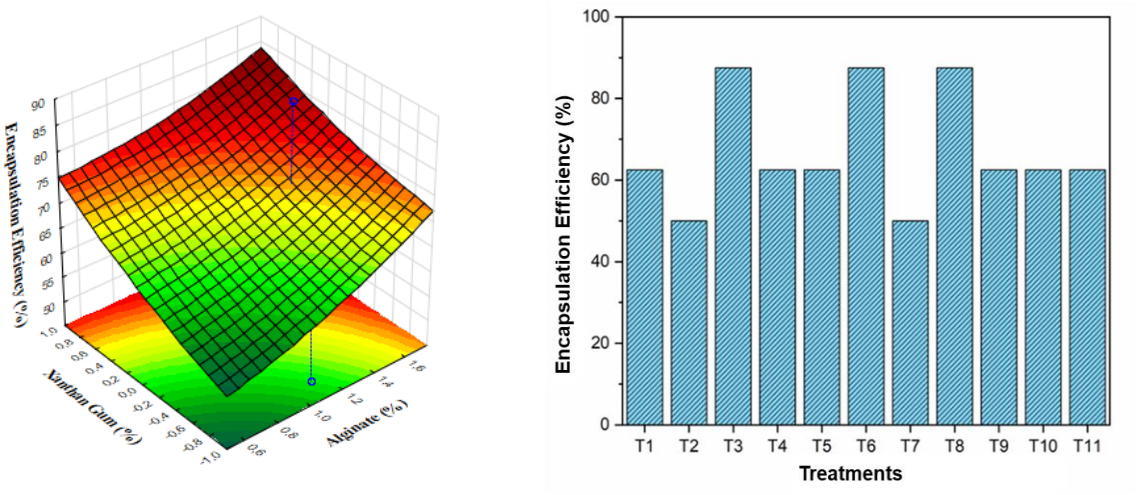

Figure 1. (A) Response surface with decoded values of alginate and xanthan gum for encapsulation efficiency. **(B)** Experimental response for efficiency.

3.2.2 Particle size distribution and sphericity

Particle size can affect the solubility, storage stability and release of core materials (Jang & Koh, 2023). The particle size varied between 4.25 ± 0.15 mm and 5.57 ± 0.25 mm, and the sphericity varied between 0.94 ± 0.04 and 0.98 ± 0.01 (Table 3). In this work, it was observed a tendency of the smallest values of size and greater dispersion were related to lower levels of xanthan gum and alginate, although had not been found a significant difference ($p > 0.05$).

These results are directly related to the viscosity of the treatments. In the conditions in which the polymeric solution had higher viscosity, there was a tendency for larger uniformity between the particles obtained. Sphericity closer to 1.0 is considered a perfect sphere and plays a key role in preventing bacterial overgrowth in encapsulated granules (Tanganurat, 2020).

Table 3. Experimental responses for Particle size, sphericity of RCCD.

Treatment (AG+XG)	Particle size (mm)	Sphericity
T1 (0.86+0.15)	4.25 ± 0.15	0.97 ± 0.02
T2 (1.39+0.15)	4.63 ± 0.09	0.96 ± 0.02
T3 (0.86+0.43)	4.65 ± 0.27	0.94 ± 0.04
T4 (1.39+0.43)	4.89 ± 0.25	0.97 ± 0.02
T5 (0.75+0.25)	5.12 ± 0.26	0.96 ± 0.04
T6 (1.50+0.25)	4.97 ± 0.11	0.98 ± 0.01
T7 (1.125+0)	5.57 ± 0.25	0.95 ± 0.03
T8 (1.125+0.50)	5.46 ± 0.15	0.97 ± 0.02
T9 (1.125+0.25)	5.39 ± 0.20	0.97 ± 0.02
T10 (1.125+0.25)	5.30 ± 0.16	0.97 ± 0.02
T11(1.125+0.25)	5.30 ± 0.19	0.98 ± 0.02

3.2.3 Viscosity of polymeric solutions

The combination of polysaccharides is a strategy to create functional materials with synergistic properties and low cost (Kondaveeti et al., 2022), providing the formation of a complex matrix that can help in the viability of probiotics (Bekhit et al., 2016). However, a study of the proportion of each polymer needs to be realized to achieve the maximum potential of the combination.

There was a significant difference between the viscosity parameter ($p \leq 0.05$) of the polymeric solutions of alginate and xanthan gum. The dynamic viscosity varied between 212.4 Pa.s (T3) and 1670.8 Pa.s (T8), (figure 2). These values were higher with the increase in the percentage of xanthan gum, which can be explained by hydrogen bonds between gum and alginate.

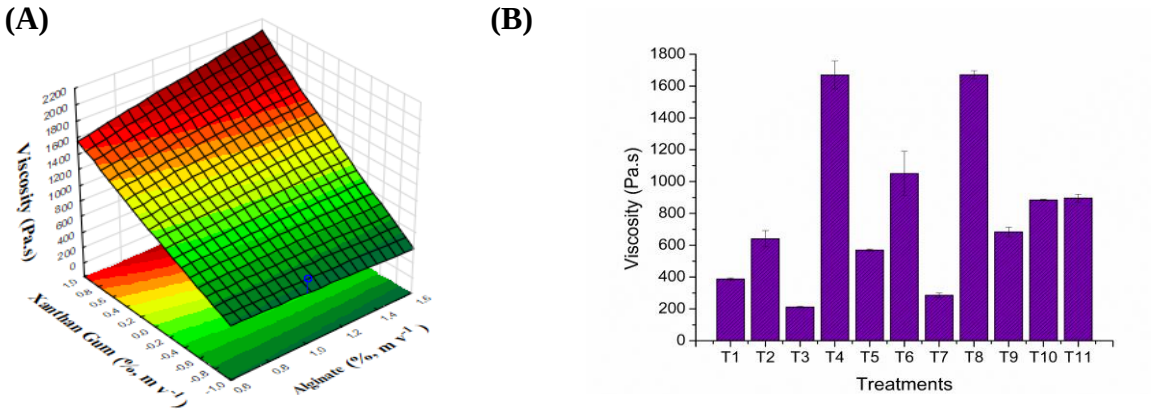

Figure 2. (A) Response surface with decoded values. **(B)** Experimental response for viscosity.

During encapsulation, the treatments that presented lower viscosities, and therefore, more fluids, were easily dripped into the reticulant. However, there was greater heterogeneity of the macrospheres, while those with higher viscosities were dripped more slowly, generating greater homogeneity of the macrospheres. Khoshdouni Farahani et al. (2023), by encapsulating jujube extract with alginate and gellan gum, noticed that the increase in the total solids content of alginate and gellan generated greater viscosity of the drink since these biopolymers increase the binding capacity with water and reduce the flow rate, forming a stronger network of gels.

Through the results found previously, the T8 treatment (alginate 1.125% m v^{-1} and xanthan gum 0.50% m v^{-1}) was chosen as the best condition for the macrospheres. This treatment showed good EE, viscosity and homogeneity in particle size and sphericity, exhibiting attractive sensory characteristics for applicability in new probiotic food products.

3.3 Resistance of encapsulated probiotics at different pH values

From the chosen treatment, the encapsulated probiotics added to soy beverage and to water at different pH values were broken and counted in plates. Figure 4 shows the effect of pH on the survival of encapsulated and free probiotics.

