UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS RURAIS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA
DOS ALIMENTOS

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CO-ENCAPSULAÇÃO DE *Lactobacillus rhamnosus* E COMPOSTOS ANTOCIÂNICOS EXTRAÍDOS DO MIRTILO (*Vaccinium myrtillus*)

Santa Maria, RS
2021

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Dissertação apresentada ao Curso de Pós-Graduação em Ciência e Tecnologia dos Alimentos, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Mestre em Ciência e Tecnologia dos Alimentos**.

Orientador: Profº Drº. Cristiano Ragagnin de Menezes
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Aprovado em 22 de janeiro de 2021:

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Santa Maria, RS
2021

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RESUMO

CO-ENCAPSULAÇÃO DE *Lactobacillus rhamnosus* E COMPOSTOS ANTOCIÂNICOS EXTRAÍDOS DO MIRTILO *Vaccinium myrtillus*

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O consumo regular de probióticos e alimentos ricos em antioxidantes como o mirtilo é reconhecidamente benéfico para a manutenção da saúde. Esse efeito é atribuído às propriedades terapêuticas dos microrganismos probióticos e à presença dos compostos fenólicos do mirtilo. No entanto, a estabilidade físico-química e biodisponibilidade destas substâncias ativas têm representado um grande desafio para a indústria de alimentos. A encapsulação consiste na técnica de envolver materiais sólidos, líquidos ou gasosos em pequenas cápsulas que liberam seu conteúdo sob condições controladas. Esta tecnologia tem sido empregada para viabilizar a proteção destes compostos nos alimentos, além de melhorar a sua utilização promovendo a entrega bem-sucedida destas substâncias bioativas no trato gastrointestinal. Desta forma, o objetivo deste estudo consistiu em avaliar se a co-encapsulação de diferentes concentrações de extrato de mirtilo teria efeito positivo sobre a sobrevivência das bactérias probióticas de *Lactobacillus rhamnosus*. As microcápsulas foram produzidas por spray drying, utilizando como agentes encapsulantes inulina, goma arábica e maltodextrina. As concentrações utilizadas foram de 0% (cápsula controle; ME-0%), 10% (ME-10%), 50% (ME-50%) e 100% (ME-100%) de extrato de mirtilo. Posteriormente, as microcápsulas foram avaliadas quanto a morfologia, tamanho e distribuição de partícula, eficiência de encapsulamento, conteúdo de antocianinas, cor, resistência ao tratamento térmico, simulação do sistema gastrointestinal e viabilidade ao armazenamento. Obteve-se uma eficiência maior que 80% de encapsulação. O tamanho médio das microcápsulas ficou entre 1,74 ɥm e 6,29 ɥm e o formato apresentado foi esférico e com presença de concavidades. As microcápsulas com maior concentração de extrato de mirtilo apresentaram maior resistência dos *Lactobacillus rhamnosus* a tratamentos térmicos, maior liberação destes no intestino durante a passagem pelo sistema gastrointestinal simulado, bem como maior vida útil durante o armazenamento. Por fim, os resultados deste estudo mostraram que a co-encapsulação de extrato de mirtilo melhorou a viabilidade, e consequentemente, a sobrevivência de *Lactobacillus rhamnosus*, demonstrando o efeito protetor do extrato de mirtilo para a cultura probiótica microencapsulada em spray drying. Além disso, a encapsulação de bactérias probióticas juntamente com extratos de frutas fora pouco estudado.

Palavras-chave: Microencapsulação. Bactérias probióticas. Latobacillus. Compostos fenólicos. Antocianinas

ABSTRACT

CO-ENCAPSULATION OF *Lactobacillus rhamnosus* AND ANTOCYANIC COMPOUNDS EXTRACTED FROM THE BLUEBERRY *Vaccinium myrtillus*

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Regular consumption of probiotics and foods rich in antioxidants such as blueberries are known to be beneficial in maintaining health. This effect is attributed to the therapeutic properties of probiotic microorganisms and the presence of phenolic compounds in blueberry. However, the physicochemical stability and bioavailability of these active substances have represented a great challenge for the food industry. Encapsulation is the technique of wrapping solid, liquid or gaseous materials in small capsules that release their contents under controlled conditions. This technology has been used to enable the protection of these compounds in food, in addition to improving their use by promoting the successful delivery of these bioactive substances in the gastrointestinal tract. Thus, the aim of this study was to evaluate whether co-encapsulation of different concentrations of blueberry extract would have a positive effect on the survival of probiotic bacteria of *Lactobacillus rhamnosus*. The microcapsules were produced by spray drying, using inulin, gum arabic and maltodextrin as encapsulating agents. The concentrations used were 0% (control capsule; ME-0%), 10% (ME-10%), 50% (ME-50%) and 100% (ME-100%) of blueberry extract. Subsequently, the microcapsules were evaluated for morphology, particle size and distribution, encapsulation efficiency, anthocyanin content, color, resistance to heat treatment, gastrointestinal system simulation and storage viability. An efficiency greater than 80% of encapsulation was obtained. The average size of the microcapsules was between 1.74 µm and 6.29 µm and the shape presented was spherical and with the presence of concavities. Microcapsules with higher concentration of blueberry extract showed greater resistance of *Lactobacillus rhamnosus* to heat treatments, greater release of these in the intestine during passage through the simulated gastrointestinal system, as well as longer shelf life during storage. Finally, the results of this study showed that the co-encapsulation of blueberry extract improved the viability, and consequently, the survival of *Lactobacillus rhamnosus*, demonstrating the protective effect of blueberry extract for microencapsulated probiotic culture in spray drying. Furthermore, the encapsulation of probiotic bacteria together with fruit extracts has been little studied.

Keywords: Microencapsulation. Probiotic bacteria. Latobacillus. Phenolic compounds. Anthocyanins.

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1 INTRODUÇÃO

A busca pela qualidade de vida e a relação entre alimentação balanceada e promoção da saúde tem estimulado a procura por alimentos saudáveis. A alimentação saudável fornece fontes de micro e macro nutrientes necessários para o funcionamento adequado do organismo. Porém, nas últimas décadas, o alimento deixou de ter apenas a função nutricional básica e passou a ser considerado como fonte de proteção à saúde. Certos alimentos podem estimular o metabolismo, melhorando o funcionamento do organismo, prevenindo o surgimento de determinadas doenças e proporcionando o bem-estar aos indivíduos (MUHAMMAD et al., 2021). Nesse contexto, destacam-se os produtos probióticos e os compostos bioativos presentes em extratos de frutos como o mirtilo.

Os probióticos estão entre as espécies vivas de microrganismos capazes de proporcionar benefícios à saúde do seu hospedeiro quando ingeridos em quantidades adequadas (FAO/OMS, 2002). Eles são responsáveis, principalmente, pelo equilíbrio da microbiota intestinal normal e aumento da resposta imunológica, entre outros (PATEL; SHUKLA; GOYAL, 2015), agindo na prevenção e tratamento de diversas doenças, especialmente àquelas relacionadas ao tratogastrointestinal (AZAM et al., 2020). Isso se deve ao potencial dos probióticos de inibirem a colonização do intestino por bactérias patogênicas ou prejudiciais através da produção de substâncias antimicrobianas, competição pela adesão na parede intestinal, competição por nutrientes, acidificação do local, aumento da secreção de mucina, entre outros (SZYMÁNSKI et al., 2006).

O mirtilo possui altas concentrações de compostos antioxidantes, como antocianinas e flavonoides, além de vitaminas C, B, E e A (CASTAGNINI et al., 2015). Estudos sugerem que os diferentes polifenois presentes no mirtilo podem apresentar efeitos benéficos à saúde (BASU; RHONE; LYONS, 2010; HOWARD et al., 2012), o que tem estimulado o consumo deste fruto em todo o mundo (SINELLI et al., 2008).

A sobrevivência dos microrganismos probióticos pode ser afetada por diversos fatores, incluindo pH, temperatura, tempo de estocagem e exposição às condições adversas encontradas no trato gastrointestinal. Além disso, durante o processamento e armazenamento de frutos, os compostos fenólicos presentes nos mesmos são propensos a processos de oxidação, degradação e polimerização. Nos últimos anos várias pesquisas estão sendo realizadas para preservar estes compostos da

deterioração durante o processamento (HOWARD et al., 2012). A aplicação de uma barreira física que confere proteção contra essas condições adversas é uma opção valiosa para manter a viabilidade celular, bem como a preservação destes compostos.

O empacotamento de substâncias ativas em uma matriz polimérica, visando protegê-las durante o processamento, armazenamento e a passagem pelo trato gastrointestinal é definido como microencapsulação. É considerada uma alternativa promissora para aumentar a viabilidade dos microrganismos probióticos e preservar a estabilidade dos compostos bioativos presentes em extratos vegetais.

Um grande desafio para os alimentos funcionais que contêm probióticos é fornecer um número suficiente de microrganismos probióticos para sobreviver aos ambientes hostis durante o processamento e armazenamento, de modo que possam conferir seus benefícios à saúde. Desta forma, o objetivo deste trabalho consiste em produzir microcápsulas contendo *Lactobacillus rhamnosus* juntamente com a adição de extrato de mirtilo em diferentes concentrações, caracterizar e avaliar se a adição de extrato apresenta efeito positivo na preservação dos probióticos, verificando a viabilidade e estabilidade dos probióticos sob diferentes condições térmicas, de armazenamento e passagem pelo sistema gastrointestinal simulado.

2 REVISÃO DE LITERATURA

2.1 PROBIÓTICOS

Associados a uma alimentação equilibrada e hábitos de vida saudáveis, os probióticos são definidos como microrganismos vivos que, quando administrados em níveis adequados promovem benefícios a saúde do hospedeiro (FAO/WHO, 2002). Para isso, é importante salientar que para um microrganismo ser considerado probiótico é necessário que o mesmo tenha como habitat natural o trato gastrointestinal, seja capaz de permanecer metabolicamente ativo no intestino, resistindo antes a passagem pelo sistema gástrico, bem como às enzimas digestivas (DE MENEZES et al., 2013; SONG; IBRAHIM; HAYEK, 2012).

A ação benéfica dos probióticos sobre o organismo se deve principalmente à modulação da microbiota intestinal humana, por um mecanismo de "exclusão competitiva", o que impede a adesão de microrganismos patogênicos, favorecendo a multiplicação de bactérias benéficas e a colonização por probióticos (DE MENEZES et al., 2013; GUARNER; MALAGELADA, 2003). Além disso, os probióticos são capazes de estimular uma resposta imunológica no seu hospedeiro, atuando na prevenção e tratamento de doenças infecciosas e inflamatórias (LEE et al., 2014). Possuem propriedades anticarcinogênicas (NAGPAL et al., 2012), antialérgicas (LEE et al., 2014), anti-hipertensivas (LOLLO et al., 2015), auxiliam na digestão da lactose através da enzima bacteriana lactase (PAKDAMAN et al., 2016), no tratamento de diarreias (FOX et al., 2015; LEE et al., 2015; SOHAIL et al., 2012; WANG; GAO; FANG, 2013) e outras doenças, como hepatopatias (MA et al., 2013) e cânceres gástricos e intestinais (NAGPAL et al., 2012).

Os gêneros *Lactobacillus* e *Bifidobacterium* são as bactérias ácido lácticas mais amplamente estudadas e utilizadas em produtos probióticos (ASGARI et al., 2020). *Bifidobacterium longum*, *Bifidobacterium breve*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus* e *Lactobacillus fermentum* são exemplos de espécies destes gêneros, os quais desempenham um importante papel na preservação da saúde humana (ASGARI et al., 2020). Lactobacillus spp. e Bifidobacterium spp. são capazes de converter a lactose e/ou outras fontes de carboidratos em ácido láctico, que é usado por outras bactérias intestinais para produzir ácidos graxos de cadeia curta, favorecendo o ambiente ácido do intestino, desfavorável às bactérias patogênicas

(ASGARI et al., 2020). Vale ressaltar que, a viabilidade dos probióticos deve ser mantida durante o processamento, armazenamento (temperatura, oxigênio, umidade e etc.) e passagem pelo sistema gastrointestinal. Diante disso, estratégias que forneçam proteção são importantes para preservar os probióticos de condições ambientais adversas e para garantir que estes cheguem ao local alvo em quantidades adequadas (ASGARI et al., 2020).

2.1.1 *Lactobacillus*

Os *Lactobacillus* são bastonetes gram-positivos, não esporulados, imóveis, anaeróbios ou anaeróbios facultativos (SLOVER; DANZIGER, 2008). O gênero *Lactobacillus* empregado na produção alimentar faz parte do grupo das bactérias ácido-lácticas, cujo principal produto metabólico é o ácido lático através da fermentação de carboidratos (BURITI; SAAD, 2007).

Lactobacillus é um gênero altamente heterogêneo, compreendendo mais de 50 espécies, e que recentemente tem sido utilizado para oferecer benefícios à saúde de quem os consome por meio das cepas probióticas (BERNARDEAU et al., 2008; RAIZEL et al., 2011). A bactéria probiótica *Lactobacillus rhamnosus* GG foi isolada pela primeira vez a partir de fezes de humanos sadios. Devido ao seu potencial probiótico relatado, cepas de *Lactobacillus rhamnosus* foram identificadas em diversas matrizes alimentares e suplementos probióticos nos últimos anos (MOAYYEDI et al., 2018). Em humanos, o probiótico demonstrou ser um agente profilático eficaz contra infecções primárias por rotavírus e dermatite atópica, bem como na prevenção e tratamento da diarreia (JIANG et al., 2020; ZHANG et al., 2010).

Segundo Valik, Medved'ova e Liptakova (2008), o *Lactobacillus rhamnosus* GG existe no intestino em seu estado de biofilme, e devido as suas propriedades antimicrobianas, além das probióticas, é introduzido em suplementos, alimentos fermentados e não fermentados. Um atributo muito importante, a formação de biofilmes por bactérias probióticas contribui para a resistência ao pH gástrico, antibióticos e estresse mecânico (KUMAR e MICALLEF, 2017, pp. 107-130). Por conta disso, bactérias de *Lactobacillus rhamnosus* GG apresentaram o potencial de impedir a adesão de *Clostridium histolyticum*, *C. difficile* e *Salmonella enterica* ao muco do intestino humano in vitro devido à sua alta capacidade de formação de biofilme (COLLADO; GRZEŚKOWIAK; SALMINEN, 2007). Song et al. (2019) relatam também

a inibição de formação de biofilme de *Escherichia coli* na cocultura, com uma taxa de inibição de 79% por microcápsulas de *Lactobacillus rhamnosus* GG. Aliado a isso, em uma meta-análise de estudos realizada por Sazawal et al. (2006), os autores concluíram que a ingestão de *Lactobacillus rhamnosus* GG, bem como de outras cepas probióticas, diminuiu em 52% a incidência de diarreia associada a antibióticos e em 57% o risco de diarreia aguda entre crianças.

2.2 MIRTILO (*Vaccinium myrtillus* L.)

O mirtilo, também conhecido como "*blueberry*" (do inglês), é uma fruta de clima temperado pertencente à família Ericaceae e ao gênero *Vaccinium* (RASEIRA; ANTUNES, 2004). É uma espécie nativa do hemisfério Norte e Europa, que tem seu cultivo em franca expansão em países da América do Sul. A América do Sul é o terceiro território com maior produção de mirtilo (10.720 toneladas), ficando atrás apenas da América do Norte com 103.495 toneladas e da Europa com 11.320 toneladas de mirtilo (ANTUNES; PAGOT, 2016).

Na América do Sul, o Brasil é o quarto país com maior produtividade de mirtilo (118 hectares de área plantada), sendo o estado do Rio Grande do Sul o de maior produção, apresentando 83 hectares para cultivo, os quais concentram-se nas cidades de Vacaria, Pelotas, Erechim e Caxias do Sul (ANTUNES; PAGOT, 2016). Estados como Minas Gerais, São Paulo, Santa Catarina e Paraná também se destacam no cultivo de mirtilo, proporcionando ao país um quadro produtivo de aproximadamente 300 toneladas de mirtilo (ANTUNES; PAGOT, 2016).

O mirtilo é uma fruta do tipo baga, de formato achatado e coloração azul intenso (WANG et al., 2017). É conhecida como a fruta da longevidade em virtude da sua composição nutricional, sendo uma rica fonte de fibras, vitaminas e minerais (PAES et al., 2014). Além disso, são frutas com elevado teor antioxidante, cuja atividade está relacionada à presença de compostos fenólicos (WANG et al., 2017). Dentre os compostos fenólicos tem-se grande predominância dos flavonoides (campferol, quercetina e miricetina) e ácidos fenólicos (ácidos cafeíco, ácido ferúlico, ácido clorogênico e ácido cumárico) (CHENG et al., 2020; PERTUZATTI et al., 2021).

O grupo majoritário dos compostos presente no mirtilo são as antocianinas, correspondendo de 35-74% do total de compostos fenólicos da fruta (GAVRILOVA et al., 2011). As antocianinas pertencem a classe dos flavonoides e são amplamente

encontradas na natureza em frutas ou flores (NILE; PARK, 2014). No mirtilo, as antocianinas são responsáveis pela sua coloração característica e são encontradas predominantemente na casca da fruta. Também se encontra antocianina na polpa da fruta, porém em menor proporção (NILE; PARK, 2014; PAES et al., 2014).

Na natureza, as antocianinas são encontradas em sua grande maioria na forma glicosilada (ex. glicosídeo, galactosídeo, arabinosídeo), ou seja, contendo uma ou mais moléculas de açúcar na sua estrutura (SINOPOLI; CALOGERO; BARTOLOTTA, 2019). Também se encontram antocianinas na sua forma acilglicosiladas (ex. ácidos acético, malônico, málico), ou seja, além da molécula de açúcar, também apresentam moléculas aciladas com ácidos orgânicos em sua estrutura (ZHAO et al., 2017). Já as antocianidinas são raramente encontradas na sua forma livre em virtude da sua instabilidade (GIUSTI; JING, 2007). Porém, as antocianidinas são a estrutura base das antocianinas, diferenciando-se quanto ao número e grau de metilação dos grupos hidroxilas presentes na estrutura do anel aromático (LOPES et al., 2007).

No total existem 6 antocianidinas, sendo elas: ciadinina, delfinidina, malvidina, pelargonidina, peonidina e petunidina (LOPES et al., 2007), e o que as torna mais estáveis em relação à antocianidina é justamente sua ligação a moléculas de açúcares e ácidos (GIUSTI; JING, 2007). Entretanto, de acordo com Giusti e Wrolstad (2001), as antocianinas são muito instáveis e suscetíveis à degradação. Sua estabilidade é afetada principalmente pela estrutura química, pH, temperatura, luz, presença de oxigênio, presença de enzimas e interações entre os componentes dos alimentos, tais como ácido ascórbico, íons metálicos, açúcares e copigmentos (KUCK, 2012). Uma alternativa para ajudar na preservação das antocianinas é realizando a microencapsulação desses compostos. A microencapsulação promove a melhora da estabilidade, protegendo as antocianinas contra degradações, além de também ajudar no controle da liberação desses compostos no corpo humano (GUO; GIUSTI; KALETUNÇ, 2018).