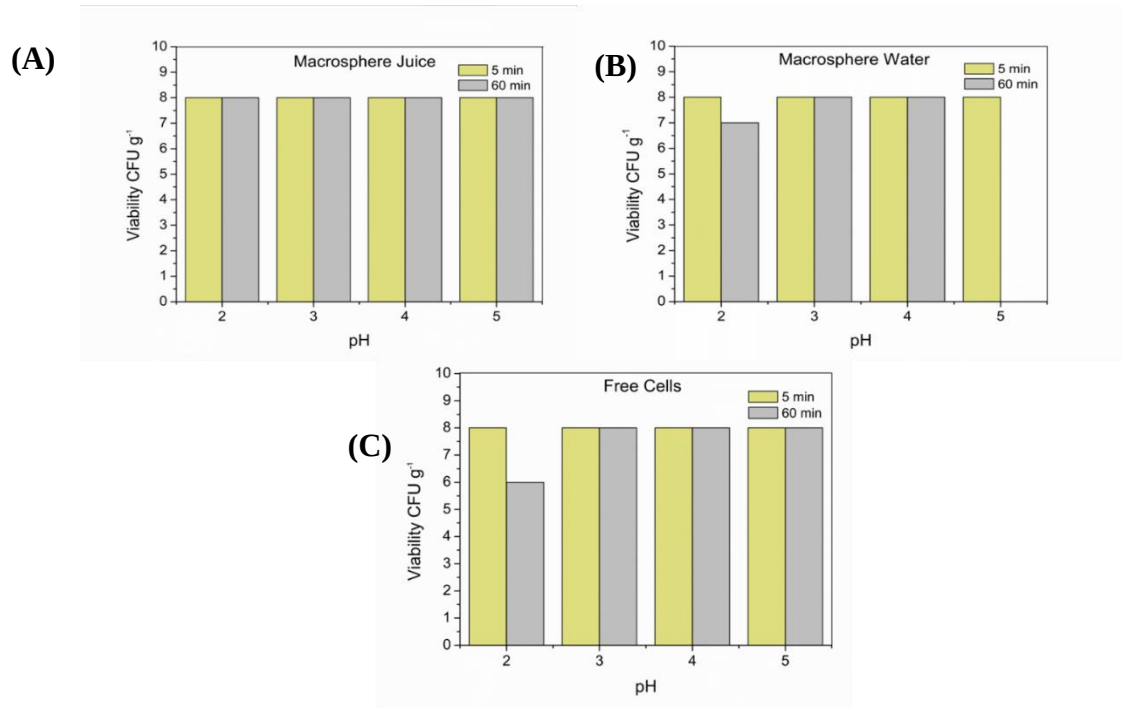

Figure 4. Probiotic viability in different pH conditions of encapsulated and free cells at times of 5 and 60 minutes. (A) Juice macrosphere; (B) Water macrosphere; (C) Free cells.

After 5 minutes, it was possible to verify that the macrospheres of juice, water and free cells presented similar behavior, with a viability of 8 log CFU g^{-1} (Figure 4). After 1 hour of immersion in different pH values, the juice macrospheres were preserved and maintained their viability at 8 log CFU g^{-1}. However, the free cells underwent a loss of 2 log CFU g^{-1} at pH 2, while the water macrospheres lost 1 log CFU g^{-1}, indicating that acidic conditions, encapsulation was able to promote greater cell protection. Studies of Chotiko & Sathivel (2016) reported the influence of encapsulation on probiotic survival in acidic conditions. The authors encapsulated *Lactobacillus plantarum* with rice bran and pectin by ionic gelation and observed a reduction of 1.0 log CFU g^{-1} in free cells subjected to pH 3.0, while encapsulated cells showed a smaller reduction (0.5 log CFU g^{-1}), indicating that the wall materials were effective in protecting the probiotics.

After 1 h in pH 5, the water macrospheres swelled and released the bacterial cells. This occurs because when the samples are above the pKa values of mannuronic acid (pKa = 3.38) and glucuronic acid (pKa = 3.65) (Wongverawattanakul et al., 2022), which are the building blocks of alginate, there is a disorder in the calcium-alginate structure (Dalponte Dallabona et al., 2020). At higher pH, when the carboxyl groups are in the COO- form, the presence of the negative charge causes repulsion of the alginate polymer chain, causing polymer swelling and, consequently, facilitating the release of the encapsulated compound (Camacho et al., 2019). The opposite happens when alginate carboxyl groups are in the COOH form when the pH is below the pKa, generating an increase in the strength of hydrogen bonds.

3.4 Evaluation of probiotic survival during storage

The viability of the probiotics encapsulated in soy juice and in water (control) under storage in their respective media (juice or water) and in dry conditions was carried out to evaluate the best storage conditions for the probiotic macrospheres (Figure 5).

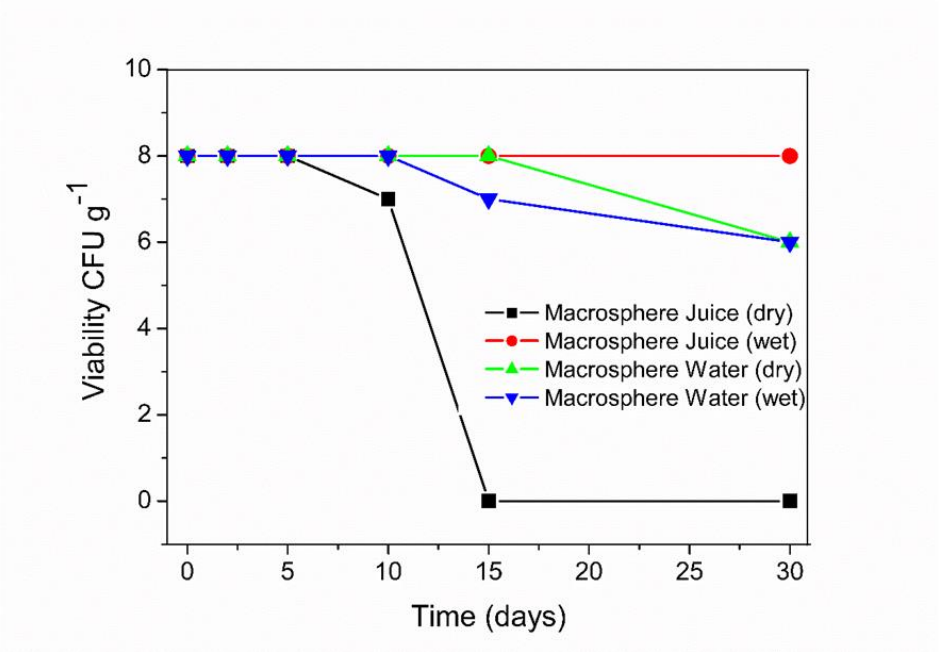

Figure 5. Probiotic viability over time. Where, juice macrospheres in dry medium; juice macrospheres stored in juice; water macrospheres stored dry and water macrospheres stored in water.

Our studies found that for 10 days, all storage conditions had viable probiotics (7 log CFU g^{-1} and 8 log CFU g^{-1}). After 15 days, probiotics encapsulated in soy and dry stored lost their viability. After 30 days of storage, soy macrospheres stored in juice maintained bacterial cells at 8 log CFU g^{-1}, indicating bacterial preservation. At the same time, after 30 days, the water macrospheres reduced the bacterial concentration, reaching 6 log CFU g^{-1}. The loss of 2 log CFU not impaired probiotic action since it still fell within the indicated amount per day.

In this work, by 30 days the probiotic macrospheres formed in soy juice and storage in this medium was the best condition found for the viability of the microorganisms. Similar results were reported by other authors. D'Alessandro et al. (2023) developed fermented soy beverages containing encapsulated and nonencapsulated probiotics. The viability of the strains remained at approximately 7 log CFU mL^{-1} of product from beginning to finish time. This finding complies with the literature, as lactic acid bacteria can reach 8-9 log CFU g^{-1} of viable cells in soy drink without the need to add other carbohydrates. Studies by Cui et al. (2021) reported the application of probiotic BB-12 in soymilk yogurt and milk yogurt of cow, and the probiotic count in soy yogurt was significantly higher, confirming our findings. This occurs because most Bifidobacteria has the enzyme α-galactosidase, capable of

fermenting raffinose (Cui et al., 2021), present in high levels in soy drinks, so this probiotic strain can use raffinose as a source of carbohydrate, preserving its viability over time. According to Kumari et al. (2022), soy beverage is an affordable and appropriate medium for growth and intended as a suitable vehicle for probiotics.