Estudos mostram que as antocianinas são consideradas compostos biologicamente ativos com muitos benefícios para a saúde humana. Nesse sentido, as antocianinas são conhecidas particularmente por suas propriedades anti-inflamatórias, anticarcinogênica e antimutagênica, podendo prevenir ou até mesmo bloquear o metabolismo celular contra doenças (CASSIDY, 2018). Além disso, outros benefícios mostram que a ingestão de mirtilo proporciona uma melhora da visão noturna, no tratamento de glaucoma e retinopatia (CAMIRE, 2002). Sua ingestão

também ajuda no controle da obesidade e diabetes (DRÓŻDŻ; ŠĖŽIENĖ; PYRZYNSKA, 2017).

Com base nisso, vê-se a importância no consumo de mirtilo, seja ele *in natura* ou na sua forma processada. Porém, o mirtilo é uma fruta altamente perecível, tendo como vida útil de 14 a 20 dias, com isso vê-se a necessidade de consumi-lo de forma rápida ou processada (FALAGÁN; MICLO; TERRY, 2020). Na forma processada encontra-se mirtilo desidratado (SHARIF et al., 2018), suco de mirtilo (ZHU et al., 2019). Além disso, hoje no mercado já se encontra a inserção de mirtilo em produtos como chá (PORTO-FIGUEIRA et al., 2015), iogurte (ŚCIBISZ et al., 2012), geleia (POIANA; ALEXA; MATEESCU, 2012), vinho (SUN et al., 2019), dentre outros. O mirtilo também está inserido na área farmacêutica, sendo usado em produtos cosméticos (FARIA-SILVA et al., 2020).

2.3 MICROENCAPSULAÇÃO

A microencapsulação é uma tecnologia que consiste no revestimento de partículas, formando cápsulas sólidas e esféricas com tamanho entre 1 e 1000 μm (RATHORE et al., 2013), podendo apresentar diferentes morfologias dependendo do método e dos materiais utilizados na sua preparação (FANG; BHANDARI, 2010). O composto a encapsular pode encontrar-se concentrado na região central da cápsula ou disperso uniformemente numa matriz contendo o agente encapsulante (DE AZEREDO, 2005).

O processo de microencapsulação visa fornecer proteção às condições ambientais adversas, tais como luz, pH, umidade, oxigênio, contribuindo assim para o aumento da estabilidade e biodisponibilidade das substâncias ativas, evitando assim os efeitos de sua exposição inadequada (SUAVE et al., 2006). Além disso, é capaz de promover a liberação controlada do material encapsulado em locais potenciais de ação (BETZ; KULOZIK, 2011). Vários mecanismos podem ser usados para seu acionamento, como mudança de pH, estresse mecânico, temperatura, atividade enzimática, tempo, força osmótica, fermentação bacteriana, entre outras (COOK et al., 2012).

Diferentes métodos são utilizados para a produção de microcápsulas (SOHAIL et al., 2011), sendo que a diferença entre eles está no envolvimento ou aprisionamento do material a encapsular pelo agente encapsulante. Os principais métodos de

encapsulação são: spray drying, spray cooling, extrusão, coacervação, liofilização e emulsificação (COOK et al., 2012). A seleção depende do tamanho desejado da microcápsula e da aplicação que será dada à mesma, do mecanismo de liberação e das propriedades físico-químicas, tanto do material ativo, quanto do agente encapsulante (ASSUNÇÃO et al., 2014; COOK et al., 2012).

2.3.1 Spray drying

A secagem por spray é considerada um dos métodos mais comumente empregados para microencapsulação (RODRIGUES et al., 2020). É definida como o processo de transformação de uma solução, emulsão ou suspensão no estado fluido em um pó por pulverização da solução de alimentação em um gás de secagem quente (PIÑÓN-BALDERRAMA et al., 2020). O tamanho de partícula dos pós secos por spray drying convencional pode ser classificado em: pequeno (1-5 µm), médio (5-25 µm) e grande (10-60 µm) (PIÑÓN-BALDERRAMA et al., 2020).

Para aplicações industriais, a secagem por spray é a mais adequada devido ao seu baixo custo, processo contínuo e alta reprodutibilidade (LIU et al., 2015). No entanto, ela confere baixa viabilidade e estabilidade na secagem de células probióticas e culturas iniciais após a secagem e durante o armazenamento (PEIGHAMBARDOUST et al., 2011). Por isso, para uma secagem bem-sucedida de organismos vivos requer-se alguns protetores para melhorar a tolerância de secagem das células (GODERSKA & CZARNECKI, 2008). A secagem desprotegida pode resultar na morte celular devido aos danos causados à frágil membrana das células e desnaturação das proteínas (MORGAN et al., 2006). A microencapsulação é um método usado com secagem por spray para proteger as células bacterianas, cobrindo-as e formando barreiras contra o ambiente prejudicial (IRAVANI et al., 2015).

Um estudo realizado por Nunes et al. (2018a) avaliou o uso de inulina, hi-maize e trealose na secagem por spray para produzir micropartículas contendo *Lactobacillus acidophilus* La-5. Os autores relataram uma eficiência de encapsulamento de 93,12% e 94,26% para as matrizes encapsulantes de inulina e hi-maize, respectivamente. Além disso, a matriz encapsulante de trealose proporcionou maior proteção térmica para os probióticos; trealose e hi-maize apresentaram maior estabilidade ao armazenamento; e para condições gastrointestinais simuladas, as micropartículas ofereceram maior proteção.

Segundo Piñón-Balderrama et al. (2020), diversos fatores influenciam o processo de secagem por spray, alterando as características finais dos produtos secos, como morfologia, distribuição e tamanho de partícula, teor de umidade, eficiência de encapsulamento, rendimento e qualidade do produto. Dentre os fatores tem-se:

- Temperatura de entrada: é a temperatura do fluxo de gás utilizado na secagem, a qual determina a capacidade de evaporação do solvente. Esta deve ser escolhida a mais alta possível para obter um produto com menor teor de umidade sem que ocorra a degradação térmica dos componentes bioativos. A alta temperatura de entrada ajuda a evitar a aglomeração de partículas, a adesão destas à parede da câmara de secagem e ruptura da microestrutura. Durante o processo, à medida que o gás de secagem é submetido às gotículas atomizadas, a temperatura do ar de entrada diminui, o que influência a temperatura de saída. Além do que, à medida que a temperatura de entrada aumenta, o tamanho das partículas aumenta e o teor de umidade diminui, aumentando assim o rendimento do processo.

- Temperatura de saída: é uma variável experimental, a qual não pode ser controlada manualmente no equipamento, e sim uma consequência da temperatura do ar de entrada do gás, taxa de fluxo, entalpia de evaporação e concentração de sólidos no fluido.

- Taxa de alimentação: refere-se ao fluxo volumétrico (mL / min) do fluxo de fluido, o qual tem um efeito direto no tamanho das partículas do produto final obtido. Então, quanto maior for a taxa do fluxo de alimentação, maior será o tamanho de partícula do pó obtido. Ademais, o teor de umidade é aumentado com o uso de altas taxas de alimentação.

- Taxa do aspirador: é uma medida do volume de gás comprimido que entra na câmara de secagem através do bico de atomização. Um maior grau de separação dentro do cilindro é obtido com altas taxas de aspiração. Além disso, uma baixa taxa de aspiração resulta em baixa umidade residual.

- Tempo de residência: é o tempo transcorrido desde a entrada do fluido atomizado na câmara de secagem até a sua saída na forma de partículas secas. Um tempo de residência mais curto (10-15 s) é recomendado para obtenção de uma grande quantidade de partículas mais finas, livres de umidade, enquanto que um tempo de residência médio (25-35 s) deve ser aplicado para obtenção de partículas

finas a semi-grossas. Ademais, para secar fluidos de alta viscosidade é necessário um tempo de residência maior.

- Concentração da solução de alimentação: refere-se à concentração inicial de sólidos dissolvidos no fluido alimentado. Quanto maior a concentração de sólidos na solução de alimentação, maior será a viscosidade e, consequentemente, partículas aglomeradas e de formato irregular podem ser formadas. Além disso, aumentando-se a concentração de sólidos, aumenta-se o tamanho da partícula, uma vez que acarreta na diminuição do solvente de evaporação, o qual também está relacionado a temperatura de saída.

Nunes et al. (2018b) avaliaram a influência de diferentes temperaturas por spray drying na microencapsulação de células probióticas de *Lactobacillus acidophillus* La-5 e *Bifidobacterium* Bb-12 e relataram uma maior eficiência de encapsulação com a temperatura de secagem de 130 °C. Os autores também relataram tamanhos de partícula de 4,85 µm para *Lactobacillus acidophillus* La-5 e 8,75 µm para *Bifidobacterium* Bb-12, estando de acordo com os valores desejáveis para produtos obtidos por spray drying.

2.3.2 Agentes encapsulantes

Os agentes encapsulantes ou materiais de parede atuam como uma barreira física, protegendo os ingredientes bioativos das condições adversas do meio, além de proporcionarem aumento da estabilidade e preservação das propriedades funcionais (PIÑÓN-BALDERRAMA et al., 2020). Por isso, a seleção do agente encapsulante utilizado na microencapsulação é um fator importante, uma vez que influencia na eficiência do processo e na estabilidade das microcápsulas (MAHDAVI et al., 2014).

A escolha do agente encapsulante depende de uma série de fatores, entre eles: a não reatividade com o material a ser encapsulado durante o processo e estocagem; o processo utilizado para a formação da microcápsula; o mecanismo e liberação do material encapsulado; suas propriedades reológicas; a habilidade de dispersar ou emulsificar; a capacidade de prover máxima proteção para o material a ser encapsulado contra condições desfavoráveis, como alimentos com alta atividade de água (WEINBRECK; BODNÁR; MARCO, 2010), temperatura e presença de oxigênio atmosférico (ANAL; SINGH, 2007), das condições ácidas do estômago e dos sucos

biliares do intestino delgado (KENT; DOHERTY, 2014) e ser economicamente viável (ASSUNÇÃO et al., 2014).

Vários polímeros são utilizados como encapsulantes, tais quais alginato, agarose, quitosana, carboximetilcelulose, goma arábica, carragena, gelatina, caseína, pectina e amidos (DE MENEZES et al., 2013; GASPERINI; MANO; REIS, 2014).

A maltodextrina é um polissacarídeo comumente utilizado como agente encapsulante, uma vez que possui baixo custo, boa solubilidade, baixa viscosidade e elevada eficiência de encapsulação (PIECZYKOLAN; KUREK, 2019). A inulina é um carboidrato de reserva, naturalmente encontrado em diversos vegetais. Dentro dos polissacarídeos, ela faz parte do grupo dos frutanos e sua extração ocorre principalmente a partir de raízes como a chicória (BIEDRZYCKA; BIELECKA, 2004; KAWAI et al., 2011). A inulina tem recebido bastante atenção devido aos benefícios para a saúde atribuídos às fibras dietéticas não digeríveis pelo trato gastrointestinal e pela sua natureza prebiótica (BURITI et al., 2007; KIM, 2002). No entanto, devido principalmente a baixa temperatura de transição vítria, a inulina apresenta um comportamento glutinoso, limitando a sua aplicação como material de parede no encapsulamento de bactérias probióticas. A adição de agentes de alto peso molecular é capaz de alterar a temperatura de transição vítria, minimizando a viscosidade durante a secagem por spray (MOAYYEDI et al., 2018).

Araujo-Díaz et al. (2017) avaliaram o uso de inulina e maltodextrina como agentes encapsulantes na microencapsulação de suco de mirtilo por secagem em spray, a fim de conservar as propriedades antioxidantes. Os autores relatam que ambos os agentes transportadores foram adequados para encapsular o suco de mirtilo, mas que a maltodextrina com baixo equivalente de dextrose apresentou maior teor de antioxidantes. Isso demonstra que, diferenças na estrutura química do agente encapsulante ou material de parede podem levar a afinidades diferentes e interações com os antioxidantes.

3 OBJETIVOS

3.1 OBJETIVO GERAL

O objetivo geral deste trabalho consistiu em produzir microcápsulas probióticas contendo *Lactobacillus rhamnosus* juntamente com a adição de extrato de mirtilo em diferentes concentrações e avaliar se a adição de extrato contribui para uma maior eficiência de encapsulação, viabilidade e estabilidade das microcápsulas.

3.2 OBJETIVOS ESPECÍFICOS

- Verificar se a adição de diferentes concentrações de extrato de mirtilo às formulações de alimentação apresenta algum efeito sobre a eficiência de encapsulamento quanto a sobrevivência das bactérias probióticas;

- Verificar se as microcápsulas formadas contendo *Lactobacillus rhamnosus* e diferentes concentrações de extrato de mirtilo apresentam características de produtos obtidos por spray drying quanto ao tamanho, forma e distribuição, bem como verificar se há efeito da adição do extrato na morfologia das microcápsulas;

- Determinar se as bactérias probióticas de *Lactobacillus rhamnosus* livres e microencapsuladas apresentam resistência às condições do trato gastrointestinal simuladas, e se há efeito positivo na resistência das microcápsulas pela adição do extrato de mirtilo;

- Determinar se as bactérias probióticas livres e microencapsuladas sobrevivem ao armazenamento pelo período de 120 dias, sob temperatura ambiente (25 °C), refrigerada (7 °C) e de congelamento (-18 °C), bem como determinar se há efeito positivo na sobrevivência dos *Lactobacillus rhamnosus* ao armazenamento sob diferentes temperaturas pela adição do extrato de mirtilo;

- Determinar se as bactérias probióticas livres e microencapsuladas resistem ao tratamento térmico a 63 e 72 °C, bem como se há efeito positivo na proteção térmica das microcápsulas pela adição do extrato de mirtilo;

- Avaliar se a cor das microcápsulas tem relação com a concentração de extrato de mirtilo adicionado às formulações de alimentação; e

- Avaliar se após o processo de encapsulamento as microcápsulas apresentam boa retenção de antocianinas.

4 ARTIGO 1 – INCREASED STABILITY OF BLUEBERRY PHENOLIC COMPOUNDS BY MICROENCAPSULATION

Increased stability of blueberry phenolic compounds by microencapsulation

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Abstract

Blueberries are a rich source of bioactive compounds, including vitamin C and phenolic compounds. The growing interest in these berries is associated with the beneficial effects that consuming them provides consumers and their significant amount of anthocyanins, which are responsible for their intense blue color. However, the compounds are highly unstable and susceptible to degradation, and anthocyanins are known to be sensitive to light, oxygen, temperature, and pH variations. Therefore, microencapsulation is an efficient and promising method to increase the stability and improve the half-life of biologically active compounds, promoting their incorporation and protection. This review article discusses the phenolic composition present in blueberry fruits by focusing mainly on the composition of anthocyanins, which represent the most widely studied class of blueberry phenolic compounds. Additionally, we provide an overview of the main factors affecting anthocyanin stability and the importance of microencapsulation to increase blueberry phenolic compound stability during processing and storage before their application in food and improve bioavailability and controlled release in the gastrointestinal system.

Keywords: *Vaccinium myrtillus*. Bioactive compounds. Anthocyanins. Bioavailability. Encapsulation.

Resumo

Mirtilo é uma rica fonte de compostos bioativos, incluindo vitamina C e compostos fenólicos. O crescente interesse nestas bagas está associado aos efeitos benéficos que o seu consumo proporciona aos consumidores e à sua significativa quantidade de antocianinas, responsáveis pela sua intensa cor azul. No entanto, os compostos são altamente instáveis e suscetíveis à degradação, e as antocianinas são conhecidas por serem sensíveis à luz, oxigênio, temperatura e variações de pH. Portanto, a microencapsulação é um método eficiente e promissor para aumentar a estabilidade e melhorar a meia-vida dos compostos biologicamente ativos, promovendo a incorporação e proteção. Este artigo de revisão discute a composição fenólica presente nos frutos de mirtilo focando principalmente na composição das antocianinas, que representam a classe de compostos fenólicos do mirtilo mais amplamente estudada. Além disso, fornecemos uma visão geral dos principais fatores que afetam a estabilidade das antocianinas e a importância da microencapsulação para a estabilidade dos compostos fenólicos do mirtilo durante o processamento e armazenamento antes da sua aplicação em alimentos, e melhorar a biodisponibilidade e liberação no sistema gastrointestinal.

Palavras-chave: *Vaccinium myrtillus*. Compostos bioativos. Antocianinas. Biodisponibilidade. Encapsulamento.

1. Introduction

The blueberry (*Vaccinium myrtillus*) is a fruit belonging to the genus *Vaccinium*, of the Ericaceae family, which has about 450 species that includes trees, shrubs, and others around the world, especially in the colder regions of the northern hemisphere (COLAK et al., 2016; PIRES et al., 2020). Berry-type fruits have different flavors and are usually consumed fresh, and their high durability leads them to be frozen, dried, and processed in numerous ways (PIRES et al., 2020; SATER et al., 2020). Blueberry storage time varies between 14 and 20 days at 0 °C, depending on factors such as cultivar, maturation stage, harvesting method, and storage conditions (FALAGÁN; MICLO; TERRY, 2020).

Also known as the "superfruit," blueberries have gained great notoriety and become an important part of the typical diet, in addition to being used to produce

medicine (PIRES et al., 2020; SILVA et al., 2020). Moreover, blueberries are recognized for promoting health benefits given they are rich in polyphenolic compounds, vitamins, fibers, and minerals. Consuming blueberry fruits confers countless health benefits, including improved metabolic function and cognitive performance (WHYTE et al., 2021), reduced effects of oxidative stress (BHATT; DEBNATH, 2021), and neuroprotective (TRAN; TRAN, 2021), cardioprotective (SUN et al., 2019), anti-inflammatory (DRISCOLL et al., 2020), anti-cancer (SEZER et al., 2019), anti-obesity (JIAO et al., 2019), hypoglycemic (HUANG et al., 2018), ocular (LOU et al., 2021), antiproliferative (LAMDAN et al., 2020), and antibacterial properties (ZHOU et al., 2020b).

Among the class of polyphenols, anthocyanins are the most abundant and responsible for the dark blue color of this fruit. According to the literature, fifteen main compounds characterize the anthocyanin profile of the blueberry, which are derived from the aglycones delphinidin, cyanidin, petunidin, peonidin, and malvidin and are linked to sugar moieties of glucosides, galactosides, and arabinosides (PIRES et al., 2020). Nonetheless, anthocyanins are quite unstable and susceptible to degradation (GIUSTI; WROLSTAD, 2001), and their stability is affected by several factors, including chemical structure, pH, oxygen action, temperature variation, incidence of light, presence of enzymes, and interactions between food components, such as ascorbic acid, metal ions, sugars, and copigments (CASTAÑEDA-OVANDO et al., 2009). Anthocyanins have low bioavailability due to their susceptibility to changes in pH, and at pH values equal to or below 3.5, anthocyanins are generally stable in stomach conditions. However, anthocyanins are easily degraded at higher pH values (e.g., in the gastrointestinal system), thus reducing their nutritional value (MAHDAVI et al., 2014).