4. Conclusion

The combination of xanthan gum and alginate proved to be efficient in the encapsulation and viability of probiotics *Bifidobacterium animalis* BB-12. The chosen treatment for formation of the spheres was 1.125 g mL^{-1} alginate and 0.5 g mL^{-1} xanthan gum. For this treatment, the morphological characteristics of particle size and sphericity were homogeneous, with potential future food applications. In addition, the macrospheres were efficient in protecting the probiotics in acidic conditions close to those of the stomach. The probiotics encapsulated and stored in juice were viable for 30 days, showing that the soy beverage was important in the viability of the probiotics for a longer period.

CRediT authorship contribution statement

Joana de Barros Alexandre: Methodology, Investigation, Analysis, Validation, Writing – original draft. **Luana Carvalho da Silva:** Investigation, Validation, Writing – original draft. **Rachel Menezes Carvalho:** Investigation, Analysis, Writing – original draft. **Gabrielle Albuquerque Freire:** Investigation, Analysis, Writing – original draft. **Tiago Linhares Cruz Tabosa Barroso:** Investigation, Writing – original draft. **Kelvi Wilson Evaristo Miranda:** Investigation, Writing – original draft. **Adriano Lincoln Albuquerque Mattos -** Investigation, Analysis. **Laura Maria Bruno -** Conceptualization, Supervision, Resources. **Roselayne Ferro Furtado:** Conceptualization, Supervision, Resources, Project administration, Funding acquisition, Writing - review & editing.

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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4.2 Artigo 2

Particles of beetroot extract (*Beta vulgaris* L.) obtained by internal and external ionic gelation: A comparative study

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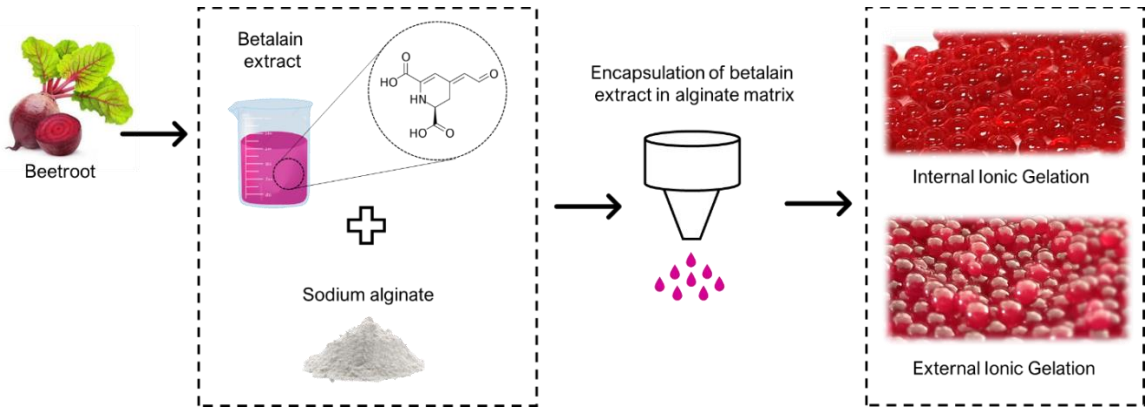
Graphical abstract



Highlights

- The mechanism of ionic gelation influences the final characteristics of the particles;
- The internal ionic gelation generated particles with higher encapsulation efficiency and rehydration capacity;
- Particles generated by external ionic gelation had higher hardness.

Abstract

Natural pigments, such as betalains present in beets, are sensitive to environmental conditions, accelerating the reactivity of compounds. This sensitivity makes it difficult for the food industry to apply pigments. Microencapsulation is an alternative to enable the delivery of these compounds, as it protects the active agent through a polymeric matrix. The aim of this study was to compare two ionic gelation mechanisms, external (EG) and internal (IG) in the encapsulation of beet aqueous extract. The particles were obtained by mixing sodium alginate with the aqueous extract of beetroot and crosslinking with sodium chloride solution using the extrusion method. Encapsulation characteristics and physical, morphological, and colorimetric analyses were evaluated. The particles showed 10.72% and 89.90% encapsulation efficiency for EG and IG, respectively. The loading capacity was of 18.90% in the EG and 25.60% in the IG. For rehydration, those obtained by IG had a greater capacity to absorb water. Texture analysis indicated that the EG particles showed greater hardness. The release kinetics indicated that the EG particles followed the Korsmeyer-Peppas model, while the IG particles followed the Higuchi model. In conclusion, the encapsulation technique must be selected depending on the food matrix to be added, and the objective of delivering the active molecules encapsulated.

Keywords: Alginate; Encapsulation; Controlled release.

1. Introduction

Beetroot (*Beta vulgaris* L.) is rich in betalains compounds, water-soluble pigments containing nitrogen, which are characterized by their high antioxidant activity and have potential applications in the industrial field, food, cosmetic, or pharmaceutical (Rodríguez-Félix et al., 2022; Yang et al., 2021). Betalain derivatives can be classified as betacyanins (red–violet color) and betaxanthins (yellow-orange color) (Celli and Brooks, 2017; Luiza Koop et al., 2022).

Natural food dyes and pigments, such as betalains, have poor thermal stability and may be unstable in the presence of oxygen and light (Li et al., 2022). In addition, beetroot betalains have a limitation related to their earthy taste (Luiza Koop et al., 2022). Given this, encapsulation technology is an alternative to protect bioactive compounds from adverse conditions and mask unpleasant flavors/odors since the encapsulating agent forms a protective barrier with the encapsulated compound, extending the shelf-life of substances and the release controlled under specific conditions (Alexandre et al., 2019; Barroso et al., 2021).

Among the encapsulation techniques, ionic gelation is used to obtain particles from nano to macro sizes and exhibits advantages over many others because it is simple, low cost, and does not require high temperatures or organic solvents (Kurtulbaş et al., 2022; Otálora et al., 2018). In gastronomy, the ionic gelation technique is commonly called basic spherification (external ionic gelling) and reverse spherification (internal ionic gelation). These are used to prepare caviar, ravioli, and pasta and can be used in teas and other beverages.

In this study, beetroot particles obtained by external ionic gelation (EG) and internal gelation (IG) techniques were compared to their characteristics. EG occurs from the interaction of the dripped polymeric solution in an ionic solution, for example, calcium chloride, under constant agitation. The compound to be encapsulated is mixed with the polymeric solution, and the drops that reach the ionic solution form spherical gel structures (Da Silveira Cáceres de Menezes et al., 2015; Kurozawa and Hubinger, 2017). On the other hand, IG produces particles from an insoluble calcium salt, for example, calcium carbonate, which will be dispersed in a polymeric solution containing the active agent. The solution is dripped into a medium with acidified oil where Ca2+ ions are released and, consequently, the crosslinking of the alginate (Kurozawa and Hubinger, 2017). Studies have reported that the encapsulation of hydrophilic compounds by IG can be an alternative to avoid diffusion to the crosslinking solution, increasing the encapsulation efficiency and promoting the formation of a more homogeneous gel (Belščak-Cvitanović et al., 2016; Kurozawa and Hubinger, 2017a; Wenjuan Wang, Rui Sun, 2023).

In this work, sodium alginate was chosen as the polymer to encapsulate betalain. Alginate is a polysaccharide normally extracted from the cell wall of brown algae and widely used in ionic gelling techniques due to its biodegradability, filmogenic properties, non-toxicity, non-interaction with the active principle and, in the presence of bivalent cations, such as $Ca^{2+,}$ alginate undergoes a transition from solution to gel (Dodero et al., 2019; Kurozawa and Hubinger, 2017; Kurtulbaş et al., 2022; Noor et al., 2022).

This study aimed to encapsulate the betalains in beetroots extract using the ionic gelation technique. The performance of the particles formed by both EG and IG was evaluated and compared to encapsulation efficiency, morphology, mechanical properties, pH resistance, and release kinetics.

2. Materials and Methods

2.1 Materials

Beetroot and canola oil were purchased at a local market in Fortaleza. The chemical reagents: alginate, acetic acid, citric acid, sodium citrate, calcium chloride, and calcium carbonate were purchased from Dinâmica Química Contemporânea Ltda (São Paulo, Brazil).