Hence, one way of improving the bioavailability of anthocyanins and protecting them against degradation is through encapsulation techniques. Microencapsulation allows sensitive bioactive compounds to be stored, decelerates degradation processes, and improves delivery to specific locations (BORA et al., 2018; PIECZYKOLAN; KUREK, 2019). This low-cost technology is rapidly spreading and highly advantageous (LAOKULDILOK; KANHA, 2015). In the food industry, there are different microencapsulation methods and types of coating and core materials. However, choosing the appropriate coating method and material is essential to ensure

the effective protection of the encapsulated compounds and guarantee the safe and controlled release of the active materials (BORA et al., 2018; LAOKULDILOK; KANHA, 2015).

Given the above, this review aimed to highlight the bioactive components of the blueberry and describe the known compounds in the fruit to address the main factors that affect anthocyanin stability and demonstrate the importance of microencapsulation in the bioavailability and preservation of these bioactive components during storage and processing.

2 Blueberry bioactive compounds

Blueberries are small berries and important sources of bioactive compounds such as phenolic compounds and anthocyanins, which are responsible for the high antioxidant activity of these fruits (SHI et al., 2017). Regularly consuming blueberries provides a series of health benefits, including weight loss, higher insulin resistance, lower insulin levels, LDL, total cholesterol, and uric acid (ISTEK; GURBUZ, 2017).

The bioactive composition of blueberries and other fruits depends on several factors, such as the species and cultivar, maturation, climatic conditions, location of the plant, and year of cultivation (GÜNDÜZ; SERÇE; HANCOCK, 2015). Phenolic compounds are found in blueberries and a class of chemical substances derived from the secondary metabolism of plants, having at least one phenyl group in their chemical structure with one or more hydroxy substituents such as esters or glycosides (RAHAIEE et al., 2020). The numerous possibilities of ligands in the aromatic ring generate several phenolic compounds, where more than ten thousand different structures have already been identified (TAIZ and ZEIGER, 2006). Additionally, numerous studies have sought to clarify which blueberry compounds are responsible for their bioactive activity as there are many phenolic compounds.

Research has described a broad range of phenolic compounds in different species and blueberry cultivars, identifying compounds from different classes of phenolics and emphasizing the class of flavonoids (Table 1). Regarding flavonoids, anthocyanins can be found at concentrations of 3.14 to 301 mg/100 g of fruit (DEBNATH-CANNING et al., 2020; GRACE et al., 2019; LI et al., 2017; LI; MENG; LI, 2016; WANG et al., 2017; ZHOU et al., 2020a). This vast difference between

anthocyanin content may be correlated with the color of the fruits, which may vary depending on the species (Figure 1) and blueberry cultivar, and pink-colored blueberries have lower anthocyanin content, while blueberries with high anthocyanin content are bluish (GRACE et al., 2019). In addition, the color of the fruits can also be influenced by the degree of anthocyanin hydroxylation and methylation, where the hydroxyl groups are responsible for increasing the blue tint, while the methoxyl groups increase the red tint (RODRIGUEZ-AMAYA, 2019). Other non-anthocyanin flavonoids are found in these fruits at concentrations ranging from 0.262 to 27.10 mg/100 g of fruit. In addition to flavonoids, other phenolic compounds have been identified, including phenolic acids at concentrations between 0.12 and 63.74 mg/100 g of fruit (GRACE et al., 2019; WANG et al., 2017).

Research has usually focused on identifying anthocyanins in blueberries. This is mainly because anthocyanins are the major phenolic compounds in these fruits (GRACE et al., 2019; WANG et al., 2017), and blueberry anthocyanins provide beneficial effects, such as neuroprotective (GRACE et al., 2019) and antibacterial properties (SHAO et al., 2019; SUN et al., 2018), prebiotic potential (MAR et al., 2020), and protect retinal cells against oxidative stress and inflammation induced by diabetes (SONG; HUANG; YU, 2016). Nonetheless, anthocyanins are sensitive to several factors, making it necessary to use technologies that improve their protection to reach the intestine and be adequately absorbed to exert their bioactive functions.

Table 1 - Phenolic compounds identified in blueberries (Vaccinium)

		Compound	References
Flavonoids	Anthocyanins	Cyanidin-3-O-acetylglucoside	(GRACE et al., 2019; PERTUZATTI et al., 2016)
		Cyanidin-3-O-acetylhexoside	(LIN et al., 2020)
		Cyanidin-3-O-acetylgalactoside	(PERTUZATTI et al., 2016)
		Cyanidin-3-O-arabinoside	(GRACE et al., 2019; LIN et al., 2020; PERTUZATTI et al., 2016; WU et al., 2017; ZHOU et al., 2020a)
		Cyanidin-3,5-dihexoside	(LIN et al., 2020)
		Cyanidin-3-O-galactoside	(GRACE et al., 2019; LIN et al., 2020; PERTUZATTI et al., 2016; WU et al., 2017; ZHOU et al., 2020a)
		Cyanidin-3-O-glucoside	(GRACE et al., 2019; LIN et al., 2020; PERTUZATTI et al., 2016; WU et al., 2017; ZHOU et al., 2020a)
		Cyanidin-3-malonylgalactoside	(PERTUZATTI et al., 2016)
		Cyanidin-3-malonylglucoside	(PERTUZATTI et al., 2016)
		Cyanidin-3-O-rutinoside-5-glucoside	(GRACE et al., 2019; PERTUZATTI et al., 2016; WU et al., 2017)
		Cyanidin- 3-xyloside	(PERTUZATTI et al., 2016)
		Delphinidin-3-acetylgalactoside	(PERTUZATTI et al., 2016)
		Delphinidin-3-O-acetylglicosídeo	(ZHOU et al., 2020a)
		Delphinidin-3-O-arabinoside	(GRACE et al., 2019; LIN et al., 2020; PERTUZATTI et al., 2016; WU et al., 2017; ZHOU et al., 2020a)
		Delphinidin-3-O-galactoside	(GRACE et al., 2019; LIN et al., 2020; PERTUZATTI et al., 2016; WU et al., 2017; ZHOU et al., 2020a)
		Delphinidin-3-O-glucoside	(GRACE et al., 2019; LIN et al., 2020; PERTUZATTI et al., 2016; WU et al., 2017; ZHOU et al., 2020a)
		Delphinidin-3-rutinoside	(LIN et al., 2020)
		Delphinidin-3-malonylgalactoside	(PERTUZATTI et al., 2016)
		Delphinidin-3-malonylglucoside	(PERTUZATTI et al., 2016)
		Delphinidin-3-O-rutinoside-5-glucoside	(MAR et al., 2020)
		Delphinidin-3-O-rutinoside-5-pentoside	(MAR et al., 2020)
		Delphinidin-3-xyloside	(PERTUZATTI et al., 2016)
		Malvidin-3-O-acetylhexoside	(LIN et al., 2020)
		Malvidin-3-O-acetylgalactoside	(GRACE et al., 2019; PERTUZATTI et al., 2016; WU et al., 2017)
		Malvidin-3-O-acetylglucoside	(GRACE et al., 2019; PERTUZATTI et al., 2016; WU et al., 2017)
		Malvidin-3-O-arabinoside	(GRACE et al., 2019; LIN et al., 2020; PERTUZATTI et al., 2016; WU et al., 2017; ZHOU et al., 2020a)

		Malvidin-3-O-galactoside	(GRACE et al., 2019; LIN et al., 2020; PERTUZATTI et al., 2016; WU et al., 2017; ZHOU et al., 2020a)
		Malvidin-3-O-glucoside	(GRACE et al., 2019; LIN et al., 2020; PERTUZATTI et al., 2016; WU et al., 2017; ZHOU et al., 2020a)
		Malvidin-3-malonylgalactoside	(PERTUZATTI et al., 2016)
		Malvidin-3-malonylglucoside	(PERTUZATTI et al., 2016)
		Malvidin-3-xyloside	(PERTUZATTI et al., 2016; ZHOU et al., 2020a)
		Peonidin-3-acetylgalactoside	(GRACE et al., 2019)
		Peonidin-3-acetylglucoside	(GRACE et al., 2019; PERTUZATTI et al., 2016)
		Peonidin-3-arabinoside	(PERTUZATTI et al., 2016)
		Peonidin-3-O-galactoside	(GRACE et al., 2019; PERTUZATTI et al., 2016; WU et al., 2017; ZHOU et al., 2020a)
		Peonidin-3-O-glucoside	(GRACE et al., 2019; PERTUZATTI et al., 2016; WU et al., 2017; ZHOU et al., 2020a)
		Peonidin-3-xyloside	(PERTUZATTI et al., 2016)
		Petunidin-3-O-acetylgalactoside	(GRACE et al., 2019)
		Petunidin-3-O-acetylglucoside	(GRACE et al., 2019; WU et al., 2017)
		Petunidin-3-acetylhexoside	(LIN et al., 2020)
		Petunidin-3-O-arabinoside	(GRACE et al., 2019; LIN et al., 2020; WU et al., 2017)
		Petunidin-3-O-galactoside	(GRACE et al., 2019; WU et al., 2017; ZHOU et al., 2020a)
		Petunidin-3-O-glucoside	(GRACE et al., 2019; LIN et al., 2020; WU et al., 2017; ZHOU et al., 2020a)
		Catechin	(GRACE et al., 2019; WANG et al., 2017)
		Epicatechin	(GRACE et al., 2019)
		Resveratrol	(GRACE et al., 2019; WANG et al., 2017)
		Epigallocatequin	(MAR et al., 2020)
		Epigallocatequin gallate	(MAR et al., 2020)
		Isorhamnetin	(PERTUZATTI et al., 2021)
		Isorhamnetin-3-(6″-rhamnosyl)galactoside	(PERTUZATTI et al., 2021)
		Isorhamnetin-3-(6″-rhamnosyl)glucoside	(PERTUZATTI et al., 2021)
Other flavonoids		Isorhamnetin-3-acetylhexoside	(PERTUZATTI et al., 2021)
		Isorhamnetin-3-galactoside	(PERTUZATTI et al., 2021)
		Myricetin-3-(6″-rhamnosyl)galactoside	(PERTUZATTI et al., 2021)
		Myricetin-3-(6″-rhamnosyl)glucoside	(PERTUZATTI et al., 2021)
		Myricetin-3-galactoside	(PERTUZATTI et al., 2021)
		Myricetin-3-glucoside	(GRACE et al., 2019; PERTUZATTI et al., 2021)
		Myricetin-3-glucuronide	(PERTUZATTI et al., 2021)
		Myricetin-3-pentoside	(PERTUZATTI et al., 2021)
		Myricetin-3-rhamnoside	(PERTUZATTI et al., 2021)

		Kaempferol-glucoside	(GRACE et al., 2019; PERTUZATTI et al., 2021)
		Laricitrin	(PERTUZATTI et al., 2021)
		Laricitrin-3-galactoside	(PERTUZATTI et al., 2021)
		Laricitrin-3-glucuronide	(PERTUZATTI et al., 2021)
		Laricitrin-3-glucuside	(PERTUZATTI et al., 2021)
		Quercetin	(GRACE et al., 2019; PERTUZATTI et al., 2021; WANG et al., 2017)
		Quercetin-3-(6″-rhamnosyl)galactoside	(PERTUZATTI et al., 2021)
		Quercetin-3-(6″-rhamnosyl)glucoside	(PERTUZATTI et al., 2021)
		Quercetin-3-acetylhexoside	(PERTUZATTI et al., 2021)
		Quercetin-arabinoside	(GRACE et al., 2019)
		Quercetin-galactoside	(GRACE et al., 2019; PERTUZATTI et al., 2021)
		Quercetin-glucoside	(GRACE et al., 2019; PERTUZATTI et al., 2021)
		Quercetin-3-pentoside	(PERTUZATTI et al., 2021)
		Quercetin-3-*O*-[4″-(3-hydroxy-3-methylglutaroyl)]-α-rhamnoside	(PERTUZATTI et al., 2021)
		Quercetin-3-O-rhamnoside	(MAR et al., 2020; PERTUZATTI et al., 2021)
		Hydroxytyrosol	(WU et al., 2017)
		Syringetin	(PERTUZATTI et al., 2021)
		Syringetin-3-acetylhexoside	(PERTUZATTI et al., 2021)
		Syringetin-3-galactoside	(PERTUZATTI et al., 2021)
		Syringetin-3-glucoside	(GRACE et al., 2019; PERTUZATTI et al., 2021)
		Syringetin-3-glucuronide	(PERTUZATTI et al., 2021)
		Syringetin-3-pentoside	(PERTUZATTI et al., 2021)
		Syringetin-3-rhamnoside	(PERTUZATTI et al., 2021)
		Procyanidin B1	(GRACE et al., 2019)
		Procyanidin B2	(GRACE et al., 2019)
		2,4 Hyroxybenzoic acid	(GRACE et al., 2019)
		3-(carboxymethyl)-3- (hydroxymethyl)-pentanedioic acid	(WU et al., 2017)
		5-O-Caffeoylquinic acid	(WU et al., 2017)
		Caffeic acid hexose (I)	(WU et al., 2017)
Non-flavonoids	Phenolic acids	Caffeic acid hexose (II)	(WU et al., 2017)
		Caffeic acid	(GRACE et al., 2019; WANG et al., 2017)
		Chlorogenic acid	(GRACE et al., 2019; PERTUZATTI et al., 2021)
		Dicaffeoylquinic acid	(WU et al., 2017)
		Dihydroferulic acid-4'-sulfate	(WU et al., 2017)
		Ferulic acid	(WANG et al., 2017)
		Ferulic acid hexose	(WU et al., 2017)

Feruloylquinic acid	(WU et al., 2017)
Gallic acid	(GRACE et al., 2019; MAR et al., 2020)
p-coumaric acid	(WANG et al., 2017)
p-coumaric acid hexose	(WU et al., 2017)
Pyrogallol	(WU et al., 2017)
Protocatechuic acid-3-O-rhamnose	(WU et al., 2017)
Salicylic acid	(WANG et al., 2017)
Trans-5-O-caffeoylquinic acid isomer	(WU et al., 2017)

Source: "Scopus" data search platform with the keywords "blueberry," and "phenolic compounds" since 2016.

Figure 1 - Different blueberry colors

A) Pink-colored fruits. B) Blue-colored fruits.
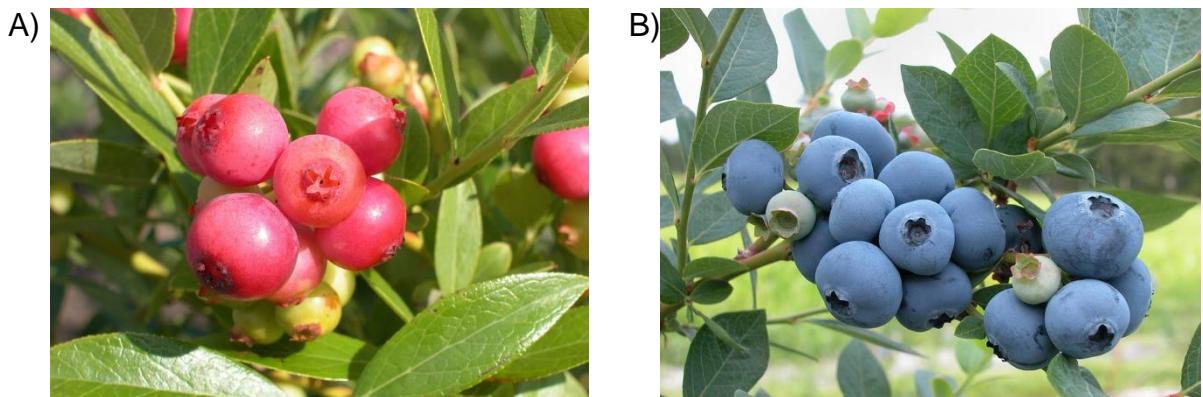
Source: Pixabay (2020)

3 The main factors that affect anthocyanin stability

Temperature

Temperature is a pivotal factor in anthocyanin stability, and temperatures above 25 ºC increase instability, consequently leading to degradation (FRACASSETTI et al., 2013). Many studies have demonstrated a logarithmic relationship between the destruction of anthocyanins and the arithmetic increase in temperature (FRACASSETTI et al., 2013; LIU et al., 2018; PATRAS et al., 2010). Sun et al. (2011) reported that the thermal degradation of anthocyanins occurs in three ways: the hydrolysis of the glycosidic bond, which forms a highly unstable aglycone anthocyanidin and proceeds through an intermediate α-diketone pathway to form aldehydes and benzoic acid derivatives. Another route involves the hydrolytic opening of the pyrrolic ring and subsequent hydrolysis of the glycosidic bond to generate the pseudo-base carbinol. The third form is the route in which the pseudo-carbinol is transformed into chalcone, and later, into glycosides of coumaric acid, which are both colorless.

Experiments involving anthocyanin degradation have indicated the stability of blueberry juices at temperatures of up to 60 ºC and highlighted the acceleration of degradation at higher temperatures. Samples placed at 80, 90, 100, 110, and 115 ºC

had a 50% loss of anthocyanins at the time intervals of 180, 115, 40, 15, and 10 min, respectively (BUCKOW et al., 2010). The main anthocyanins of blueberry fruits are malvidin-3-glycoside and delphinidin-3-glycoside and small amounts of peonidin-3-glycoside and petunidin-3-glycoside. Buckow et al. (2010) also reported that the stability of different juices in relation to temperature varies with the different anthocyanins found in each fruit. Thermal stability decreases according to the number of hydroxyls linked to anthocyanin and the position of the glycosides, thus indicating the relationship between the chemical structure and anthocyanin stability. Still, anthocyanin stability against temperature is influenced by the degree of acylation, and acylated anthocyanins are more stable than non-acylated anthocyanins (ZHAO et al., 2017). This is because the acyl groups protect the flavylium cation from the nucleophilic attack of water, preventing them from becoming colorless pseudobase or chalcone structure (ZHAO et al., 2017).

Fracassetti et al. (2013) studied the effects of storage time and temperature on anthocyanin decomposition and antioxidant activity in blueberry powder and observed that storage reduced anthocyanin content at all temperatures, being slower at 25 °C (-3% after two weeks) and faster at 60 °C (-60%) and 80 °C (-85%) after three days. No significant results were observed for antioxidant activity. This is likely due to low molecular weight antioxidant polymers, such as procyanidins, or the formation of degradation products of anthocyanins or phenolic acids, which also present antioxidant activity (FRACASSETTI et al., 2013). In addition, the authors revealed that the anthocyanins bound to galactose were more heat-stable, these compounds being Cy-gal, Mv-gal, Pt-gal, and Pe-gal, while Pt-glc and Cy-ara were the most unstable. According to Trost et al. (2008), anthocyanins linked to glucose and galactose are more stable than those linked to arabinose, and this is due to the steric impediment, which is greater for hexose sugars.

Consistent with the results above, Zorenc et al. (2017) evaluated the effects of different temperatures (4 °C, room temperature, 50, 75, and 100 °C for 2 h) on the stability of blueberry purees and reported that samples prepared at higher temperatures were characterized by an 11 to 32% decrease in total phenolic content and 38 to 64% reduction in total anthocyanins. Thus, anthocyanin stability is significantly influenced by temperature (PATRAS et al., 2010).

Hydrogenionic potential (pH)

Anthocyanin stability is highly affected by pH and more stable at lower pH. In highly acidic conditions, anthocyanins exist as flavylium cations (red color at pH <2). At higher pH, the anthocyanins undergo hydration through the nucleophilic attack of water, where the cation form changes to a pseudo-base form of colorless carbinol and later to the colorless chalcone form. In slightly acidic to neutral conditions (>5), the flavylium cation undergoes deprotonation, generating a neutral (blue) quinoidal base (CHEN; INBARAJ, 2019). Hence, the change in pH leads to a shift in the structural configuration of anthocyanins, in which as the pH of the medium increases, the number of conjugated double bonds decreases, characterizing the loss of color due to cleavage by pyrylium ring (ring C) (TARONE; CAZARIN; JUNIOR, 2020).

Liu et al. (2018) investigated the effects of temperature and pH on the stability of total and individual anthocyanin in blueberries and noted a rapid decrease in the total anthocyanin content and the half-life of only 4 h at pH 8.0 and 9.0. The authors also demonstrated that the anthocyanins malvidin-3-O-galactose and malvidin-3-O-arabinose showed alkaline resistance. Furthermore, malvidin-3-O-arabinose content increased after heating, differently from the other blueberry anthocyanins under the condition of pH 5.0-6.0, and anthocyanin stability was relatively better at pH below 3.0 (LIU et al., 2018). Similar effects were also reported by Jamei and Babaloo (2017), who demonstrated that higher pH values caused significant destruction of anthocyanins.

In response to the processing of blueberry juice, the effects of acidification (pH 2.1, 2.5, and 2.9 (control)) were studied by Howard et al. (2016) regarding changes in anthocyanins and the percentage of polymeric color during storage at room temperature and refrigerated. Higher levels of total anthocyanins and lower percentages of polymeric color were observed in the acidified juice at pH 2.1 after pasteurization. In addition, after eight months of storage, the acidified juice at pH 2.1 showed, on average, 11 and 22% higher levels of total anthocyanins than the juice at pH 2.5 and the control juice stored at 4 ºC, and 26 and 59% of total anthocyanins higher than juices at pH 2.5 and the control stored at 25 ºC. Furthermore, the authors observed that the anthocyanin arabinoside compounds were more susceptible to thermal degradation than glycosides, galactosides, and acetylated derivatives. In

summary, the study findings showed that low temperatures and pH are beneficial for blueberry anthocyanin stability.

Exposure to light

When exposed to ultraviolet and visible light or other sources of ionizing radiation, anthocyanins are generally unstable (MALACRIDA; MOTTA, 2005). As in thermal degradation, the same final products are found in light-induced degradation, although through a different kinetic pathway involving the excitation of the flavylium cation, resulting in a decrease in the colored forms (FURTADO et al., 1993); (TARONE; CAZARIN; JUNIOR, 2020). Peng, Liu and Wang (2016) analyzed the effects of light intensity on the anthocyanin stability of blueberry peels and found that anthocyanins were stable without the presence of light in indoor, but decreased when exposed in indoor or outdoor with increase of light intensity. A similar effect was observed by Liu et al. (2018) when light accelerated the breakdown of blueberry anthocyanins. Song et al. (2018) suggested that blueberry juice be kept out of the light as they observed a rapid color reduction rate in the first seven days during exposure to light and that the colors were continually destabilized after three weeks. In contrast, more than 90% of the color could be seen in the dark after seven days.

Exposure to oxygen

Another significant factor in anthocyanin degradation is the presence of oxygen in the medium, albeit in the absence of light and at all pH values. This degradation occurs through direct or indirect oxidation mechanisms when oxidized constituents of the medium react with anthocyanins (LOPES et al., 2007). The formation of hydrogen peroxide (H_2O_2), formed by the oxidation of ascorbic acid in the presence of oxygen and copper ions, causes the anthocyanins to discolor. Malacrida and Motta (2005) suggested that anthocyanin degradation under these conditions is mediated by H_2O_2. Evidence indicates that the condensation reaction between ascorbic acid and anthocyanin is the primary degradation mechanism, forming unstable products that degrade into colorless compounds (DE ROSSO; MERCADANTE, 2007; WROLSTAD; DURST; LEE, 2005). Santos-Buelga and González-Paramás (2018) revealed that anthocyanin degradation occurred when induced by ascorbic acid in the presence of

oxygen due to cleavage of the pyrylium ring as well as by the photo-oxidative mechanism when exposed to light. In addition, Patras et al. (2010) reported that, in the presence of oxygen, enzymes such as polyphenol oxidase (PPO) catalyze the oxidation of chlorogenic acid to the corresponding o-quinone (chlorogenoquinone), which reacts with anthocyanins to form brown condensation products.

Zheng et al. (2003, 2010) analyzed the influence of atmospheres with high oxygen content during blueberry fruit storage and noted that, in the initial stage of maturation, treatments with high oxygen had few effects on phytochemicals. In the first days of storage, the authors could observe higher levels of total anthocyanins, total phenolics, and several individual flavonoid compounds, in addition to the increased antioxidant capacity. Nevertheless, these effects diminished with prolonged storage. According to Zheng et al. (2003), the effects of high oxygen exposure on total anthocyanins, total phenolics, and antioxidant potential may vary depending on the product, oxygen concentration, storage time, and temperature. Costa, Almeida and Pintado (2015) also evaluated the effects of atmospheric composition on the content of blueberry phenolic compounds during storage and observed improved preservation of anthocyanins with low oxygen content (2% O_2).

4 Microencapsulation of the blueberry bioactive compounds

Microencapsulation is defined as a process by which bioactive agents are trapped within a coating material to protect the core material and deliver it at a specific time and place (SHARIF; KHOSHNOUDI-NIA; JAFARI, 2020). Several benefits to the bioactive agent are attributed to microencapsulation, such as improved oxidative stability (SVANBERG et al., 2019), thermal and photoprotective properties (XU et al., 2019), bioavailability (MUELLER et al., 2018), controlled release (CAI et al., 2019), ease of manipulation, among others.

Several studies have reported the use of microencapsulation to protect blueberry bioactive compounds (Table 2). Microencapsulation helps maintain stability and improve the half-life of molecules and compounds sensitive to light and heat, as is the case with anthocyanins (CAI et al., 2019). Therefore, in order to improve the stability of blueberry anthocyanins, Xu et al. (2019) investigated the effects of microencapsulation and observed better anthocyanin retention rates (>75%) after microencapsulation and exposure to higher temperatures and extended heating times.

In addition, blueberry anthocyanins were more resistant to light after microencapsulation. A similar reaction was found by Guo, Giusti and Kaletunç (2018) when encapsulation significantly reduced anthocyanin degradation rates after exposure to light. Still, Cai et al. (2019) demonstrated that, during *in vitro* release, anthocyanins were primarily retained in the microcapsules in the stomach phase and released in the intestine. Da Rosa et al. (2019) also observed better anthocyanin delivery in gastrointestinal system simulation. Wu et al. (2017) reported adequate storage time and temperature of three months and -18 ºC, respectively, for blueberry anthocyanin microcapsules coated with polysaccharide-protein as coating material.

Different coating materials can be used in microencapsulation, including proteins, carbohydrates, gums, fibers, and the mixture of these compounds in various proportions (ROCHA et al., 2019; TAO et al., 2017). However, these must be food-grade, have low hygroscopicity and biodegradability, and above all, protect the bioactive material from adverse conditions and maintain its stability, forming a barrier between the external and internal phases during processing and storage (ROCHA et al., 2019). Maltodextrin, alone or combined with other biopolymers, was the most used coating material, and 13 of the 30 (43.33%) studies, followed by whey protein and gum arabic, with 12 (40%) and 8 (26.67%) of the 30 cases. Maltodextrin is widely used in microencapsulation because it has good solubility, low viscosity, and high encapsulation efficiency, in addition to being a low-cost and easily accessible material (PIECZYKOLAN; KUREK, 2019). Bora et al. (2018) demonstrated that maltodextrin has good film formation capacity, oxygen protection, and agglomeration resistance. Whey proteins, in particular, have proven to be excellent coating materials to microencapsulate bioactive compounds, as in addition to their nutritional value, they are capable of forming a gel with covalent and/or electrostatic complexes with the molecules of interest (ROCHA et al., 2019). There are still controversies about using the coating material individually or with other biopolymers. For example, Lu et al. (2020) and Lim, Ma and Dolan (2011) used maltodextrin alone to encapsulate blueberry bagasse extracts, highlighting even greater retention of the nutraceutical compounds in a blueberry extract with increased maltodextrin levels.

Furthermore, Flores et al. (2015) described using whey protein isolate (WPI) to encapsulate blueberry extracts, while Tao et al. (2017) observed improved encapsulation efficiency by combining the encapsulating materials and the influence

of the composition on the properties of blueberry anthocyanins powder, including density, particle size, and glass transition temperature. In addition, the authors highlighted the whey protein isolate as the main component of ideal formulations. Wu et al. (2017) emphasized that whey proteins can improve the stability of malvidin-3-glycoside. In addition, Cai et al. (2019) developed a system of carboxymethyl starch (CMS) and xanthan gum (XG) to improve blueberry anthocyanin stability. The study results revealed an encapsulation efficiency above 96%, in addition to higher thermal anthocyanin stability, with retention of 76.11 and 90.47% after 30 days of storage at 37 and 5 °C, respectively. Nonetheless, the tests performed by Rocha et al. (2019) with maltodextrin, gum arabic, WPI, and combinations provided maximum anthocyanin encapsulation efficiency using separate encapsulating agents. Therefore, based on these results, it is possible to conclude that the composition of the encapsulating agents has a significant influence on encapsulation productivity, encapsulation efficiency, and the physicochemical properties of the resulting anthocyanin powders.

Spray drying

Among the different microencapsulation methods, spray drying is the most used technique in the food industry (Table 2), and 60.0% of the cases (18 out of 30 studies) were based on this method. The popularity of this method is due to its low cost, simplicity of operation, the possibility of continuous production, high stability of the powders obtained (high dehydration speed), significant reduction in volume, and ease of handling and storage of the formed microcapsules (ROCHA et al., 2019). The process consists of dissolving, dispersing, or emulsifying the bioactive agent in an aqueous solution containing the coating material and then spraying it in hot air. During the drying of the liquid droplets, a coat is formed on their surface, and while the smaller water particles evaporate, the larger particles of the coating material remain in the system and are recovered at the end of the process in the form of powder. The method also allows the sample to be sprayed with a high-pressure nozzle and to operate simultaneously with air and particle flow, thus guaranteeing a minimal overheating of the particles (PIECZYKOLAN; KUREK, 2019). Because of this, spray drying is the most common process to microencapsulate extracted anthocyanins since they are susceptible to heat (MAHDAVI et al., 2014; PIECZYKOLAN; KUREK, 2019).

The size of the particles formed in spray drying is generally on a micro-scale and dramatically affects the application and performance of microcarriers (SHARIF; KHOSHNOUDI-NIA; JAFARI, 2020). Mahdavi et al. (2014) reported that spray-dried particle sizes are mainly affected by the size and position of the spray nozzle, feed flow rate, atomizer pressure, and solution viscosity, making its control challenging. Because of this, Turan, Cengiz and Kahyaoglu (2015) studied a new technology to microencapsulate blueberry bioactive compounds using an ultrasonic spray drying nozzle. In the study, the microparticles produced by the ultrasonic nozzle were more uniform in terms of size and shape. In addition, improved protection of the bioactive compounds was observed compared to a conventional nozzle.

The increase in temperature during the drying process is an important factor in obtaining spray-dried particles since the anthocyanin retention decreases at high temperatures (CAI et al., 2019; SHARIF; KHOSHNOUDI-NIA; JAFARI, 2020). In this sense, Lu et al. (2020) evaluated the effects of the incoming air temperatures (150, 160, 170, 180, and 190 °C) during the spray drying process of blueberry bagasse extract encapsulation. The encapsulation efficiency was not altered with the studied parameters, and the yield was similar in the temperature range of 150 to 180 °C. However, the encapsulation yield decreased significantly when the temperature increased to 190 ºC, and the loss of anthocyanins increased to 62.65%. Nevertheless, Silva et al. (2013) demonstrated that when the inlet air temperature is very low, the material is easily stuck on the drying chamber walls. Therefore, optimizing processing conditions is a critical step to encapsulate anthocyanins (SHARIF; KHOSHNOUDI-NIA; JAFARI, 2020).

Freeze drying

Freeze drying or lyophilization is a standard microencapsulation method based on eliminating water by sublimating the frozen product (TURAN; CENGIZ; KAHYAOGLU, 2015). Considered promising for encapsulating wild fruit extracts since it uses low temperatures, lyophilization represents 26.67% of the cases (8 of 30 studies; Table 2). Unlike other encapsulation methods such as spray drying, where the active substance is coated with an external material, the particles formed by lyophilization present the embedded bioactive compound dispersed throughout the

polymeric matrix (CELLI et al., 2016). In addition, damage to the product, such as changes in structure, appearance, texture, and taste, can be reduced by freeze-drying, which in spray drying can occur due to high drying temperatures (TURAN; CENGIZ; KAHYAOGLU, 2015). However, the process poses several disadvantages, including lengthy drying times and high energy consumption, making it a more expensive process at an industrial scale (4 to 5 times more expensive than spray drying) (TURAN; CENGIZ; KAHYAOGLU, 2015).

The anthocyanin powders obtained by freeze-drying have adequate properties in terms of angle of rest, apparent density, anthocyanin retention, and higher product yield (CAI et al., 2019). Wilkowska et al. (2016) reported that lyophilized powders showed better anthocyanin retention, while spray drying caused more significant losses of total phenolics (up to 78%). Still, Tao et al. (2017) encapsulated blueberry extract by lyophilization and reached encapsulation productivity values and encapsulation efficiency above 96 and 82%, respectively.

Ionic or ionotropic gelation

Another encapsulation method shown in Table 2 is ionic or ionotropic gelation, with 13.33% of the cases (4 out of 30 studies). This method consists of dripping or atomizing a polymeric solution containing the active compound to be encapsulated in an ionic solution under constant agitation. Upon reaching the ionic solution, the drops immediately form hydrogel granules with a three-dimensional structure through the interaction of divalent ions with the negatively charged biopolymer chains (BITTENCOURT et al., 2018; KUROZAWA; HUBINGER, 2017; OZKAN et al., 2019). Ionic gelation has a low cost compared to other techniques and does not require specialized equipment, high temperatures, and organic solvents, making it a simple process (KUROZAWA; HUBINGER, 2017; PATIL et al., 2010). In addition, alginate, low methoxylation pectin, chitosan, chitin, and Ca^{+2} ions as crosslinking agents are generally employed as coating materials (KUROZAWA; HUBINGER, 2017). Alginate is the most commonly used polymer in crosslinked gel systems due to its superior gelling properties, low toxicity, biodegradability, and biocompatibility (OZKAN et al., 2019). Bittencourt et al. (2018) tested sodium alginate as wall material in blueberry residue encapsulation by ionotropic gelation and reported a retention of 67.01% of

phenolics and 68.2% of release of phenolic compounds after 120 min of *in vitro* dissolution.

Despite the ionic gelation particles being suitable for encapsulation, they present a drawback due to the porosity of the matrix, which determines the release of the encapsulated substance. To overcome this limitation, different types of biopolymers can be combined to modify the gel structure or interaction between the active compound and polymers, thus guaranteeing the best use of the encapsulated substance (KUROZAWA; HUBINGER, 2017; PATIL et al., 2010). In this sense, Guo, Giusti and Kaletunç (2018) encapsulated extracts of purple corn and blueberry in alginate-pectin hydrogel particles and reported an improved encapsulation efficiency by increasing the concentration of alginate and total gum. In addition, the authors emphasized that blueberry encapsulation efficiency is higher than that of purple corn, which is likely due to the difference in the chemical structure of anthocyanins, and consequently, their interaction with alginate-pectin.

Liposomes

Liposome microencapsulation has gained increasing attention in recent years by the food, cosmetic, and biomedical industries for the most varied applications. This technique allows both hydrophilic and hydrophobic ingredients to be encapsulated because they are made up of bilayers consisting mainly of phospholipids, which can be simple or multiple (OZKAN et al., 2019; ZHAO; TEMELLI; CHEN, 2017). In addition to protecting active ingredients against adverse conditions, liposomes also increase absorption and bioavailability, and this is due to the properties of biocompatibility, biodegradability, amphiphilicity, immunogenicity, non-toxicity, and high permeability of the cell membrane (OZKAN et al., 2019; ZHAO; TEMELLI; CHEN, 2017). However, this technology has some limitations, such as costs at acceptable levels with an increase in the production scale, low physical and chemical stability, inhomogeneity, varied particle size distribution, lipid oxidation, and complex post-treatment steps (OZKAN et al., 2019; TRUCILLO; CAMPARDELLI; REVERCHON, 2018). Some supercritical-based processes have been developed to overcome these disadvantages, including supercritical carbon dioxide (SC-CO_2). The SC-CO_2 method uses carbon dioxide in processing conditions above its critical point (31.1 °C, 74 bar), offering solvency power similar to liquid organic solvents.

Nevertheless, SC-CO_2 is an inert, non-toxic, and ecological solvent, with the potential to encapsulate different compounds without thermal degradation and the presence of organic solvent residues (ZHAO; TEMELLI, 2017; ZHAO; TEMELLI; CHEN, 2017). During the process, the phospholipids are dissolved in ethanol and supercritical CO_2, forming a fluid phase where a water solution is atomized in the formation vessel, producing small droplets covered by the phospholipids and forming the liposomes (TRUCILLO; CAMPARDELLI; REVERCHON, 2018). Zhao, Temelli and Chen (2017) analyzed the effects of anthocyanin and sterol concentrations on blueberry anthocyanin encapsulation in liposomes using supercritical carbon dioxide and demonstrated that the greater anthocyanin addition (30-40%) may have contributed to structural and packaging in loaded liposomes. In addition, the authors observed a slow release of anthocyanins from liposomes in the simulated gastric fluid, while rapid release and degradation were noted in the simulated intestinal fluid, demonstrating the need for additional protection.