2.2 Extraction of compounds from beetroot

The beetroots were sanitized, cut with peels, and added to water in a ratio of 1:2 (m/v). Then, they were crushed in a blender, and the mixture was adjusted to pH 5.5 with 0.2 M citric acid. Afterward, the solution was submitted to ultrasound with a 22 mm diameter probe (Hielscher Ultrasonics HmnH, model UP 400S, 24 kHz) for 20 minutes. Finally, the extract was frozen in an ultrafreezer (Sanyo VIP, Temperature Freezer Model MDF-U33V,) at -85 °C for freeze-drying (CHRIST, model 1-8 LSC basic). The dry mass obtained was ground in a knife mill and stored in propylene bags away from light.

2.3 Formation of particles by external and internal gelation

The particles were obtained by external ionic gelation (EG), according to Castelo et al. (2020), with adaptations. For encapsulation, 10 mL of alginate solution 2% (m/v) was mixed with 3.3 mL of aqueous beet extract (1:20) (m/v) to obtain a final concentration of 1.5% (v/v). The solution was inserted into the encapsulator (Büchi, model B-395, Essen, Germany) with a 120 µm drip nozzle, flow rate of 5 mL/min, and frequency of 1800 Hz. The solution was dropped into a calcium chloride solution of 2% (m/v). Then, the microparticles were washed with distilled water to remove excess salt.

The methodology to obtain particles by internal ionic gelation (IG) was adapted from Basu et al. (2018). For encapsulation, 13 mL of alginate solution 2% (m/v) was mixed with 3.3 mL of aqueous beet extract (1:20) (m/v) and 1 mL of calcium carbonate 3.6% (m/v) to obtain a final concentration of 1.5% (v/v). The solution was inserted into the encapsulator

(Büchi, model B-395,) with a 120 µm drip nozzle, a flow rate of 5 mL/min, and a frequency of 1800 Hz. The solution was dropped into 50 mL of canola oil with 80 µL of glacial acetic acid PA. Then, the microparticles were washed with distilled water to remove excess oil.

2.4 Evaluation of encapsulation of beetroot extract

2.4.1 Encapsulation efficiency

The encapsulation efficiency (EE) of microparticles was determined by weighing 0.5 g of particles and immersing them in 25 mL of sodium citrate solution 3% (m/v) according to Silva et al (2022) with adaptations. The samples were stirred for 10 minutes on a magnetic stirrer and then read on a spectrophotometer (Cary 50 Conc, California). The values were expressed in betalains from the sum of the concentrations of betacyanins (535 nm) and betaxanthins (490 nm). For quantification, standard curves were made for betacyanins (y= 0.0005x + 0.0153; R^2 = 0.99) and betaxanthin (y= 0.0003 + 0.0212; R^2 =0.99) of the beetroot extract.

$$EE= \left(\frac{Tb}{Ti}\right) x100 \qquad \text{(Equation 1)}$$

Where: *Tb* is the total betalain content, representing the amount of betalain recovered after particle rupture; *Ti* is the initial content of betalains used in encapsulation.

2.4.2 Loading capacity

Loading capacity (LC) can be defined as the amount of active ingredient loaded per unit mass of carrier particle. The (LC) was determined by weighing 0.5 g of microparticles, and LC was calculated from Equation 2.

$$LC = \left(\frac{mb}{mt}\right) x100 \qquad \text{(Equation 2)}$$

Where: *m*b is the mass of the active ingredient (betalain) and *m*t is the total mass of the particle (active ingredient + carrier material).

2.4.3 Colorimetry

The color parameters L*, a* and b* of EG and IG microparticles in the presence and absence of light in 24 h intervals were measured using a digital colorimeter (Konica MINOLTA, model CR-400) calibrated with a white calibration plate. The result obtained was the average of the tests performed in quintuplicate (Equation 3).

$$\Delta E: (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2} \quad \text{(Equation 3)}$$

Where: ΔL* is the difference in luminosity between the samples and Δa* and Δb* are the differences in red and yellow colors, respectively.

2.5 Morphological and mechanical characterization of particles

2.5.1 Optical morphology, particle size and sphericity

The particles size was determined by images obtained with an optical microscope (Zeiss, model Axio Imager A2). Micrographs were captured from random samples of five particles. Measurements of transverse and longitudinal particle diameters were performed and the average size was calculated using the Ferret diameter (Zanetti et al., 2002), according to Equation 4.

$$TM = \frac{d+D}{2} \quad \text{(Equation 4)}$$

Where: TM is the average size; d is the most minor diameter of an inscribed circle; and D is the largest diameter of the circumscribed circle, both concerning the most significant cross-section of the particle.

The degree of sphericity of the particles was determined using the Riley method (Riley, 1941) according to Equation 5.

$$\Phi_0 = \sqrt{\frac{d}{D}} \quad \text{(Equation 5)}$$

Where: Φ0 is the sphericity; d is the smallest diameter of an inscribed circle; and D is the largest diameter of the circumscribed circle, both in relation to the largest cross section of the particle.

2.5.2 Determination of mechanical properties

Texture analysis of microcapsules was performed as described by Deladino et al. (2008), with adaptations. Seven grams of particles were weighed and placed in a petri dish with a diameter of 50 mm to fill the entire surface, forming a single layer. A texturometer (Stable Micro Systems Ltd., model TA-TX2i) was used with 30 kg load cell. A probe of 0.5 mm diameter was used to perform compression with a test speed of 1 mm/sec and 2 g trigger. The results were expressed in Newtons (N).

2.5.4 Swelling analysis

The swelling degree (SD) of the microparticles after drying was determined according to the methodology proposed by Xu et al. (2003). The total mass of the initial sample (M_i) was quantified in a Gooch filter, and this was immersed in distilled water for 24 h. Excess water was removed, and the final wet mass (M_f) was determined. The swelling degree was calculated based on the initial mass of the sample, according to Equation 6.

$$SD = \frac{Mf - Mi}{Mf} \qquad \text{(Equation 6)}$$

Where: SD is the swelling degree, Mf is the final wet mass and Mi is the initial total mass.

2.5.5 Resistance to different pH values

The resistance of the microparticles was determined at pH from 2, 3, 4, 5, 6 and 7. Each sample was immersed in 25 mL of water, and the pH was adjusted with 0.1 M hydrochloric acid or 0.1 M sodium hydroxide. Then, the reading was realized in a spectrophotometer (Cary 50 Conc, California) at wavelengths of 490 nm for betaxanthins and 535 nm for betacyanins.

2.6 Release study and kinetic evaluation

The release was performed varying the times from 0 to 120 minutes. A total of 0.5 g of microparticles was added to 25 mL of water. An aliquot of the solution was removed at different times for reading in a spectrophotometer (Cary 50 Conc,) at wavelengths of 490 nm for betaxanthins and 535 nm for betacyanins.

For the assessment of the release mechanism, the data were fitted to mathematical models that describe zero-order, first-order, Higuchi and Korsmeyer-Peppas kinetics (Eqs. 7, 8, 9, and 10) as defined by Silva et al. (2022):

Zero order: $Q = Q_0 - K_0\, t$ (Equation 7)

First order $lnQ = lnQ_0 - K_1\, t$ (Equation 8)

Higuchi: $Q = K_H\, t^{1/2}$ (Equation 9)

Korsmeyer-Peppas: $Q = K_{KP}\, t^n$ (Equation 10)

Where: Q is the amount of oil released at time t, Q_0 is the initial amount of oil in the solution, n is the diffusion exponent indicative of the transport mechanism of the active ingredient, and K_0, K_1, K_H, and K_{KP} are release rate constants for the zero-order, first-order, and kinetic models of Higuchi and Korsmeyer-Peppas, respectively.

2.7 Statistical analysis

Analysis of variance (ANOVA) with repeated measures was used to evaluate statistically significant variables and their interactions. Using Tukey's test ($p \leq 0.05$), significant differences between the samples were determined. Statistica® software was used for the statistical analysis (version 10.0, StatSoft Inc.).