Table 2 - Microencapsulation studies of blueberry phenolic compounds

METHOD	COATING MATERIAL	RESULTS	REFERENCES
Spray drying	Micellar casein and whey protein isolate (WPI)	Improved anthocyanin retention in the gastrointestinal tract with micellar casein due to low solubility and curd formation; rapid dissolution and slow release of anthocyanins from microcapsules with WPI	(LIAO et al., 2021)
Spray drying	Maltodextrin DE20, hi-maize, inulin and gum arabic	Higher concentration of anthocyanins, less loss during storage, and longer half-life (t ½) by combining inulin and gum arabic; lower anthocyanin loss at lower storage temperatures; EE 96.80-98.83%	(DA ROSA et al., 2021)
Ultrasonic spray nozzle	Modified starch (HI-CAP 100) and whey protein isolate (WPI)	Apparent viscosity affected by the self-heating of the ultrasonic nozzle with increased power; higher glass transition temperature with maximum coating concentration (30%) and minimum power level (5W); WPI structures not affected by the power of the nozzle and a slight change of the HI-CAP 100 in 10W	(TURAN; KAHYAOGLU, 2021)
Spray drying	Maltodextrin (MD)	Anthocyanin degradation at temperatures above 60 °C; core/wall material ratio and optimized temperature of 50:50 and 160 °C; EE 98%	(LU et al., 2020)
Electrospraying	Corn prolamin zein, whey protein concentrate and agave fructans	52.65% EE achieved with corn prolamin zein, with homogeneous and smooth morphology	(GONZÁLEZ-CRUZ et al., 2020)
Freeze drying	Maltodextrin (MD) of different dextrose equivalents (DE)	Satisfactory thermal and storage stability; better retention and stability of bioactive compounds with lower DE carrier; MD10 resulted in better stability and antioxidant potential; EE 87.8-99.8%; RH 22%	(MAR et al., 2020)
Freeze drying	Gelatin, soy bean lecithin, soy pro-tein isolate, whey protein isolate, synanthrin, β-cyclodextrin, mal-todextrin and gum arabic	Greater colonic accessibility and delay in anthocyanin release during fecal fermentation *in vitro*, mainly with soy; increase in the intestinal microbial metabolism of phenolics; AGCC production; change in the Firmicutes/Bacteroidetes ratio	(WU et al., 2020)
Freeze drying	Carboxymethyl starch (CMS) and xanthan gum (XG)	Increased storage stability (76.11% after 30 days at 37 °C); increased thermal stability; superior antioxidant stability with the CMS/XG ratio of 30/1; better protection against anthocyanins from gastric digestion, facilitating their release in the intestine; EE >96%	(CAI et al., 2019)
Spray drying	Maltodextrin DE20 e hi-maize	Better protection; lower anthocyanin degradation and longer half-life with T3 (9% DE20 maltodextrin and 9% hi-maize) at 140 °C; EE 74.4-85.22%; improved anthocyanin delivery	(DA ROSA et al., 2019)

Spray drying	Maltodextrin, gum arabic and whey protein concentrate (WPC)	High concentration of anthocyanins and phenolics; better rehydration profiles and particle size with maltodextrin and WPI; 70-100% EE	(ROCHA et al., 2019)
Spray drying	β-cyclodextrin, whey protein and gum arabic	More stable to light and temperature (>75%) than native anthocyanins; EE 95%	(XU et al., 2019)
Ionotropic gelation	Sodium alginate	Microspheres with superficial invagination; good retention of phenolic compounds (67.01%); 68.2% phenolic release after 120 min in *in vitro* dissolution	(BITTENCOURT et al., 2018)
Ionic gelation	Alginate – pectin (Al-P)	Best EE with increased concentration of alginate and total gum; better anthocyanin retention at low temperatures and a high proportion of particle weight to volume of solution; reduction of degradation when exposed to light	(GUO; GIUSTI; KALETUNÇ, 2018)
Spray drying	Inulin (I) and maltodextrin (MX)	Unclustered particles; glass transition temperature decreased with water activity; microstructure unchanged with maltodextrin and from spherical to irregular with inulin; better conservation of antioxidant content with maltodextrin	(ARAUJO-DÍAZ et al., 2017)
Spray drying, freeze drying and vacuum oven drying	Wheat flour, chickpea flour, coconut flour and soy protein isolate	Higher content of polyphenols (156.2 mg GAE/g), anthocyanins (13.4 mg/g), and DPPH sequestering activity (714.1 µmol TE/g) with matrices produced with spray-dried soy protein isolate; protection from degradation for 16 weeks at 4 and 20 °C from spray-dried protein complexes	(CORREIA et al., 2017)
Freeze drying	Maltodextrin (MD), β-cyclodextrin (β-CD), whey protein isolate (WPI) and gum arabic (Gum-A)	EE improved by the combination of wall materials; main WPI component; properties of anthocyanin powder affected by the composition of wall materials; better thermal stability; EP >96%; EE >82%	(TAO et al., 2017)
Ionic gelation	Chitosan (CH) and cellulose nanocrystal (CNC)	Chitosan (CH) -CNC microcapsules showed better EE and stability; EE ~94%	(WANG; JUNG; ZHAO, 2017)
Spray drying	Whey protein isolate, maltodextrin, β-cyclodextrin and gum arabic	Downward trend in total anthocyanins and total phenolics; increased stability at -18 °C; increased size of the particles with the decline of the glass transition temperature; decreased antioxidant capacity; 3-month storage time	(WU et al., 2017)
Liposomes using supercritical carbon dioxide (SC-CO$_2$)	Soy lecithin	EE 50.6%; structural change with increased anthocyanin level; reduced surface load with increased cholesterol level; anthocyanin protection during simulated gastric fluid	(ZHAO; TEMELLI; CHEN, 2017)
Freeze drying	Maltodextrin with different dextrose equivalents	Different physicochemical properties; half-life of up to 108 days; degraded anthocyanin degradation during storage	(CELLI et al., 2016)

Technique	Wall material	Main results	Reference
Spray drying and freeze drying	HP-β-cyclodextrin and β-cyclodextrin	Spray drying caused more significant losses of total phenolics (76-78%) and anthocyanins (57%); better anthocyanin retention by lyophilization (1.5 times greater); high antioxidant activity	(WILKOWSKA et al., 2016)
Ultrasonic nozzle, spray drying (conventional nozzle) and freeze drying	Maltodextrin of 4-7 dextrose equivalents and gum arabic	The microspheres produced by an ultrasonic nozzle showed better uniformity in terms of size and shape; greater protection to bioactive compounds	(TURAN; CENGIZ; KAHYAOGLU, 2015)
Spray drying	Whey protein isolate	Greater antioxidant capacity; release properties similar to lyophilized juice	(FLORES et al., 2015)
Spray drying	Whey protein isolate and gum arabic	Gradual release of phenolics and assured antioxidant activity; high release of phenolics with WPI and high antioxidant activity with gum arabic during the gastric phase	(FLORES et al., 2014)
Spray drying	Whey protein isolate	Degradation kinetics of first-order anthocyanins; increase in total phenolics and antioxidant activity; EE 70%	(FLORES; SINGH; KONG, 2014)
Cross-link	Oxidized potato starch	High anthocyanin absorption capacity at low pH and low salt concentration (62 mg/g at pH 3) by the microgel with a high degree of oxidation and high crosslinking density; protection of anthocyanins from degradation during passage through gastric fluid and delivery to the intestine	(WANG et al., 2013)
Spray drying	Maltodextrin, shellac and pectin	The addition of pectin did not influence the structure of the microcapsules; citric pectin with a high degree of esterification and sugar beet pectin showed greater water-binding capacity and, consequently, less anthocyanin release in the simulated gastric fluid	(BERG et al., 2012)
Ionic gelation	Whey protein isolate	Diffusive release mechanism without the influence of protein content and blueberry extract load; interactions between whey proteins and constituents of microcapsules strongly dependent on pH; harmful effects at pH 3; greater stability at pH 1.5	(BETZ; KULOZIK, 2011)
Spray drying	Mesquite gum	Good color protection; reduction of anthocyanin degradation when exposed to light and temperatures of 4 and 25 °C; minimal changes in color at 4 °C and in the dark after four weeks of storage (ΔE = 5)	(JIMÉNEZ-AGUILAR et al., 2011)
Spray drying	Maltodextrin	Greater retention of nutraceutical compounds with the increase of maltodextrin; high antioxidant capacity; anthocyanidins and stable phenolics	(LIM; MA; DOLAN, 2011)

EE: Encapsulation efficiency; EP: Encapsulation productivity; MD10: Maltodextrin equivalent to dextrose; DE20: Dextrose equivalents; GAE: Gallic acid equivalents; DPPH: Radical 2,2-diphenyl-1-picryl-hydrazil; TE: Trolox equivalent; RH: relative humidity; AGCC: short-chain fatty acid; ΔE: total color difference.

Source: "Scopus" data search platform with the keywords "microencapsulation," "phenolic compounds," and "blueberry" between the years 2011 and 2021.

5 Conclusions

Blueberries are known for their high levels of phenolic compounds, which provide potential health benefits. However, phytoactive components can be easily degraded with exposure to heat, light, and oxygen, and anthocyanin stability, in particular, is highly susceptible to pH changes, among other factors. Therefore, microencapsulating blueberry bioactive compounds is an effective and promising way to solve the challenges that these compounds face during processing and storage and provide controlled release in the human body. For this, choosing the appropriate microencapsulation process and coating materials is essential for successful encapsulation in order to increase bioavailability and reduce degradation and nutritional loss. Given the above, this study provides important information for the food industry and shows different methods of microencapsulating blueberries without losing the health-promoting effects.

Acknowledgements

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5 ARTIGO 2 – EFFECTS OF BLUEBERRY EXTRACT CO-MICROENCAPSULATION ON THE SURVIVAL OF *Lactobacillus rhamnosus*

Effects of blueberry extract co-microencapsulation on the survival of *Lactobacillus rhamnosus*

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Abstract

Probiotics are used as dietary supplements with beneficial health effects mainly due to modulating the human intestinal microbiota, although they must withstand adverse environmental conditions and arrive at the target site in sufficient quantities. Co-microencapsulating alternative compounds such as those extracted from blueberries, besides enriching the phytochemical microcapsules, protect the probiotic cells of *Lactobacillus rhamnosus*. The microcapsules were produced by spray drying using the concentrations of 0% (control; ME-0%), 10% (ME-10%), 50% (ME-50%), and 100% (ME-100%). The *L. rhamnosus* release profile of the microcapsules after exposure to the simulated gastrointestinal system increased at 100% concentration with intestinal counts of 6.97 log CFU/g, while the lowest survival rate was observed using ME-50%. The microcapsules also showed improved thermal resistance compared to free cells,

and the ones with 100% extract (ME-100%) had better protective effects. In addition, co-microencapsulation increased the storage stability of probiotics with stability of up to 148 days at -18 °C. In short, this study demonstrates that the co-microencapsulation of the blueberry extract provided positive effects on *L. rhamnosus* survival and demonstrates its promising application in food.

Keywords: encapsulation; water-soluble extract; *Vaccinium myrtillus*; anthocyanins; probiotics; viability.

1 Introduction

Driven by modern lifestyles, the functional food sector has shown significant growth due to increased nutritional awareness of consumers seeking healthier and more balanced diets. Natural or processed, functional foods contain biologically active compounds that provide numerous health benefits in addition to the basic nutritional ones (Min et al., 2019; Tripathi & Giri, 2014). Probiotics are among the safest species of living microorganisms that provide health benefits to their host when administered in adequate amounts (Azam et al., 2020; FAO/WHO, 2001), in addition to being responsible for maintaining intestinal microbiota balance, improving the immune response (Patel et al., 2015), and acting in the prevention and treatment of several diseases, especially those related to the gastrointestinal tract (Azam et al., 2020), among others.

Lactobacillus are among the species of probiotic bacteria most commonly used in food products (Azam et al., 2020). However, probiotics must remain metabolically active in the intestine and resist the acidic conditions and digestive juices of the stomach to exert their beneficial effects (Anal & Singh, 2007; de Menezes et al., 2013; Quintana et al., 2017). Additionally, probiotics must be viable during food storage and processing to claim functional property (Min et al., 2019), and a minimum of 10^6 CFU/g or mL of probiotics in the ready-to-eat product is recommended to provide a daily dose of 10^8-10^9 viable cells in the intestine (Min et al., 2019; Tripathi & Giri, 2014).

Blueberries are a rich source of bioactive compounds such as anthocyanins, which are the most concentrated polyphenols with about 35-74% of the phenolic compounds in fruit (Gavrilova et al., 2011), and therefore the object of this study. In addition, blueberry anthocyanins have proven health attributes, including anti-

inflammatory and anticancer activity and antioxidant potential (Panche et al., 2016), preventing cardiovascular, neurological, and vision diseases (Khoo et al., 2017; Liu et al., 2020; Wallace & Giusti, 2015).

Recently, anthocyanins have also received significant attention due to their prebiotic function, and research has shown that anthocyanins improve the diversity of the intestinal microbiota (Hidalgo et al., 2012; Li et al., 2019; Pan et al., 2017), promoting the growth of beneficial bacteria and inhibiting potentially harmful ones, and increasing the concentration of short-chain fatty acids (Garcia-Mazcorro et al., 2018; Li et al., 2019). Nonetheless, like probiotics, anthocyanins are sensitive to high temperatures, the presence of light and oxygen, pH variations, among other factors (Turan et al., 2015).

Hence, microencapsulation is a promising alternative to stabilize these compounds, prolong their shelf life while maintaining their bioactive properties, and promote their release in the human body, thus expanding functional food production (Guo et al., 2018; Holkem et al., 2017). Spray drying is currently the most used technique in the food industry because it is a continuous technology, cost-effective, and requires short residence times (Guo et al., 2018; Turan et al., 2015). Several studies on encapsulating polyphenolic substances extracted from blueberries have been carried out (Araujo-Díaz et al., 2017; Flores et al., 2015; Jiménez-Aguilar et al., 2011; Liu et al., 2020; Rocha et al., 2019), as well as probiotics (Azam et al., 2020; Holkem et al., 2017; Nunes, Etchepare, et al., 2018; Sohail et al., 2012). However, few studies are based on co-microencapsulating these compounds, and little is known about how anthocyanins interact with probiotics.

Given the above, this study aimed to evaluate whether co-microencapsulating *Lactobacillus rhamnosus* with blueberry extracts of different concentrations exert positive effects on preserving probiotics and verify probiotic viability and stability under different thermal and storage conditions and through the simulated gastrointestinal system.

2. Materials and methods

2.1 Materials

'O'Neal' blueberries were provided by Embrapa Clima Temperado (Pelotas, Rio Grande do Sul State, Brazil), and the *Lactobacillus rhamnosus* probiotic culture was supplied by Coana Importação e Exportação Ltda (Florianópolis, Santa Catarina State Brazil). These products were stored at -18 °C until extraction and inoculum preparation. For microcapsule formation, gum arabic (CNI, São Paulo, Brazil), maltodextrin (Ingredion, São Paulo, Brazil), and inulin (Cia Natural, Rio Grande do Sul, Brazil) were used.

2.2 Extract preparation

The extracts were obtained by microwave hydrodiffusion and gravity (MHG; model NEOS-GR, Italy) according to the method described by Ravi et al. (2018). For this, frozen blueberries (300 g) were placed in an extraction container, and 600 W was used for MHG extraction (2 W/g). The extraction time was 8 min until complete extraction of the colored fraction (*in situ* water with polyphenols), which was carried out by a spiral condenser outside the microwave cavity, cooled, and collected. The collected extract was centrifuged at 10,000 rpm for 3 min at 10 °C, and the supernatant was frozen at -18 °C until later analyses.

2.3 Inoculum preparation

The probiotic bacteria *Lactobacillus rhamnosus* were activated in MRS broth (Kasvi, Paraná, Brazil) and incubated for 16 h at 37 °C. The bacteria were harvested in the stationary growth phase by centrifugation for 15 min at 2470 x g and 4 °C, washed with sterile 0.85% saline (NaCl), and resuspended in an equal volume of sterile 0.85% NaCl solution. The final suspension of probiotic bacteria had a cell density of approximately ~10 log CFU/mL (Nunes, Etchepare et al., 2018).

2.4 Microcapsule production by spray drying

Four feeding solutions were prepared and contained *Lactobacillus rhamnosus* and different blueberry extract concentrations: ME-0% (0% added extract; microencapsulated control), ME-10% (10% extract), ME-50% (50% extract), and ME-100% (100% extract) using gum arabic, inulin, and maltodextrin as wall materials

(Nunes, Etchepare et al., 2018) (Table 1). The microencapsulation process was carried out in a mini-dryer B-290 (Büchi, São Paulo, Brazil) with a 1.2-mm nozzle and inlet air temperature of 130 °C (Nunes, Motta et al., 2018). The different feeding solutions were kept under agitation and introduced into the drying chamber through a peristaltic pump with a feeding rate of 0.8 L/h, drying airflow rate of 40 L/min, and air pressure of 1.5 mPa. The microcapsules were then collected at the cyclone base in sterile flasks and stored at different temperatures (-18, 7, and 25 °C).

Table 1 - Composition of feeding solutions containing *Lactobacillus rhamnosus*

Encapsulating matrices	ME-0%	ME-10%	ME-50%	ME-100%
Gum arabic (g)	8	8	8	8
Inulin (g)	8	8	8	8
Maltodextrin (g)	2	2	2	2
Blueberry extract (mL)	0	10	50	100
Sterile distilled water (mL)	100	90	50	0

2.5 Cell viability

The viable probiotic cell count after microencapsulation was determined by completely releasing the cells from the capsules based on the method of Gul et al. (2019). For this, microcapsules (0.1 g) were suspended in sterile phosphate buffer (9.9 mL and pH 7.5) for 15 min at room temperature (25 °C) using a magnetic stirrer. For the microcapsules, the viable probiotic cells in the feed solution were determined by performing appropriate 10-fold serial dilutions in 0.85% saline before plating on MRS agar (Merck, Germany, USA). The plates were incubated in anaerobic jars containing an anaerobic generator (Oxoid, São Paulo, Brazil) at 37 °C for 48 h. The results were expressed in a colony-forming unit log per g of the product or mL of solution.

2.6 Encapsulation efficiency (EE)

The probiotic survival rate after microencapsulation was evaluated according to the encapsulation efficiency (EE) and method proposed by Martin et al. (2013) using Equation (1):

$$EE = (N / N_0) \times 100 \tag{1}$$

Where N is the number of viable cells (log CFU/g) released from the microcapsules and N_0 is the number of viable cells (log CFU/g) present in the feed solution before microencapsulation.

2.7 Particle size morphology and distribution

The morphology of the dry microcapsules was evaluated using a scanning electron microscope (Sigma 300 VP, Zeiss, Germany). The average particle size distribution (D [4, 3]) was measured using Mastersizer 2000 laser diffraction equipment (Malvern, Germany) and water as the dispersion medium.

2.8 Resistance to heat treatment

Resistance to heat treatment was assessed as proposed by Zhang et al. (2015), with some modifications. For this, microcapsules (0.1 g) and free culture (1 mL) were transferred to test tubes containing sterile peptone water (9 mL). The content was then subjected to different thermal conditions: 72 °C for 15 s, 72 °C for 5 min, 63 °C for 30 min, 63 °C for 60 min, and 63 °C for 120 min. After heating, the tubes were immediately cooled by immersion in ice for 10 min. Finally, aliquots were collected and cell viability was assessed.

2.9 Survival of free and microencapsulated *Lactobacillus rhamnosus* under simulated gastrointestinal conditions

The survival of free and microencapsulated *Lactobacillus rhamnosus* was evaluated under simulated human gastrointestinal conditions according to a modified method of Madureira et al. (2011). The transit tolerance of probiotics was determined by exposing free cells (1 mL) and microcapsules (0.1 g) to 37 °C and 10 mL of simulated gastric juice (addition of pepsin, pH 2.0 adjusted with HCl 0.1 N); 10 mL of pancreatic juice and simulated bile (addition of pancreatin and bile salts, pH 5.0 adjusted with $NaHCO_3$); and finally 10 mL of simulated intestinal juice (pH 7.5 adjusted with $NaHCO_3$). Aliquots (1 mL) were removed after 90, 110, and 200 min to simulate

the stages of the esophagus/stomach, duodenum, and ileum, and plated on MRS agar under anaerobic conditions at 37 °C for 48 h.

2.10 Stability evaluation of *Lactobacillus rhamnosus* during storage

The storage stability of the free and microencapsulated probiotic was determined by transferring the cell suspensions and powder samples to Eppendorf microtubes and stored at -18, 7, and 25 °C for 120 days. *Lactobacillus rhamnosus* viability was determined at 1, 15, 30, 45, 60, 75, 90, 105, and 120 days of storage. The samples were serially diluted with sterile phosphate buffer pH 7.5 and seeded on MRS agar for enumeration. The plates were incubated in anaerobic pots at 37 °C for 48 h.

2.11 Total monomeric anthocyanin determination

To quantify the total monomeric anthocyanin content, 250 mg of microcapsules were dissolved in 5 mL of ethanol:acetic acid:water (50:8:42 v/v/v). The mixture was vortexed and centrifuged at 1080 g for 15 min. Subsequently, the supernatant was collected and analyzed by the differential pH method proposed by Giusti and Wrolstad (2001). Sample absorbance was measured at 520 and 700 nm with a UV-visible spectrophotometer, and the results were expressed as delphinidin-3-glucoside equivalent per 100 g of microcapsules.

2.12 Anthocyanin separation and quantification by HPLC-DAD

Among the phenolic compounds, anthocyanins are the major class of blueberry extracts and, consequently, the predominant class of the obtained microcapsules. Its separation and quantification occurred by high-performance liquid chromatography coupled to a diode-array detector (HPLC-DAD, Shimadzu, Kyoto, Japan) according to the method of Barcia et al. (2014). Anthocyanin separation was performed using a Zorbax Eclipse XDB C-18 reverse phase column (150 mm, 2.1 mm, 3.5 μm particle size) with a flow rate of 0.19 mL min^{-1}, temperature of 40 °C, and injecting 10 μL of the sample. The two mobile phases used to separate the compounds consisted of water, acetonitrile, and formic acid (88.5:3:8.5, v/v/v) as mobile phase A, and water, acetonitrile, and formic acid (41.5:50:8.5, v/v/v) as a mobile phase B. The linear

gradient of separation was 0-8 min, 3% B; 8-28 min, 30% B; 28-34 min, 50% B; 34-38 min, 100% B; 38-40 min, 100% B; 40-46 min, 3% B, totaling 46 min. A calibration curve of delphinidin-3-glucoside was used for anthocyanin quantification at concentrations ranging from 0.25 to 2.25 mg/L (R^2 = 0.992). The curve and samples were read at 520 nm wavelength, and the results were expressed in mg of delphinidin-3-glucoside per liter of extract.

2.13 Colorimetric analysis

The instrumental color analysis of the microcapsules was performed in a colorimeter (CR-300, Minolta, Kyoto, Japan). The color scale of the International Commission on Illumination (CIE) Lab was used to measure the parameters L* (luminosity degree in the 0-100 scale from black to white), a* (degree of red (+) to green (-)), and b* (degree of yellow (+) to blue (-)). The saturation index (C*) and tint angle (h*) were calculated according to Equations (2) and (3), respectively:

$$C^* = \sqrt{(a^*)2 + (b^*)2} \tag{2}$$
$$h^* = \arctan (b^*/a^*) \tag{3}$$

2.14 Statistical analysis

The experimental design was completely randomized with a factorial arrangement of 5 x 4 (5 types of processing x 4 digestive compartments) for the digestive compartments, totaling 20 treatments with three repetitions each. For the thermal treatments, it was 5 x 6 (5 types of processing x 6 thermal treatments) with a factorial arrangement, totaling 30 treatments with three repetitions each. Finally, for storage stability, the experimental design was completely randomized with a factorial arrangement of 5 x 3 x 9 (5 types of processing x 3 storage temperatures x 9 storage times), totaling 135 treatments with three replicates each.

The data were submitted to univariate analysis of variance (ANOVA) using the GLM procedure, their means estimated using the LSMEANS command and compared using the *Student Newman Keuls* test (SNK). In addition, contrast analysis was performed to assess the effects of microencapsulation (ME) or not (free). The different blueberry extract concentrations in the microencapsulation were evaluated for linear

and quadratic trends by orthogonal contrast analysis using the coefficients to interpolate the orthogonal polynomials estimated by the Interactive Matrix Language (IML) procedure.

Subsequently, polynomial regression analysis was also performed to investigate changes in the dependent variable as a function of the blueberry extract concentration in microencapsulation. The parameters of the adjusted regression models were estimated using the GLM procedure. The coefficient of determination (R^2) values were expressed in relation to the source treatments (regression + lack of adjustment). Statistical analyses were performed using the SAS System for Windows version 9.4 (SAS Institute Inc., Cary - NC, USA) at 5% significance level.

3. Results and discussion

3.1 Characterization of microcapsules obtained by spray drying

The scanning electron microscopy (SEM) images for the analyzed processes are shown in Figure 1. The microcapsules ME-0%, ME-10%, and ME-50% were spherical and concave in shape, which is typical of products produced by spray drying because of the particles' shrinkage due to the rapid droplet evaporation (Bustamante et al., 2020). Nunes and Etchepare et al. (2018) reported similar characteristics when encapsulating *Lactobacillus acidophilus* with gum arabic, inulin, hi-maize, and trehalose, and Lu et al. (2020) found similar findings when microencapsulating anthocyanins extracted from blueberries with maltodextrin. In contrast, the ME-100% microcapsules were spherical and had a smooth surface. It is also possible to observe that the lower the blueberry extract concentration in the microcapsules, the greater the shrinkage, which is even more significant in the control microcapsule. In addition, the SEM micrographs showed that the outer surface of dry microcapsules was free from cracks or breaks, and the trapped probiotic cells were not visible in the powder.

Figure 1 - Micrographs of *Lactobacillus rhamnosus* microcapsules produced with different blueberry extract concentrations: (a) ME-100%: 100% extract addition; (b) ME-50%: 50% extract addition; (c) ME-0%: no extract addition; (d) ME-10%: 10% extract addition
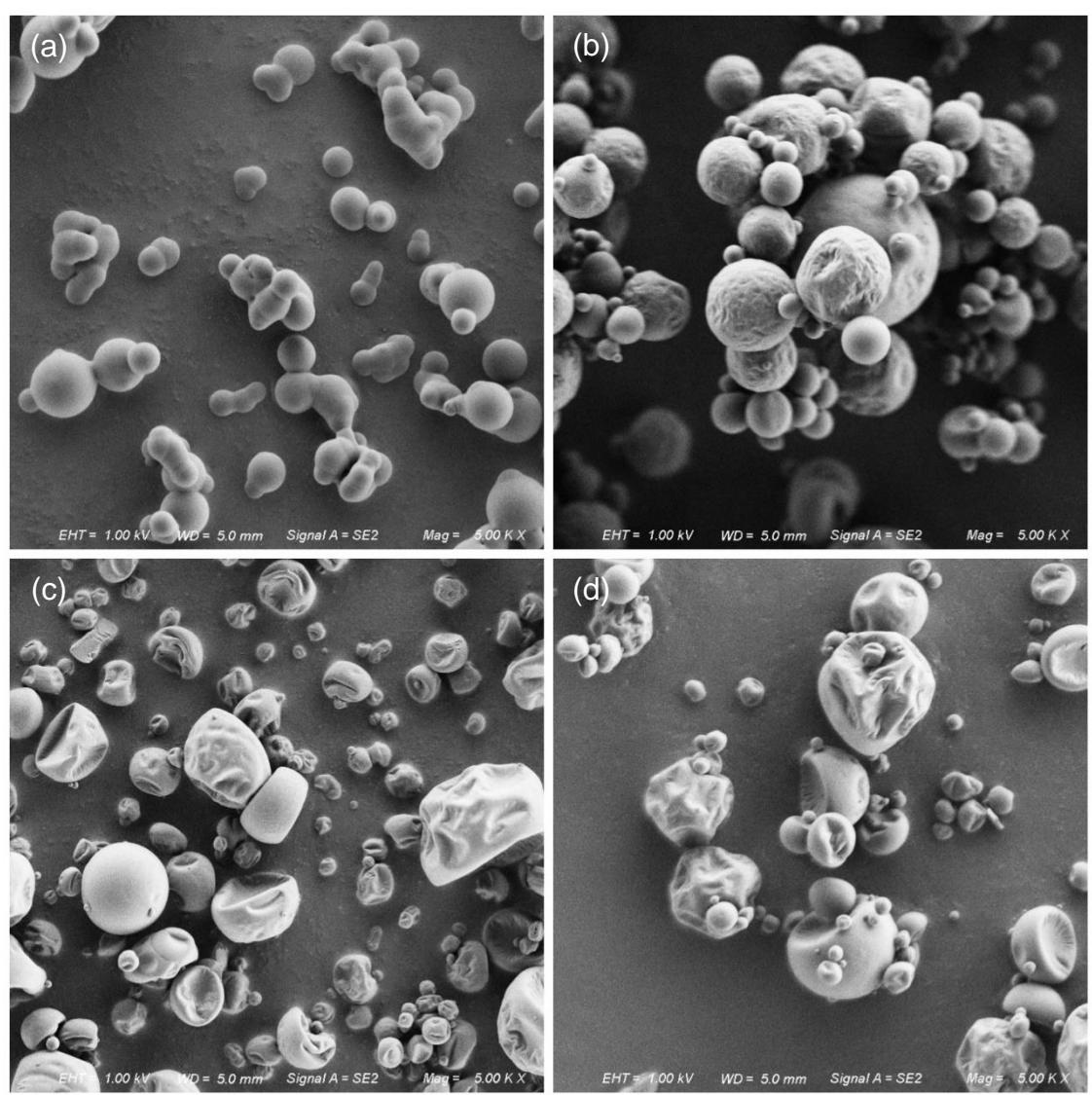
The particle sizes ranged from 1.74 to 6.29 ɥm for microcapsules containing *Lactobacillus rhamnosus* and different blueberry extract concentrations (Table 2). Lu et al. (2020) reported that the average size of the microcapsules containing anthocyanins was about 5.5 ɥm, while Bustamante et al. (2020) noted that spray-dried particle sizes contained encapsulated probiotic bacteria ranging from 1.77 to 15.5 ɥm. According to Rajam and Anandharamakrishnan (2015), the different properties of film formation and gelation of the encapsulating materials used in encapsulation may be related to the variation in particle sizes. Nevertheless, Piñón-Balderrama et al. (2020)

reported that different drying conditions and solid concentrations of the feed solution influence the particle size of the obtained powders. Although larger microcapsules may better protect the bioactive material, smaller particles are more desirable in food formulations since they guarantee better homogeneity and quality (Souza et al., 2020).

Table 2 - Viability of *Lactobacillus rhamnosus* present in the feed solution and after encapsulation, encapsulation efficiency, and particle size of the microcapsules

Treatment	CFU/g in the solution	CFU/g after encapsulation	EE (%)	Particle size (μm)
ME-0%	10.1 ± 0.1^a	8.46 ± 0.05^{ab}	84 ± 1^a	6.2 ± 0.6^a
ME-10%	10.3 ± 0.1^a	8.47 ± 0.02^a	82 ± 1^a	6.3 ± 0.2^a
ME-50%	10.2 ± 0.2^a	8.35 ± 0.04^b	82 ± 1^a	1.7 ± 0.0^b
ME-100%	10.22 ± 0.02^a	8.4 ± 0.1^{ab}	82 ± 1^a	5.7 ± 0.4^a

CFU: colony forming unit; EE: encapsulation efficiency; ME-0%: without extract addition; ME-10%: 10% extract addition; ME-50%: 50% extract addition; ME-100%: 100% extract addition. Different letters in the same column mean statistically significant differences ($p < 0.05$) by *Student Newman Keuls* test.

3.2 *Lactobacillus rhamnosus* viability and encapsulation efficiency

Lactobacillus rhamnosus viability in the feed solution, after the microencapsulation process, the EE of the microcapsules produced with gum arabic, maltodextrin, inulin, and different blueberry extract concentrations, and control microcapsules (without extract addition) are listed in Table 2. The viability of all microcapsules produced was more significant than the minimum recommended viable cell count ($>10^6$ CFU/g) for probiotics to have therapeutic effects on food products (FAO/WHO, 2002). According to the data in Table 2, spray drying caused the loss of two logarithmic units in probiotic viability, which is in line with Souza et al. (2020), who microencapsulated a mixture of acerola and ciriguela juice containing probiotic lactobacilli. This demonstrates that the technique is capable of trapping probiotic cells with relative safety. In addition, it is common for spray drying atomization operations to lose one to two logarithmic units of cell viability (Dias et al., 2018; Kalita et al., 2018; Paim et al., 2016).

The encapsulation agents promoted good encapsulation efficiency (~82% for the microcapsules with extracts and ~84% for the control), with no statistical difference between them. Fredes et al. (2018) reported that encapsulation efficiency is directly related to a higher core/coating material ratio. However, Nunes and Etchepare et al.

(2018) highlighted the potential of inulin as a protective agent and reported an EE of 93.12%. Dias et al. (2018) obtained EEs between 79.11 and 86.67% for the optimized conditions of microencapsulating bifidobacteria in passion fruit juice by spray drying.

3.3 Anthocyanin content

Blueberries are rich in phenolic compounds, including a wide range of anthocyanins with potential biological effects (Correia et al., 2017; Sun et al., 2019). The blueberry extract obtained by MHG presented a concentration of 528.4 ± 15.3 mg/L of delphinidin-3-glucoside. When co-microencapsulating the blueberry extract, a significant anthocyanin content (168.88 ± 4.79 mg/100 g of delphinidin-3-glucoside) was found in the ME-100% microcapsules, which was the highest concentration observed. The microencapsulated processes ME-50% and ME-10% showed 55.35 ± 2.89 and 15.12 ± 0.75 mg/100 g of delphinidin-3-glucoside, respectively, differing statistically between them and ME-100%. Note that the higher the proportion of blueberry extract added to the feed solution, the greater the anthocyanin concentrations in the probiotic microcapsules obtained by spray drying. In the ME-100% production, only the raw blueberry extract was added, while for ME-50% and ME-10% processing, sterile water was added with the extract (50:50 and 90:10 v/v, respectively). This was evident in the amount of anthocyanins observed in the obtained microcapsules. Spray drying promotes water loss and the consequent concentration of anthocyanin compounds, making microcapsules more stable to microbial degradation and contamination (Mahdavi et al., 2014), in addition to protecting bio-sensitive thermo-sensitive compounds (Mahdavi et al., 2016).

The results for total monomeric anthocyanins (AMT) (Figure 2) corroborate the findings from the HPLC analysis since ME-100% had higher anthocyanin content (198.7 ± 3.4 mg/100 g), followed by processing ME-50% and ME-10% with 118.22 ± 0.76 and 27.9 ± 0.6 mg/100 g of anthocyanins in the microcapsules, which differed significantly ($p < 0.05$). The crude liquid extract showed 631.93 ± 33.99 mg/L of anthocyanins. Thus, it is evident that ME-100% is a rich source of phytochemicals with relevant functional applications.

Wang et al. (2013) reported that typical American diets contain between 12.5 to 215 mg anthocyanins/day, a high amount of flavonoids found in food and beverages. Nevertheless, Roopchand et al. (2012) demonstrated that the recommended amount

of half a cup (73 g) of blueberries provides ~70 mg of anthocyanins. Therefore, equivalent anthocyanin levels can be delivered with about 35-41 g of microcapsules with the most concentrated blueberry extract (ME-100%).

Rocha et al. (2019) obtained total anthocyanin content ranging from 1186.65 to 1383.18 mg/100 g after spray-drying microencapsulation of a mixture of phenolic extracts (jabuticaba, jussara, and blueberry) using maltodextrin, gum arabic, and whey protein concentrate of milk. Paim et al. (2016) reached an anthocyanin content of 739.2 mg/100 g for probiotic jussara juice microcapsules using different combinations of maltodextrin and inulin. Correia et al. (2017) found the highest anthocyanin concentration (13.4 mg/g) in microcapsules containing spray-dried blueberry bagasse extract produced with soy protein isolate. The authors also reported a significant anthocyanin content in the concentrated blueberry extract of 166.6 mg/100 mL. The differences in the anthocyanin content herein and the literature are explained by the anthocyanin concentrations in the evaluated fruits, the difference in the preparation of the fruit (pulp or extract), the encapsulating material, the drying conditions, and the analytical method used for anthocyanin determination (Paim et al., 2016).

Figure 2 - Anthocyanin content in powders produced with different concentrations of blueberry extracts (ME-10% = 10% extract addition, ME-50% = 50% extract addition, and ME-100% = 100% extract addition)
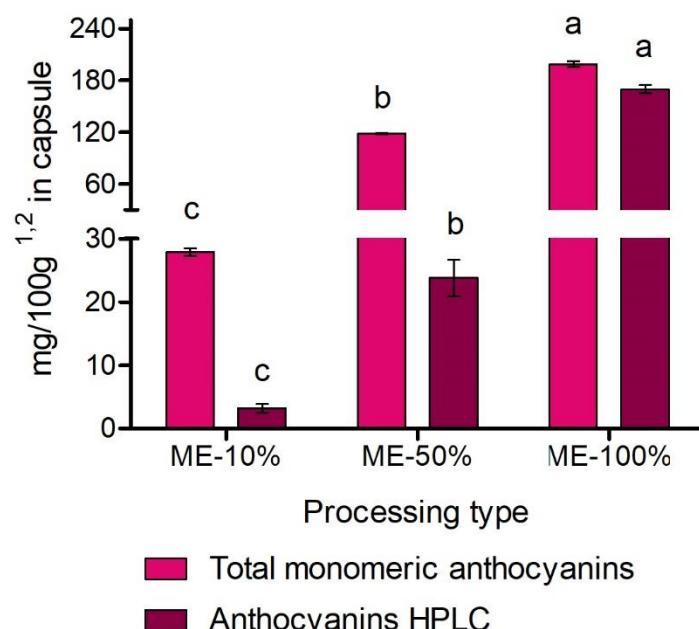
Means followed by different letters differ (p < 0.05) between processing types by *Student Newman Keuls* test.

3.4 Color

Blueberries are mainly rich in anthocyanins, which are responsible for the dark blue color of the fruits. Hence, blueberries are considered important sources of natural pigments (Sun et al., 2019). The differences in the color parameter of the microcapsules are listed in Table 3. An increase in L* (luminosity) value is observed as the extract concentration decreased in the processing, which is interpreted as an indication of discoloration (Estupiñan et al., 2011). The chromatic coordinates a* and b*, which vary from green (-) to red (+) and from blue (-) to yellow (+), presented positive and negative values, respectively. As the extract concentration in the microcapsules increased, the color parameter a* increased and the parameter b* decreased. The C* (chroma) value was proportional to the color intensity and indicated the degree of saturation (Maskan, 2001), supporting the higher intensity of the red color in the microcapsules obtained with ME-100%. The Hue angle (h*) value is the predominantly perceived color attribute (Lee, 2000), and all microcapsules had Hue angle values >330°; therefore, they can be described as red microcapsules (Lee, 2000; Rodríguez-Saona et al., 1999). These findings may affect the sensory acceptance of the food or supplement and increase the attractiveness of the product.