3. Results and discussion

3.1 Evaluation of beetroot extract encapsulation

3.1.1 Encapsulation efficiency

EE and LC are two essential parameters to evaluate in the encapsulation process. High EE values indicate a significant amount of the active compound in the microparticle. The encapsulation efficiency values varied between 10.72% and 89.90% for the EG and IG techniques, respectively. It was possible to observe that the betalain content of the EG particles was quickly diffused into the crosslinking solution, since there is a difference in concentration between the aqueous solution and capsules. However, for lipophilic compounds EG presents better encapsulation efficiency than IG, since it reduces interaction with the environment (Somacal et al., 2022). In addition, the particles produced by EG have a porous gel structure, which allows the quick and easy diffusion of water and other fluids, inside and

outside the matrix particle (da Silva Carvalho et al., 2019; Naranjo -Durán et al., 2021; Somacal et al., 2022). Our results suggest that IG can be an alternative for encapsulating hydrophilic compounds, to minimize or prevent their diffusion towards the crosslinking solution, since the encapsulation process occurs in the oil phase. Thus, a higher EE is observed in these cases (Li et al., 2023).

Particles obtained by EG and IG presented LC of 18.90% and 25.60%, respectively. The literature reports that loads >50% have disadvantages in encapsulation, since it reduces the protection of the active material facilitating its release into the media (Shaddel et al., 2018). Consequently, lower values for LC offer more excellent protection and are related to particle morphology and size (Calderón-Oliver et al., 2017).

3.4.2 Colorimetry

The colorimetry of the particles was performed as a function of time and under two different storage conditions (with and without exposure to ambient light) (Table 1). The colorimetric data were obtained using the CIELab system, which allows evaluation of the parameters L* (brightness/brightness), a* (red/green coordinate) and b* (yellow/blue coordinate). From data obtained it was possible to evaluate the total color difference (ΔE) using the initial time (0 h) based on the equation 3.

The L*, a*, and b* values of the EG and IG particles at 0 and 24 h, in the conditions with and without exposure to light are represented in Table 1. There was little color change in either evaluated conditions, although the time was short. This indicates that at the time assessed there is no perceptible evident color change of the particles with the betalain. This is a promising result, because it is one of the premises of the encapsulation of anthocyanin and betalain extracts (Castro-Enríquez et al., 2020; Sharif et al., 2020). Thus, encapsulation is an alternative to be explored for maintaining the color for a longer time and placing these particles in some food matrices (Jurić et al., 2022; Ribeiro and Veloso, 2021).

Table 1. Colorimetric analysis of IG and EG particles in an environment with and without light.

	L*	a*	b*	ΔE
GE – Exposure to light				
0 h	17.64 ± 0.57^{aA}	21.94 ± 1.33^{aA}	8.10 ± 0.83^{aA}	-
2 h	17.05 ± 0.96^{aA}	22.15 ± 3.19^{aA}	9.12 ± 1.86^{aA}	1.19
4 h	18.51 ± 0.30^{aAB}	25.97 ± 0.59^{aB}	11.75 ± 0.42^{aB}	5.51
22 h	20.33 ± 1.65^{aC}	16.18 ± 1.23^{aC}	7.80 ± 1.03^{aA}	6.37
24 h	20.13 ± 0.67^{aBC}	16.15 ± 1.40^{aC}	8.43 ± 0.60^{aA}	6.31
GE – No exposure to light				
0 h	18.24 ± 0.53^{aA}	27.32 ± 1.18^{bA}	11.16 ± 0.94^{bA}	-
2 h	19.32 ± 1.00^{bAB}	24.66 ± 1.07^{aA}	10.06 ± 0.40^{aAB}	3.07
4 h	20.37 ± 0.41^{bBC}	24.30 ± 4.35^{aA}	10.17 ± 2.42^{aAB}	3.82
22 h	23.09 ± 1.15^{bD}	16.75 ± 1.51^{aB}	7.87 ± 1.01^{aB}	7,53
24 h	21.15 ± 0.79^{aC}	16.42 ± 1.2^{2aB}	9.02 ± 0.65^{aAB}	6,60
GI – No exposure to light				
0 h	19.16 ± 0.61^{aA}	16.33 ± 2.01^{cA}	5.59 ± 0.49^{aA}	-
2 h	17.16 ± 0.88^{aB}	17.09 ± 1.24^{bA}	6.80 ± 0.69^{aB}	2.46
4 h	18.32 ± 1.10^{aAB}	14.93 ± 1.34^{bAB}	6.05 ± 0.60^{aAB}	1.70
22 h	19.25 ± 0.22^{aA}	12.04 ± 3.74^{bC}	5.56 ± 0.17^{aA}	4.29
24 h	19.03 ± 0.70^{aA}	13.72 ± 1.08^{aC}	5.71 ± 0.49^{aA}	2.62
GI – No exposure to light				
0 h	18.09 ± 0.76^{bA}	17.87 ± 0.83^{cA}	6.45 ± 0.61^{bA}	-
2 h	17.49 ± 0.97^{aAB}	16.44 ± 1.50^{bAB}	6.38 ± 0.76^{aA}	1.55
4 h	18.26 ± 0.81^{aA}	13.72 ± 1.24^{bC}	5.45 ± 0.52^{aA}	4.27
22 h	15.96 ± 0.7^{4bB}	14.76 ± 0.92^{abBC}	6.11 ± 0.45^{bA}	3.78
24 h	18.11 ± 1.16^{aA}	15.16 ± 1.73^{abBC}	6.22 ± 0.74^{aA}	2.72

Capital letters refer time variation of the technique. Lowercase letters refer to the storage conditions of the same technique. The results are expressed as the mean ± standard deviation. The analysis was conducted in quintuplicate.

The ΔE values result from the variation of one or more coordinates (Alvarez Gaona et al., 2022). These values indicate if there are samples differ from one another. The microparticles produced by EG (with and without exposure to light) showed ΔE values > 3 after 2 hours of analysis, and those produced by IG only after 4 hours.

Considering the two techniques (EG and IG), it could be observed that the microparticles produced by GE had higher ΔE values (Table 1), possibly because of the characteristics of the final particle, although be used an equal matrix. Studies such as Freire et al. (2022) and Rodrigues et al. (2022) described that values of ΔE > 3.0 make it possible to observe the color change with the naked eye (untrained and experienced), values greater than human eyes can easily detect 5.0, and ΔE greater than 12.0 may imply different color spaces.

As described before, EG and IG have different structures of their polymeric matrix, which causes a change in the encapsulation efficiency (Kurozawa and Hubinger, 2017). Since IG has a greater trapping power of the bioactive material, it causes less interaction between its material and the environment, which corresponds to ΔE with lower values than EG.

Understanding the characteristics of the particles using different techniques and under other storage conditions is a promising result, considering possible applications in foods. There is a great challenge in using food dyes/coloring of natural origin due to the low stability of the color over time during storage conditions. Thus, encapsulation becomes an alternative to be explored to protect the active compounds for longer time and enable their application in the food matrix (Jurić et al., 2022; Ribeiro and Veloso, 2021).

3.5 Morphological and mechanical characterization of capsules

3.5.1 Optical morphology

The morphological characteristics of the EG and IG particles were evaluated regarding their morphologic aspects before and after the drying and rehydration process (**Figure 1**). Particles from both encapsulation methods (**Figure 1A-B**) had smooth surfaces with no apparent cracks. After drying by lyophilization and rehydration in distilled water for 24 hours (**Figure 1C-D**), the particles were also analyzed for morphology. The particle obtained by EG had a more significant change on its surface, with a more wrinkle aspect. This is possibly due to a more porous matrice that allows more diffusion facilitated from the material to the external environment (Yousefi et al., 2020), causing particle deformity.

In contrast, the more compact matrix of the IG particle allowed the slightest degree of change in spherical appearance. Similar results were found by Lupo et al. (2015) In the study, greater porosity was observed in the microstructure of the particles produced by EG, and the samples obtained by IG showed a more compact structure. Silva Carvalho et al. (2019) using the EG technique reached a macroporous structure in alginate capsules, which

allows an extensive interaction of the active agent with the external environment, such as oxygen, which can lead to the degradation of the active compounds. This makes the selection of the encapsulation method and subsequent drying of great importance, enabling longer storage time and maintenance of the biological properties of the encapsulated material (Zhang et al., 2020). Visually, it is noticeable that the IG particle (Figure 1F) had a brighter surface than the EG particle (Figure 1E).

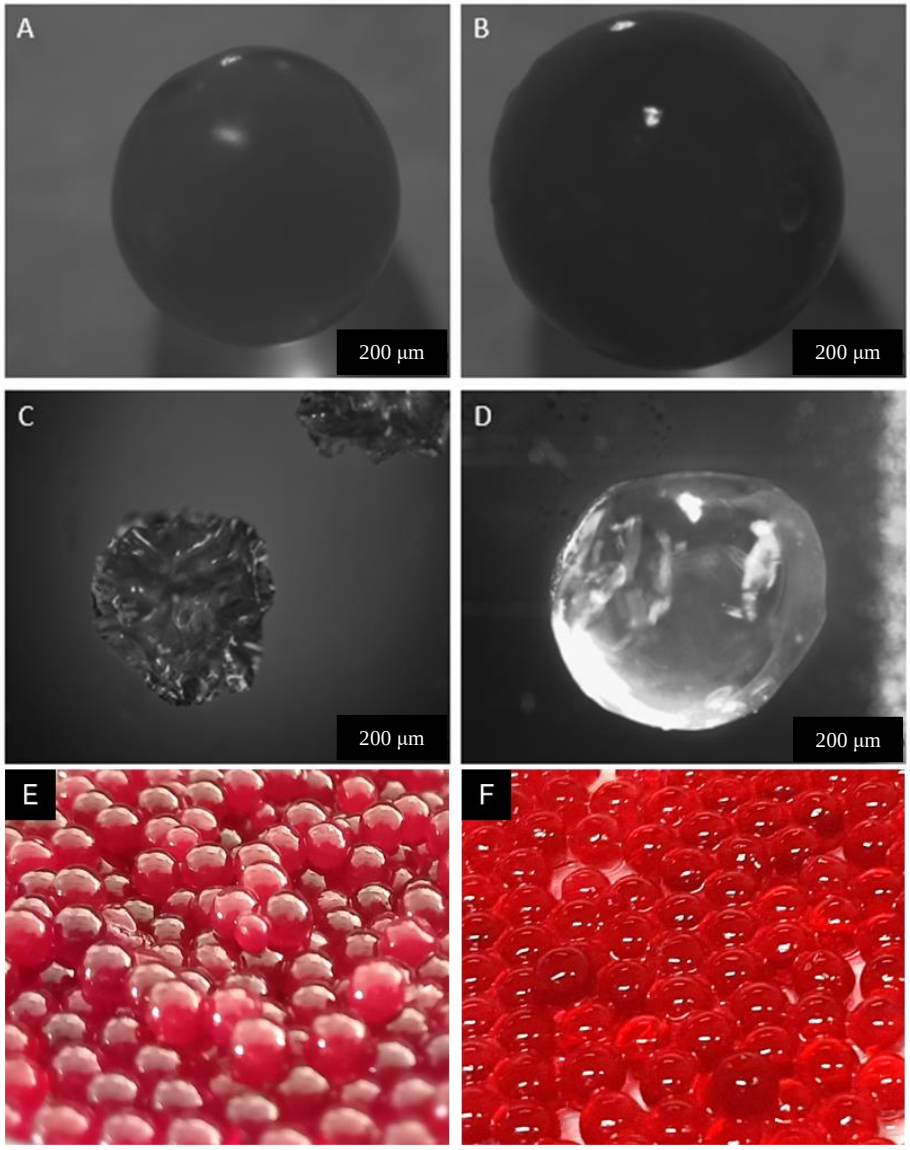


Figure 1. Optical micrographs and particles of betalain extract. (A) EG particles, (B) IG particles, (C) rehydrated EG particles, (D) rehydrated IG particles, (E) visual aspect of EG particles, and (F) visual aspect of IG particles.

3.5.2 Particle size and sphericity

The particles obtained had an average diameter (AD) of 750.17 ± 23.98 μm for the EG and 2680.23 ± 29.73 μm for the IG. Several factors may influence the size and shape of alginate particles, for example, the surface tension and viscosity of the alginate solution and the concentration of salt used in the reticulation of the encapsulating matrix (Kurtulbaş et al., 2022). The sphericity values were 0.95 ± 0.01 for EG and 0.98 ± 0.01 for GI; both closer to one (1) and it is indicative that the formed particles are spherical. Sphericity values relative to those found in this work were also reported by Castelo et al. (2020), who used the same frequency of 1800 Hz to form alginate particles to encapsulate pequi oil. The sphericity of the particles formed is desired because it indicates that both the surface tension of alginate solution and the operational parameters of encapsulation were optimized for obtaining homogenous particles (Labus et al., 2018).

3.5.3 Swelling analysis

After freeze-drying, the particles submitted to rehydration (**Figure 2**) showed swelling values between 82% and 85% for EG and from 92 to 97% for IG, attesting that the particles obtained by IG exhibited greater rehydration capacity than those obtained by EG. The IG particles exhibited rapid rehydration within the first few hours, and then the swelling rate reached equilibrium.

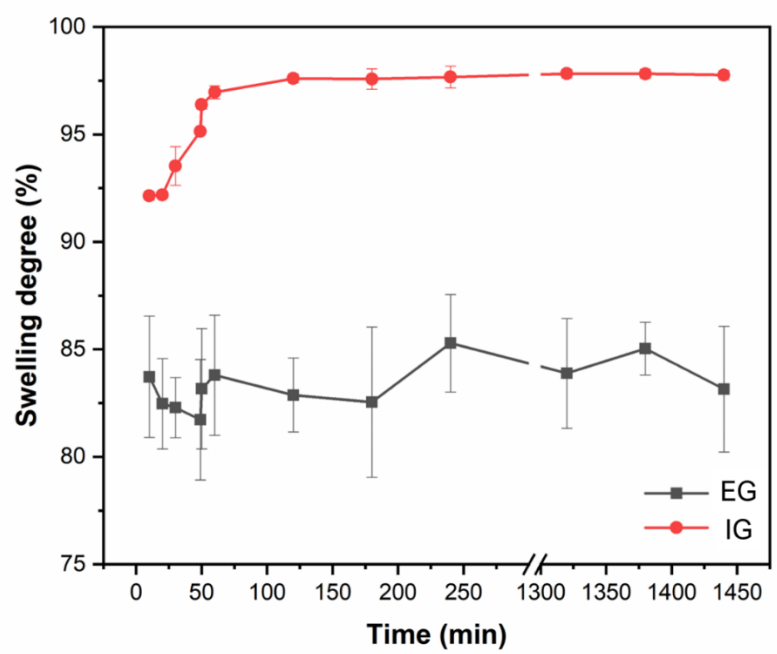


Figure 2. Swelling analysis of beet extract microparticles from EG and IG for 1450 minutes.

The swelling capacity is related to the presence of Ca^{2+} ions during the crosslinking step, which in the case of the EG particles happens in the external structure it becoming hard the absorption of water, as well as they presented greater hardness in the texture analysis. The Ca^{2+} content and the force of attraction with alginate promote decreased swelling (Günter et al., 2020; Li et al., 2019). The opposite process was observed in the IG particles (**Figure 1C-D**). The particles were obtained in the sample's presence of insoluble calcium salt before gelation in acidified canola oil (Zhang et al., 2023). This mechanism favored obtaining particles with lower hardness and greater capacity to absorb water.

3.5.4 Determination of mechanical properties

The evaluation of microparticle hardness resulted in 3.83 ± 0.54 N and 1.12 ± 0.10 N for the EG and IG, respectively. These results indicate that the hardness of the microparticles obtained by EG was significantly higher than that obtained by IG ($p < 0.05$). The results found by Rajmohan and Bellmer (2019) in the characterization of spirulina particles formed by ionic gelation, and by Lupo et al. (2015) in the comparison of the techniques of ionic gelling in cocoa extract encapsulation, corroborate our findings in which the particles obtained by external gelation showed greater hardness than those obtained by internal gelation. This behavior is related to the cross-linking of the microparticles in the presence of Ca2+ ions. In EG, ions diffuse from an external source into the polymeric solution. In turn, for IG, an insoluble calcium salt is added to the polymeric matrix containing the compound to be encapsulated, and the ion is then released in the presence of an acidic medium (Kouamé et al., 2021; Lupo et al., 2015).