Table 3 - Color parameters of microcapsules produced with 10% extract (ME-10%), 50% extract (ME-50%), and 100% blueberry extract (ME-100%)

Processing type	Parameters				
	L^*	a^*	b^*	C^*	h^*
ME-10%	77.86 ± 0.64^a	11.12 ± 0.27^a	-4.21 ± 0.15^a	11.89 ± 0.21^a	339.28 ± 1.12^a
ME-50%	60.29 ± 0.33^b	23.58 ± 0.11^b	-8.35 ± 0.04^b	25.01 ± 0.12^b	340.50 ± 0.02^a
ME-100%	55.68 ± 1.49^c	26.31 ± 0.24^c	-6.33 ± 0.18^c	27.06 ± 0.28^c	346.49 ± 0.26^b

ME-10% = 10% extract addition, ME-50% = 50% extract addition, and ME-100% = 100% extract addition. Values with different lowercase letters in the same column are statistically different ($p < 0.05$) by *Student Newman Keuls* test.

3.5 Survival of free and microencapsulated *L. rhamnosus* under simulated gastrointestinal conditions

The survival of *L. rhamnosus* during passage through the simulated gastrointestinal system was essential to claim the beneficial health effects of probiotics (Figure 3). The types of processing containing different concentrations of the blueberry extract have significant effects on *L. rhamnosus* survival in the different digestive

compartments. Furthermore, microencapsulation increased *L. rhamnosus* viability ($\bar{y}_{ME}$ $_{(0-100\%)}$ = 6.16) compared with the probiotic in the free form ($\bar{y}_{Free}$ = 4.56).

After exposure to simulated gastric juice (pH 2.0 of the stomach), the microencapsulated processes ME-0% and ME-50% showed the highest release rates in the stomach and did not differ statistically between them. According to the regression equation (Y = 4.9784 + 0.0133x - 0.0002x^2 (R^2 = 0.87)), the highest survival rate of *L. rhamnosus* in the stomach was observed with a 29% concentration of blueberry extract. In addition, the maximum protection was observed at ME-100%, while the maximum logarithmic reduction of 6.56 log CFU/g was observed in free cells.

By increasing the pH of the medium to 5.0 to simulate the passage through the duodenum in the gastrointestinal system, the release rate of *L. rhamnosus* changed except for the ME-10% processing, which statistically maintained the same rate shown in the previous compartment. The ME-50% had the highest release rate (>6 log CFU/g) with an increase of ~0.9 log CFU/g compared to the release of *L. rhamnosus* in the stomach (p < 0.05). The regression analysis (Y = 5.1020 + 0.0341x - 0.0004x^2 (R^2 = 0.63)) revealed that the highest probiotic survival rate in the duodenum is achieved with 47% of blueberry extract co-microencapsulation. Still, ME-100% and ME-10% did not differ statistically and better protected viable *L. rhamnosus* cells from the pancreatic juice and bile salts, which can colonize in the large intestine. Nonetheless, free *L. rhamnosus* was unable to maintain its viability at an effective level. Moumita et al. (2017) reported that, out of the four probiotic strains studied in free form, *Lactobacillus bulgaricus* and *Lactobacillus fermentum* cells showed moderate resistance to stress caused in the intestine, while *Lactobacillus acidophilus* and *Lactobacillus plantarum* cells showed negligible resistance. This demonstrates that the damage done to the cell depends on the type of bacteria.

The release profile of *L. rhamnosus* in the ileum (pH 7.5), after exposure to the simulated intestinal juice, increased for the ME-0%, ME-10%, and ME-100% processing, with the survival rate ME-100% being the highest compared to the other processes, with a reduction of only 1.33 log CFU/g compared to viable cells at the beginning of digestion. ME-0% and ME-10% did not differ statistically and had counts above 6 log CFU/g. However, ME-50% provided less protection (<6 log CFU/g), which may be related to particle size. According to the regression equation (Y = 6.3073 - 0.0330x + 0.0004x^2 (R^2 = 0.92), the lower survival rate of *L. rhamnosus* was observed with the co-encapsulation of 42% of blueberry extract. Given this, adding the blueberry

extract in microcapsules helped control the process of releasing *L. rhamnosus* from microcapsules. The processes with better protection took longer to release probiotics. According to Braber et al. (2020), the concentration of solids in the suspension to be dried increases the probiotic count under simulated gastrointestinal conditions, and this explains the best protection obtained with the microcapsules that received the 100% extract.

Kalita et al. (2018) produced microcapsules sprayed with lychee juice containing *L. plantarum* in different wall materials and observed that *L. plantarum* survival was more affected when exposed to gastric juice (pH 2.0 and the enzyme pepsin) than in intestinal juice (bile and pancreatin at pH 6.0), as also noted herein. In addition, free *L. plantarum* was more sensitive than the microencapsulated form. Moumita et al. (2017) evaluated four microencapsulated probiotic strains in *Pleurotus ostreatus* extract and sodium alginate by the extrusion method and observed that the encapsulated form of *L. acidophilus* showed better resistance compared to the free form. In contrast, *L. bulgaricus* and *L. fermentum* did not show increased viability after encapsulation, showing that encapsulated bacteria are more tolerant during exposure to the simulated gastrointestinal tract, albeit this protection against stress conditions depends on the type of bacteria. Thus, to improve the viability of microorganisms, the inclusion of alternative bioactive compounds, such as blueberry extract, which is effective in protecting *L. rhamnosus*, may be considered.

Figure 3 - Bacterial viability free (F) or microencapsulated (ME) with different blueberry extract concentrations on different digestive compartments
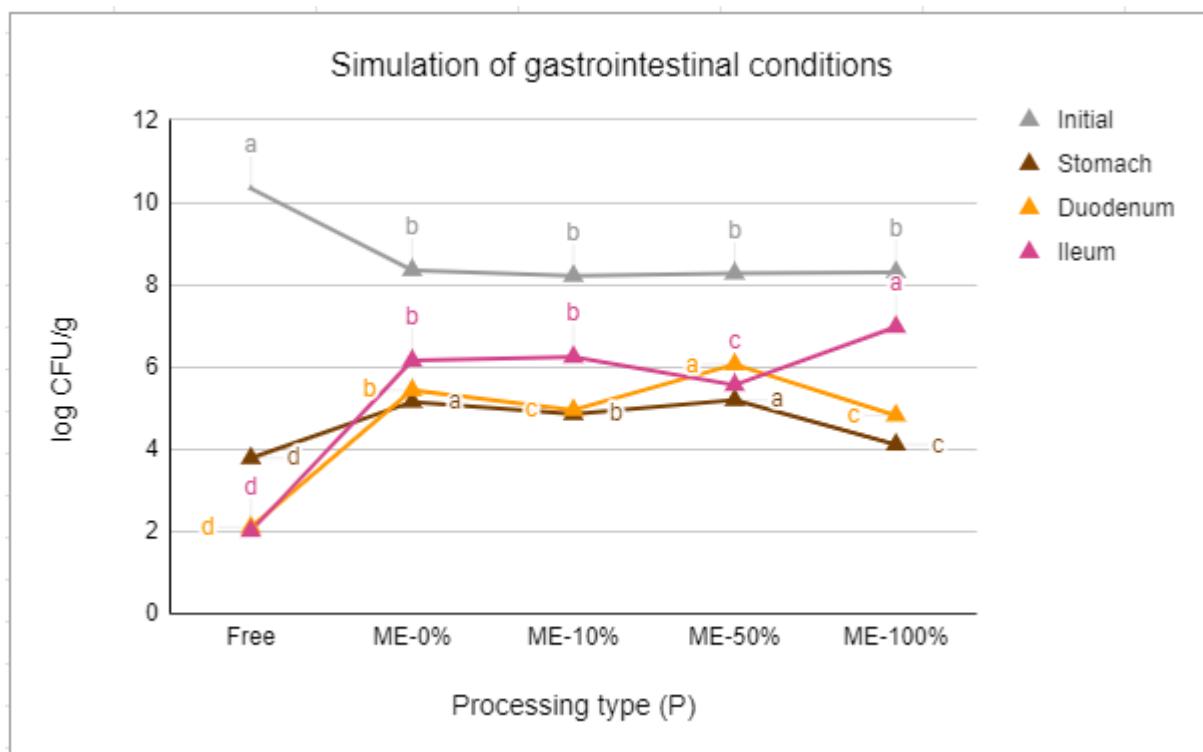
3.6 Resistance of free and microencapsulated *L. rhamnosus* to heat treatments

The thermal treatment was another critical factor, considering the decrease of the probiotic population during food processing. To investigate the effectiveness of co-microencapsulation in protecting *L. rhamnosus* from heat, cell survival was evaluated after exposure to 72 °C for 15 s and 5 min and 63 °C for 30, 60, and 120 min (Figure 4). The type of processing and different heat treatments interacted significantly. In addition, microencapsulation increased *L. rhamnosus* viability when exposed to heat ($\bar{y}_{ME\ (0-100\%)}$ = 7.05) compared to the probiotic in free form ($\bar{y}_{Free}$ = 5.27).

The free cells showed loss of viability over time of exposure to temperatures, demonstrating their high sensitivity to heat treatments. It was also possible to observe a decrease from 10 logarithmic cycles to 7 and 6 logarithmic cycles at 72 °C after 15 s and 5 min, respectively, and less than 6 logarithmic cycles at 63 °C after 30 min. After 60 and 120 min of exposure to 63 °C, the free bacterial cells lost practically all of their probiotic viability (<1 log CFU/g).

The microencapsulated *L. rhamnosus* cells showed higher cell survival rates than the free cells ($p < 0.050$) due to the thermal protection provided by the encapsulation matrices and the addition of the blueberry extract. Among the types of processing, ME-100% provided the best protection under the different heat treatments. At 72 °C for 15 s, all processings showed counts above 7 log CFU/g, and for the microencapsulated ME-0% and ME-10%, no significant differences were observed, including for the microencapsulated ME-0% and ME-50%. The smallest logarithmic reduction was observed for the microencapsulated ME-100%, with -0.71 log CFU/g, in contrast to the control microencapsulated (ME-0%), which showed the largest reduction with -1.22 log CFU/g. In contrast, the regression equation ($Y = 7.3287 - 0.0090x + 0.0001x^2$ ($R^2 = 0.88$)) showed that the minimum *L. rhamnosus* survival rate was observed with 35% blueberry extract in microencapsulation. At 72 °C for 5 min, the ME-100% processing showed greater thermal resistance with a reduction of 1.47 log CFU/g, while the ME-50% processing showed the greatest reduction with -2.59 log CFU/g. This demonstrates that the shorter exposure time enabled the longer survival of *L. rhamnosus* at 72 °C and that the particle size influenced the protection of probiotic bacteria against heat. In addition, the blueberry extract co-microencapsulation showed a protective effect, increasing the thermal resistance.

In the heat treatment at 63 °C, the ME-100% processing showed significantly higher thermal resistance than the other microencapsulated processes, reducing only 0.47, 0.84, and 1.63 log CFU/g after 30, 60, and 120 min, respectively. Higher survival rates were observed after 30 min of heating, with an average of 7 logarithmic cycles. At 63 °C for 30 min, the most significant reduction in *L. rhamnosus* cells occurred with ME-50% processing, with a decrease of 1.51 log CFU/g. The ME-0% and ME-10% processing did not differ and showed reductions of approximately 1 log CFU/g, in addition to not statistically differing from the heat treatment at 72 °C for 15 s. The regression equation ($Y = 7.4733 - 0.0281x + 0.0003x^2$ ($R^2 = 0.94$)) showed a minimum survival rate of *L. rhamnosus* with 48% blueberry extract at 63 °C for 30 min. This same percentage was observed for the treatment at 72 °C for 5 min ($Y = 6.7687 - 0.0371x + 0.0004x^2$ ($R^2 = 0.76$)). After 60 min, the number of cells remained viable, with counts above 6 log CFU/g, with the largest reduction observed for ME-50% processing, with a reduction of 2.14 log CFU/g. Nonetheless, the lowest *L. rhamnosus* survival rate was observed with 34% blueberry extract ($Y = 6.5612 - 0.0212x + 0.0003x^2$ ($R^2 = 0.94$)). Finally, except ME-100% processing, the *L. rhamnosus* probiotic cells were unfeasible

for treatment at 63 °C for 120 min, with counts below the recommended functional property claim of 6 log CFU/g. In addition, the minimum survival rate was observed with 39% blueberry extract according to the equation $Y = 5.8588 - 0.0311x + 0.0004x^2$ ($R^2 = 0.96$)). Thus, the protective effects of co-microencapsulating blueberry extract on *L. rhamnosus* survival against heat are evident.

Rosolen et al. (2019) reported a higher number of viable cells at 65 °C after 10 and 15 min and also demonstrated the effectiveness of inulin associated with whey as coating materials in protecting *Lactococcus lactis* from heat. According to the authors, long-chain inulin has high thermal stability and low solubility due to the high polymerization degree, slowing the diffusion of the material and providing greater probiotic stability.

Figure 4 - Bacterial viability free (F) or microencapsulated (ME) with different blueberry extract concentrations under different thermal treatments
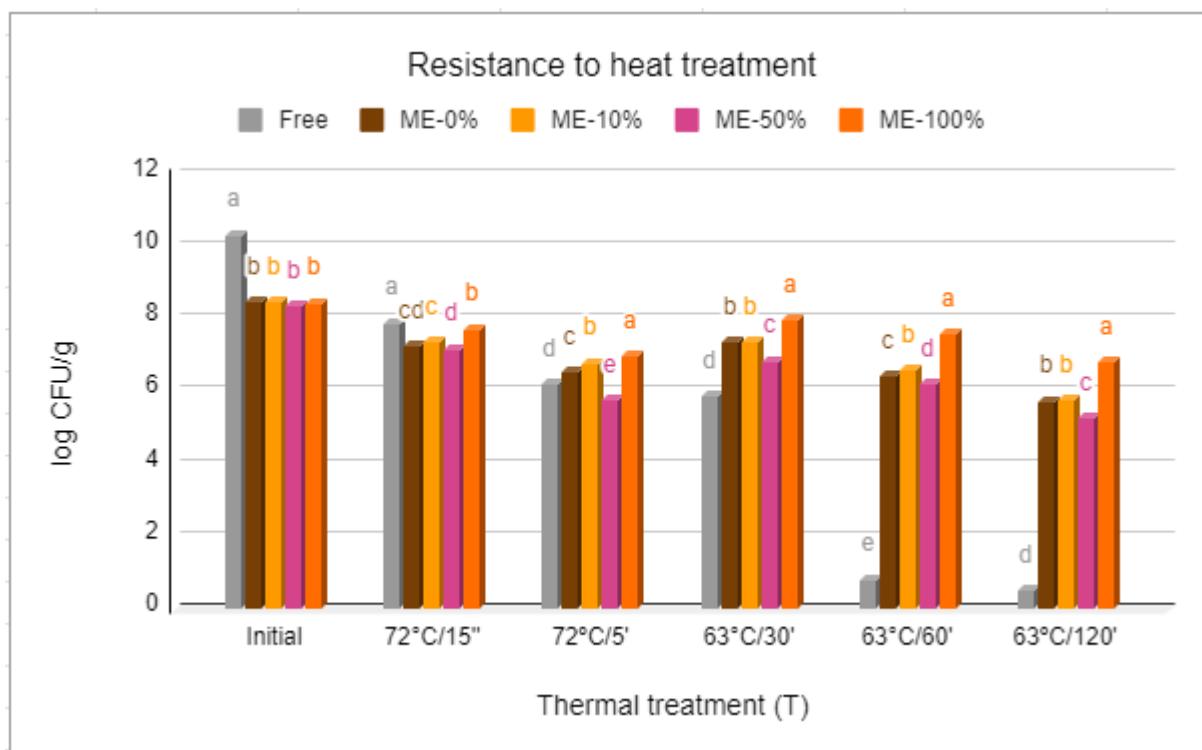
Means followed by different letters differ (p < 0.05) between processing types by *Student Newman Keuls* test.

3.7 Storage stability of free and microencapsulated *L. rhamnosus*

Cell damage and viability loss may occur during food processing and storage. Therefore, a satisfactory microencapsulation process must guarantee probiotic survivability during the different processing stages and maintain its activity during storage (Champagne et al., 2015; Oliveira et al., 2007). In addition, a minimum amount of 10^6 CFU/g of viable probiotics in the ready-to-eat product is recommended to obtain beneficial health effects (Tripathi & Giri, 2014).

In this study, there was a significant increase in the storage stability of microencapsulated *L. rhamnosus* ($\bar{y}_{ME\ (0\text{-}100\%)}$ = 7.34) compared to free probiotic cells ($\bar{y}_{Free}$ = 5.33). Azam et al. (2020) showed that non-encapsulated *L. rhamnosus* is more prone to environmental stress due to the production of different metabolites that reduce their viability. Additionally, a strong interaction between the type of processing used, temperature, and storage time was observed (p-value 0.0001). The ME-10% microencapsulated process had maximum stability with minimal loss. Storage at -18 °C for 120 days helped maintain probiotic viability, and storage at higher temperatures would reduce viability. In addition, the storage time significantly reduced *L. rhamnosus* counts.

At room temperature (25 °C) (Figure 5), the free bacteria remained viable for approximately 30 days of storage, with a cell reduction of 4.06 log CFU/g, whereas the microencapsulated *L. rhamnosus* showed viability for over 90 days with counts above 6 log CFU/g. The microencapsulated ME-10% and ME-100% had similar behavior in 45 days and from the 75th day of analysis onwards, showing viability above 6 log CFU/g after 105 days of storage and a reduction not above 2.46 log CFU/g in 120 days. Although the highest viability was achieved with ME-100% processing (5.88 log CFU/g) in 120 days of storage, the lowest log reduction was observed for ME-10% processing, with -2.38 log CFU/g. However, the lowest viability at 25 °C was observed with the micro-encapsulated ME-50%, with a decrease of 3.06 log CFU/g. According to the regression equation presented, the room temperature indicated a reduction rate of about three times higher than the freezing temperature, showing viable cell counts of up to 65 days.

Under storage conditions at refrigerated temperature (7 °C) (Figure 6), an increase in the average viability of free *L. rhamnosus* of 2.46 log CFU/g can be observed, reaching more than 60 days of viable storage. The microencapsulated processing ME-0% and ME-10% did not differ statistically at the end of the 120 days of storage, reaching counts above 6 log CFU/g. In addition, these concentrations had

similar behavior during storage, differing only on the 30th and 105th day of analysis, and this was also noted for the microencapsulated ME-100%, although there was a significant difference compared to the ME-10% and ME-0% processing on the 30th and 105th day of analysis. Still, on the 90th day of analysis, the microencapsulated ME-50% and ME-100% did not differ statistically from each other; nonetheless, they showed the lowest and highest probiotic viability, respectively, at the end of the 120 days of storage. The regression analysis showed maximum viability of up to 98 days for storage at 7 °C, (i.e., 1.5 times more viable than at 25 °C). Bustamante et al. (2020) reported that all spray-dried microcapsules using inulin and mucilage from chia and flax seeds containing probiotic bacteria showed high viability for 60 days of storage at 4 ºC, and microcapsules stored at 25 ºC had a continuous decrease in viability. In addition, *L. rhamnosus* quickly lost viability after 28 days. According to the authors, the metabolic activity is higher at 25 ºC and, consequently, nutrients are consumed more quickly, which explains the more significant reduction observed. This demonstrates that microcapsules added with blueberry extract better protected *L. rhamnosus* cells.