3.5.5 Resistance of microparticles to different pH values.

The particles obtained were also evaluated at different pH values (2, 3, 4, 5, 6 and 7) (**Figure 3**). This analysis is especially important for future applications that intend to apply these particles because the betalain stability may be pH dependent.

In general, values of betalain were more preserved in the EG particles (Figure 3A). The highest value was at pH 5, significantly different from the other pH values. This is justified because when they are above the pKa values of mannuronic acid (pKa = 3.38) and glucuronic acid (pKa = 3.65) (Wongverawattanakul et al., 2022), which are the building blocks of alginate, there is a disorder in the calcium-alginate structure (Dalponte Dallabona et al., 2020).

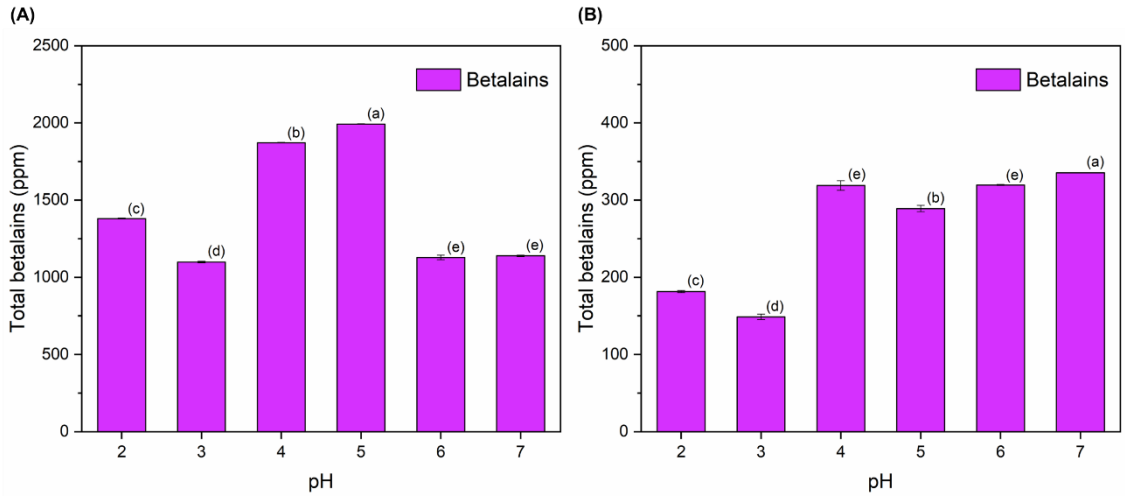


Figure 3. Release of betalain from microparticles subjected to a pH range of 2 to 7. (A) External ionic gelation - EG and (B) Internal ionic gelation – IG.

The carboxylic groups of the alginate have a COOH form when the pH is below the pKa, which causes an increase in hydrogen-bonding strength. The opposite effect occurs at higher pH when the carboxylic groups have COO^- form. The presence of this negative charge causes repulsion of the alginate polymer chain, causing swelling of the polymer and consequently facilitating the release of the encapsulated compound (Camacho et al., 2019). Thus, the IG particle had a higher release of betalains at pH 7, and this behavior is due to the conditions under which the IG particles are formed. Alginate matrices have been widely used in encapsulation as an alternative to encapsulation due to providing resistance to gastrointestinal diseases (Waqas et al., 2022).

3.6 Release study over time and kinetic evaluation

3.6.1 Study Release

The release profile of the encapsulated active material is essential to understand the release's mechanism (Silva et al., 2022). In the release of betalain particles in water (Figure 4**),** it was observed that for the EG particles, there was a rapid release of the active compound up to 120 min (“burst” effect), with a more pronounced effect between 20 and 40 min. Still, the release effect was maintained until the maximum time was analyzed (120 min). On the other hand, the IG particles obtained their “burst” effect up to 40 min, where they obtained the maximum release, with no further changes until 120 min. Therefore, for the

subsequent study of the release kinetics, values will be used up to 120 min for EG particles and up to 40 min for IG particles. The EG particles reached the final release point (120 min) 60%of releases, while the IG microcapsules reached the maximum final release of 100% in equal time (Figure 4).

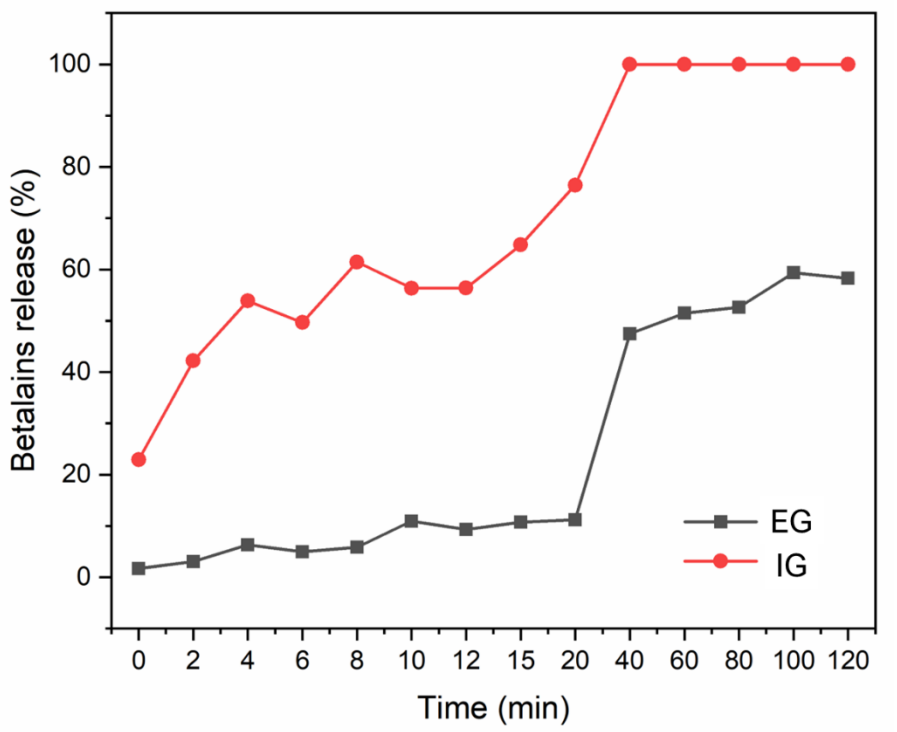


Figure 4. Release of beet extract microparticles obtained by EG and IG particles in distilled water (pH 7) over time.

Studies realized by Lin et al. (2023) found that particles prepared by external gelation had Ca^{2+} more strongly crosslinked with alginate molecules than particles prepared by internal gelation. This phenomenon also explains the lower release of betalain in EG particles because this matrix is more rigid due to its better interaction with alginate. Lupo et al. (2015) confirmed the formation of a much more rigid layer on the surface of the particles when EG was used, which provided greater external hardness, justifying its lower release. According to the authors, during EG, a superficial layer of gel was formed, delaying the diffusion of the calcium ion into the interior of the macroparticles; consequently, a heterogeneous matrix with a low cross-linking core was created due to the lack of calcium ions.

3.6.2 Kinetic study

The results obtained up to 120 min and 40 min for EG and IG particles were analyzed using mathematical models to simulate the release mechanism of the active material from the particles. The values of the kinetic parameters used for the applied models are shown in Table 2.

Table 2. Kinetic parameters for the release of EG and IG particles using mathematical models of zero order, first order, Higuchi and Korsmeyer-Peppas. Where K is the release constant, R^2 is the correlation coefficient and n is the diffusion exponent.

Mathematical model	**EG**	**IG**
Zero order		
K_0	0.56	1.64
R^2	0.88	0.87
First order		
K_1	0.03	0.03
R^2	0.76	0.67
Higuchi		
K_H	26.63	11.45
R^2	0.92	0.95
Korsmeyer-Peppas		
K_{kp}	1.56	28.02
R^2	0.95	0.85
n	0.79	0.34

The zero-order and first-order models, both for the EG and for the IG, obtained R2 values far below the standard (0.99), indicating that these models, which predict a simple linear release, were not adequate, and models such as Higuchi and Korsmeyer-Peppas were the most preferred, as shown in Table 2. For EG particles, the Korsmeyer-Peppas model obtained a high R^2 value (0.95), indicating that it is adequate to describe the release of betalain of these particles. For the IG particles, the Higuchi model presented the highest R^2 value (0.95); a similar result was also reported by Budinčić et al. (2021) e Silva et al. (2022). The Higuchi model is generally used to study the release of water-soluble and poorly soluble materials incorporated in semisolid and/or solid matrices (Costa, 2002). The Korsmeyer-Peppas model is generally used to analyze polymeric systems with release mechanisms unknown or when more than one type of release may be involved (Costa, 2002).

According to the literature, the value of n = 0.43 corresponds to the release of the active substance by diffusion according to Fick's law, while values less than 0.43 indicate pseudo-Fickian diffusion (Ferreira et al., 2019). The intermediate value n (0.43–0.89) indicated that it conformed to the non-Fick diffusion law (Zhu et al., 2022). The diffusion exponent n varied from 0.79 to 0.33 for the EG and IG particles. Thus, it can be assumed that betalain release conforms to the Non-Fick diffusion mechanism for the EG particles, that is, the dual effect of Fick diffusion and matrix dissolution Similar results were obtained by Wang et al. (2020), who obtained diffusion coefficient values between 0.47 and 0.52 following the nonfick diffusion mechanism, which is, as reported by the authors, the coexistence of fick diffusion and corrosion diffusion. For the IG particles, n being less than 0.43 indicates pseudo-Fickian diffusion, which shows similarities to a Fickian process. Lupo et al. (2015) also obtained n-values lower than 0.43, which may be related to the presence of calcium in the release medium. Alginate cross-linking may continue, delaying betalain release in releasing IG particles. For the EG particles, according to Lupo et al. (2015), the calcium concentration is located in the outer region of the particle, which requires more than one process, in addition to the diffusion process, for the release of betalain to occur.

4. Conclusion

The selection of the encapsulation method is of great importance and depends on the encapsulated active agent and the food matrix. In this study, a significantly higher amount of beet extract was encapsulated by internal ionic gelation, which can be an essential differential for the choice of this technique. It was possible to observe that the type of ionic gelling mechanism directly influences the technological characteristics of the particles. More significant, brighter, more elastic particles with greater water absorption capacity were found for those obtained by internal ionic gelation. On the other hand, the particles obtained by external ionic gelling showed a more resistant wall with a lower betalain release rate over 120 minutes. The encapsulating matrix's structure also influenced the particles' release profile in relation to the pH, with a greater tendency to release particles obtained by internal ionic gelation in a neutral medium and in an acid medium for particles obtained by external ionic gelation. Finally, EG and GI processes are promising for encapsulation and can reduce the chances of chemical compound degradation and, consequently, color change.

Implications for Gastronomy

This article proposes the encapsulation of natural dyes, betalains, and compounds susceptible to oxidation at high temperatures or in the presence of oxygen, pH, or light. For the higher stability of these compounds, encapsulation is an alternative and viable technology for future applications. Betalain macrospheres are very attractive and could be used in beverages and foods.

CRediT authorship contribution statement

Joana de Barros Alexandre: Methodology, Investigation, Analysis, Validation, Writing – original draft. **Tiago Linhares Cruz Tabosa Barroso:** Investigation, Analysis, Writing – original draft. **Luana Carvalho da Silva:** Methodology, Investigation, Validation, Writing – original draft. **Rachel Menezes Carvalho:** Investigation, Analysis, Writing – original draft. **Gabrielle Albuquerque Freire:** Investigation, Analysis, Writing – original draft. **Amanda Batista do Nascimento:** Methodology, Investigation, Analysis, Writing – original draft. **Roselayne Ferro Furtado:** Conceptualization, Supervision, Resources, Project administration, Funding acquisition, Writing - review & editing.

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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5 CONSIDERAÇÕES FINAIS

A gelificação iônica mostrou-se um método eficaz na produção de partículas probióticas e de betalaínas.

A partir da metodologia de superfície de resposta, foi definida a condição para produção das macroesferas probióticas (1,125% de alginato e 0,50% de goma xantana). Os tratamentos com maiores concentrações de alginato e goma xantana apresentaram maiores eficiência de encapsulação, maior viscosidade e homogeneidade nos tamanhos de partícula e esfericidade. Portanto, potencializando futuras aplicações, e confirmando que a combinação desses dois polissacarídeos pode melhorar os aspectos morfológicos das partículas de alginato e proteção/ retenção dos microrganismos.

A bebida de soja mostrou-se vantajosa no encapsulamento das Bifidobactérias, permitindo que elas permanecessem viáveis ao longo de trinta dias e em condições ácidas.

O mecanismo de gelificação iônica influenciou as características tecnológicas das partículas de extrato aquoso de beterraba. A gelificação iônica interna foi eficaz na encapsulação das betalaínas, proporcionando melhor eficiência de encapsulação, brilho e maior capacidade de reidratação. Já a gelificação externa proporcionou uma menor taxa de liberação e partículas mais resistentes, porém com baixa eficiência de encapsulação.

Dessa forma, a tecnologia de encapsulação por gelificação iônica mostrou-se eficiente na proteção das bactérias probióticas e das betalaínas. A técnica de gelificação interna é adequada para encapsulação de compostos hidrofílicos, uma vez que a interação com o óleo acidificado reduz a perda do material para o meio. Os resultados encontrados são promissores para futuras aplicações dos produtos, principalmente na área de alimentos devido às atrativas propriedades funcionais e sensoriais encontradas.

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APÊNDICES

APÊNDICE A – SUPPLEMENTARY MATERIAL

Frame 1 - Estimated regression coefficients for the responses of viscosity, encapsulation efficiency, particle size and sphericity

	Viscosity			Encapsulation Efficiency		
Factor	**Coeff.**	**F**	**p**	**Coeff.**	**F**	**p**
AG (L)	298.70	9.66	0.03	-0.27	0.004	0.95
AG (Q)	-47.21	0.17	0.70	4.69	0.80	0.41
XG (L)	351.37	13.36	0.01	11.32	6.64	0.05
XG (Q)	37.03	0.10	0.76	1.56	0.09	0.78
AG by XG	301.03	4.90	0.08	-3.13	0.25	0.64
	Particle size			**Sphericity**		
Factor	**Coeff.**	**F**	**p**	**Coeff.**	**F**	**p**
AG (L)	0.05	0.09	0.77	0.006	3.50	0.12
AG (Q)	-0.31	2.52	0.17	-0.003	0.58	0.50
XG (L)	0.06	0.15	0.71	0.001	0.10	0.76
XG (Q)	-0.08	0.15	0.71	-0.01	4.25	0.10
AG by XG	-0.04	0.02	0.89	0.01	4.80	0.08

Source: Prepared by the author.
Subtitle: AL(L) is linear alginate; XG (L) linear xanthan gum; AL(Q) is quadratic alginate, XG(Q) quadratic xanthan gum; and AL (L) by XG (L) is the interaction of the two variables.

Frame 2 – ANOVA for *Encapsulation Efficiency*

Source of Variation	**Sum of Squares**	**Degrees of Freedom**	**Mean Squares**	**F_{value}**	**F_{tab}**
Regression	1024,53	1	1024,53	9,85	5,12
Residual	935,70	9	103,97		
Total	1960,23	10	1356, 84		

Source: Prepared by the author.
Subtitle: % explained variation R^2= 60,63%.

Frame 3 – ANOVA for *Viscosity*

Source of Variation	**Sum of Squares**	**Degrees of Freedom**	**Mean Squares**	**F_{value}**	**F_{tab}**
Regression	2080000	2	1040000	21,73	4.46
Residual	382824	8	47853		
Total	2462079	10	246207,9		

Source: Prepared by the author.
Subtitle: % explained variation R^2= 85%.