At freezing temperatures (-18 °C) (Figure 7), free *L. rhamnosus* counts became viable for about 75 days and above 6 log CFU/g for all microencapsulated processing after 120 days of storage. The ME-10% and ME-100% processing showed similar counts after up to 60 days of storage, and at the end of 120 days, they presented the best results, with counts above 7 log CFU/g ($p > 0.05$). However, it is possible to achieve stability of up to 148 days at -18 °C by the regression equation. Nunes Etchepare et al. (2018) reached viable cell counts of spray-dried *L. acidophilus* using inulin, hi-maize, and trehalose above 6 log CFU/g after 120 days of storage. Nonetheless, the authors reported better microcapsule stability under refrigeration. According to Martín et al. (2015), different probiotic strains have different tolerances to withstand stress conditions. In addition, the conditions used in the encapsulation process are fundamental, resulting in the permanence of microorganism viability during storage (Nunes, Motta et al., 2018).

Therefore, it is clear that the addition of blueberry extract to the formulations of *L. rhamnosus* probiotic microcapsules was effective, suggesting that alternative compounds, such as the polyphenols present in blueberries, are also attractive alternatives in co-microencapsulation due to their potential benefits to human health. Efficient cultivation methods, total cost, and protection of live microorganisms are key factors that significantly affect the commercialization and action of probiotics, such as

Lactobacillus (Cai et al., 2019). Cai et al. (2019) reported that, in freeze-dried microencapsulation, blueberry extracts performed more efficiently as an additive compared to skim milk and whey protein/sodium alginate. The authors also noted that adding an aqueous blueberry extract to the culture medium improved the resistance of *Lactobacillus johnsonni* N6.2 to stress caused by lyophilization and storage for 21 weeks at 4 °C.

Figure 5 - Bacterial viability (*Lactobacillus rhamnosus*, log CFU/g) free or microencapsulated (ME) with different blueberry extract concentrations, stored under ambient temperature (25 °C), during shelf life
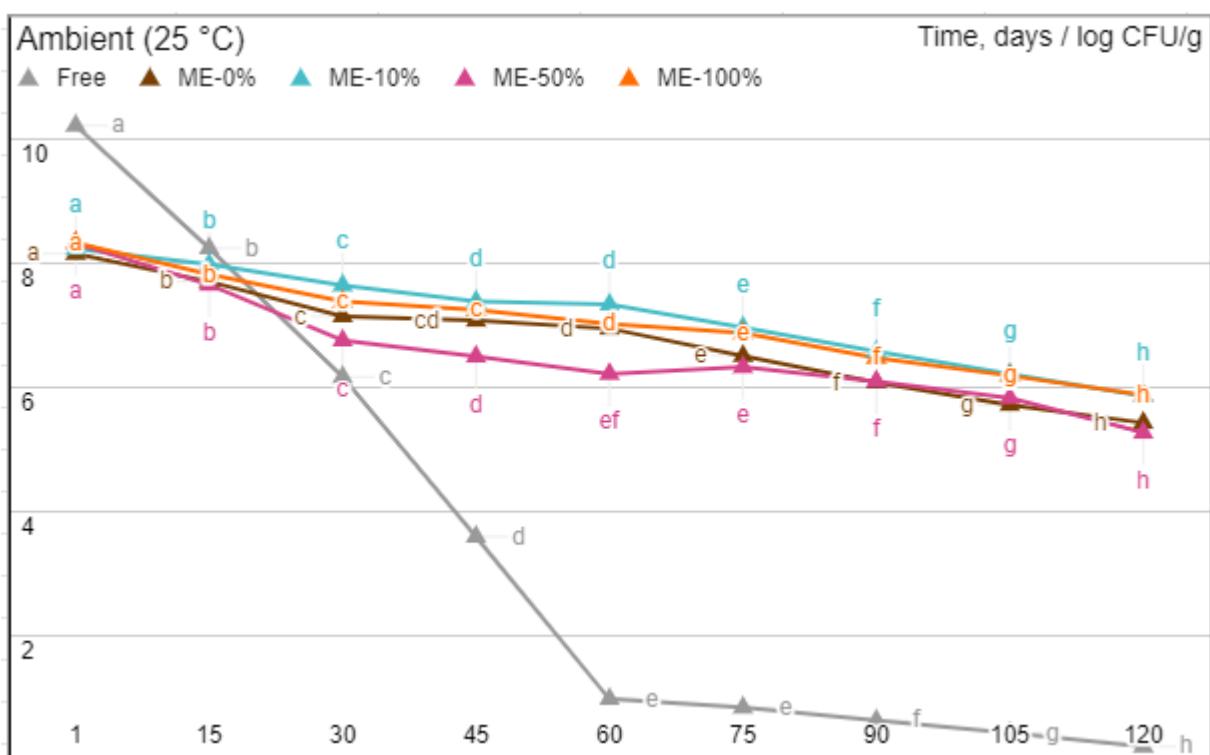
Means followed by different letters differ (p < 0.05) between times by *Student Newman Keuls* test.

Figure 6 - Bacterial viability (*Lactobacillus rhamnosus*, log CFU/g) free or microencapsulated (ME) with different blueberry extract concentrations, stored under chilled temperature (7 °C), during shelf life
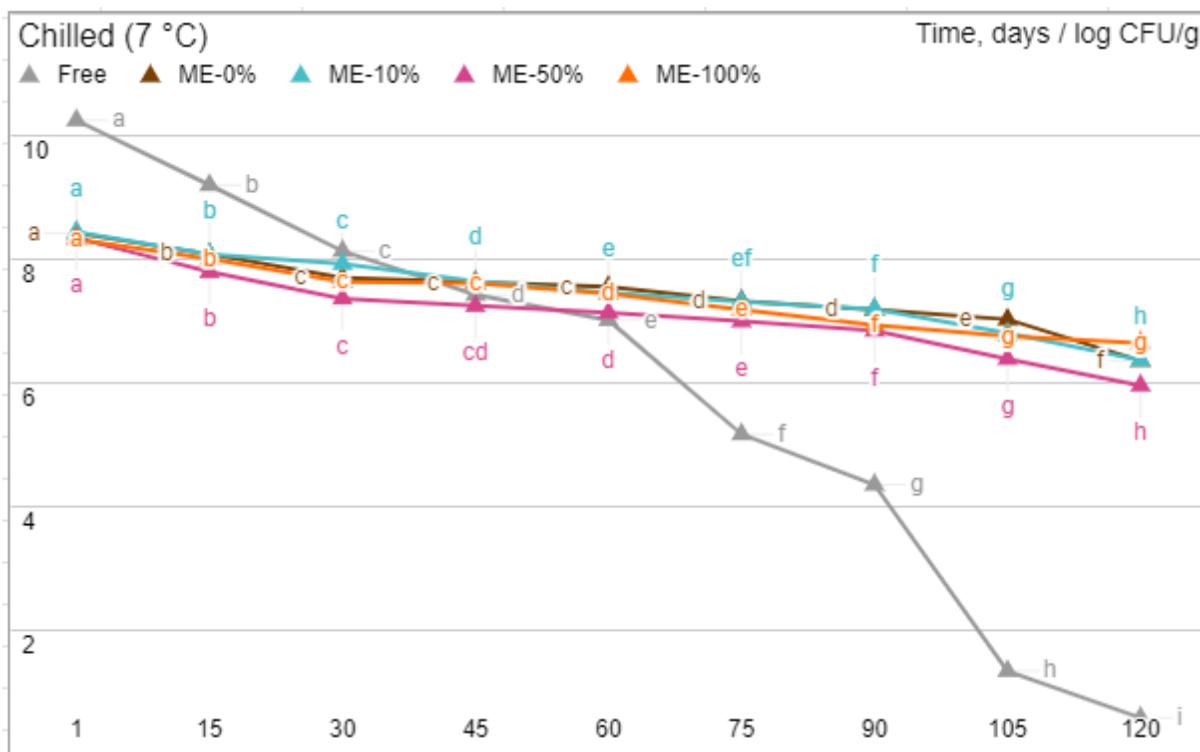
Means followed by different letters differ (p < 0.05) between times by *Student Newman Keuls* test.

Figure 7 - Bacterial viability (*Lactobacillus rhamnosus*, log CFU/g) free or microencapsulated (ME) with different blueberry extract concentrations, stored under frozen temperature (-18 °C), during shelf life
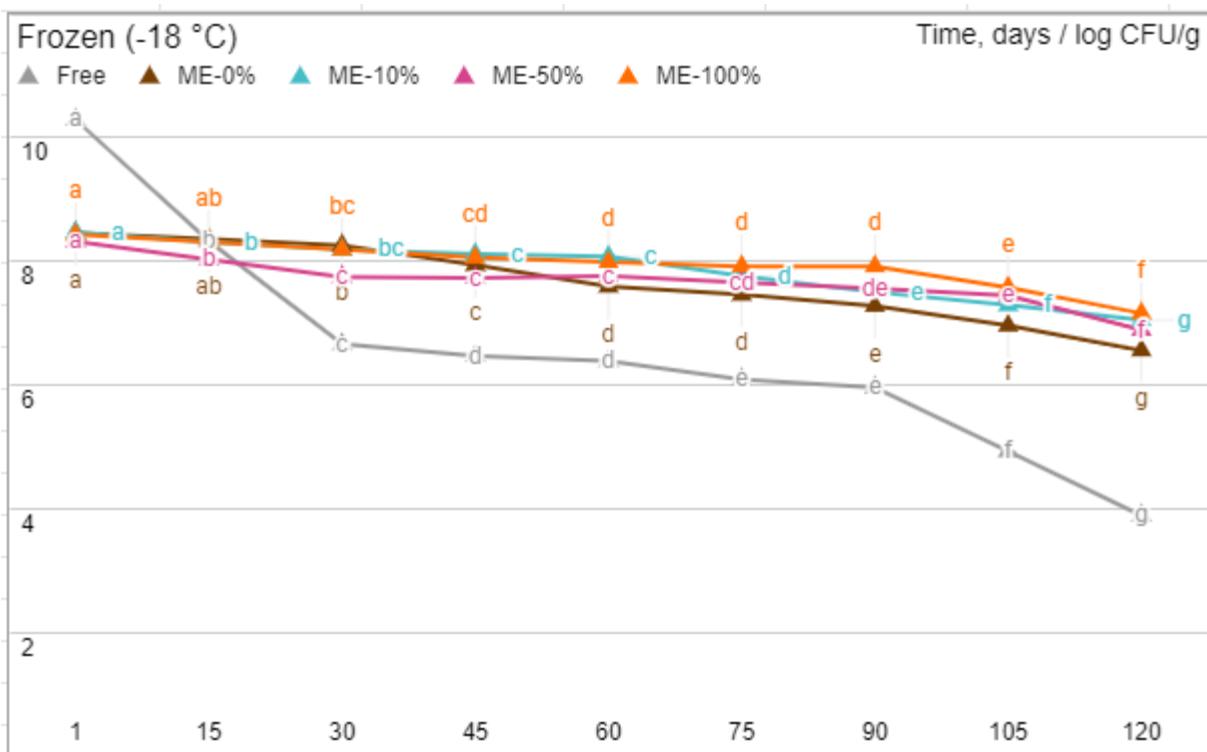
Means followed by different letters differ (p < 0.05) between times by *Student Newman Keuls* test.

4 Conclusion

Co-microencapsulating blueberry extracts has positive effects on *Lactobacillus rhamnosus* survival rates. Adding blueberry extracts to microcapsules improved *L. rhamnosus* protection during passage through the simulated gastric fluid, improving the release rate in the intestine. At the end of the simulation, microcapsules with 100% extract (ME-100%) showed maximum viability, with a loss of only 1.33 log CFU/g, while 50% of the extract (ME-50%) had the lowest survival rate. This method provides better probiotic protection when subjected to different heat treatments. The microencapsulated *L. rhamnosus* was more resistant to heat when 100% extract was added to the microcapsules, with counts above 6 log CFU/g after 120 min of heating at 63 °C. In addition, the addition of blueberry extract to microcapsules containing *L. rhamnosus* improved probiotic storage stability, and it was possible to achieve stability of up to 148 days at -18 °C, which is three times greater than at 25 °C. Therefore, the use of blueberry extract is a promising alternative to protecting micro-encapsulated *L.*

rhmanosus because, in addition to the protection, it contributes phytochemically by providing microcapsules rich in bioactive compounds.

Acknowledgements

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Supplementary material

Table 1 - Bacterial viability free (F) or microencapsulated (ME) with antioxidant levels on different digestive compartments

Digestive (D) compartment	Processing type (P)					Mean	P-value	
	Free	ME-0%	ME-10%	ME-50%	ME-100%		source	Pr > F
Lactobacillus rhamnosus, log CFU g^{-1}								
Original	10.34^{Aa}	8.35^{Ab}	8.21^{Ab}	8.27^{Ab}	8.30^{Ab}	8.69^{A}	P	0.0001
Stomach	3.78^{Bd}	5.15^{Da}	4.85^{Cb}	5.19^{Da}	4.11^{Dc}	4.62^{C}	D	0.0001
Duodenum	2.10^{Cd}	5.43^{Cb}	4.95^{Cc}	6.06^{Ba}	4.82^{Cc}	4.67^{C}	P×D	0.0001
Ileum	2.01^{Cd}	6.15^{Bb}	6.24^{Bb}	5.56^{Cc}	6.97^{Ba}	5.39^{B}	F×ME	0.0001
Mean[1]	4.56^{c}	6.27^{a}	6.06^{b}	6.27^{a}	6.05^{b}			

Means followed by different capital letters within a same column and different small letters within a same row differ (P<0.05), respectively, between digestive compartments and processing types by *Student Newman Keuls* test.

[1] $\bar{y}_{Free} = 4.56$; $\bar{y}_{ME(0-100\%)} = 6.16$.

Table 2 - Bacterial viability free (F) or microencapsulated (ME) with antioxidant levels under different thermal treatments

Thermal treatment (T)	Processing type (P)					Mean	P-value	
	Free	ME-0%	ME-10%	ME-50%	ME-100%		source	Pr > F
Lactobacillus rhamnosus, log CFU g^{-1}								
Original	10.33^{Aa}	8.46^{Ab}	8.47^{Ab}	8.35^{Ab}	8.43^{Ab}	8.81^{A}	P	0.0001
72ºC/15"	7.88^{Ba}	7.24^{Bcd}	7.37^{Bc}	7.16^{Bd}	7.72^{Cb}	7.47^{B}	T	0.0001
72ºC/5'	6.19^{Cd}	6.52^{Cc}	6.79^{Cb}	5.76^{Ee}	6.96^{Ea}	6.44^{D}	P×T	0.0001
63ºC/30'	5.87^{Dd}	7.37^{Bb}	7.37^{Bb}	6.84^{Cc}	7.96^{Ba}	7.08^{C}	F×ME	0.0001
63ºC/60'	0.82^{Ee}	6.42^{Cc}	6.58^{Db}	6.21^{Dd}	7.59^{Da}	5.52^{E}		
63ºC/120'	0.52^{Fd}	5.74^{Db}	5.75^{Eb}	5.25^{Fc}	6.80^{Fa}	4.81^{F}		
Mean[1]	5.27^{e}	6.96^{c}	7.06^{b}	6.59^{d}	7.58^{a}			

Means followed by different capital letters within a same column and different small letters within a same row differ (P<0.05), respectively, between thermal treatments and processing types by *Student Newman Keuls* test.

[1] $\bar{y}_{Free} = 5.27$; $\bar{y}_{ME(0-100\%)} = 7.05$.

Table 3 - Bacterial viability (*Lactobacillus rhamnosus*, log CFU g-1) free or microencapsulated (ME) with antioxidante levels, stored under different temperatures, during shelf life

Processing type[1]	Time, days									Mean	Overall mean
	1	15	30	45	60	75	90	105	120		
Ambient (25ºC)											6.19^C
Free	10.23^{Aa}	8.25^{Ab}	6.17^{Ec}	3.59^{Dd}	0.97^{De}	0.83^{De}	0.62^{Cf}	0.42^{Cg}	0.20^{Dh}	3.48^E	
ME-0%	8.16^{Ba}	7.71^{CDb}	7.15^{Cc}	7.08^{Bcd}	6.95^{Bd}	6.51^{Be}	6.08^{Bf}	5.72^{Bg}	5.43^{Bh}	6.75^C	
ME-10%	8.24^{Ba}	7.99^{Bb}	7.65^{Ac}	7.39^{Ad}	7.34^{Ad}	6.97^{Ae}	6.58^{Af}	6.22^{Ag}	5.86^{Ah}	7.14^A	
ME-50%	8.33^{Ba}	7.66^{Db}	6.76^{Dc}	6.50^{Cd}	6.22^{Cef}	6.33^{Ce}	6.10^{Bf}	5.83^{Bg}	5.27^{Ch}	6.56^D	
ME-100%	8.34^{Ba}	7.83^{Cb}	7.39^{Bc}	7.25^{Ac}	7.03^{Bd}	6.88^{Ae}	6.48^{Af}	6.19^{Ag}	5.88^{Ah}	7.03^B	
Mean[3]	8.66^a	7.89^b	7.02^c	6.36^d	5.70^e	5.50^f	5.17^g	4.87^h	4.53^i		
Chilled (7ºC)											7.08^B
Free	10.26^{Aa}	9.21^{Ab}	8.14^{Ac}	7.43^{Bd}	7.02^{Be}	5.17^{Cf}	4.35^{Cg}	1.33^{Dh}	0.57^{Di}	5.94^D	
ME-0%	8.43^{Ba}	8.09^{Bb}	7.71^{Cc}	7.63^{Ac}	7.56^{Ac}	7.33^{Ad}	7.19^{Ad}	7.03^{Ae}	6.36^{Bf}	7.48^A	
ME-10%	8.45^{Ba}	8.09^{Bb}	7.93^{Bc}	7.65^{Ad}	7.46^{Ae}	7.32^{Aef}	7.20^{Af}	6.81^{Bg}	6.37^{Bh}	7.47^A	
ME-50%	8.35^{Ba}	7.80^{Cb}	7.37^{Dc}	7.25^{Ccd}	7.14^{Bd}	7.00^{Be}	6.85^{Bf}	6.39^{Cg}	5.96^{Ch}	7.12^C	
ME-100%	8.33^{Ba}	8.01^{Bb}	7.64^{Cc}	7.62^{Ac}	7.45^{Ad}	7.19^{Ae}	6.94^{Bf}	6.76^{Bg}	6.65^{Ag}	7.40^B	
Mean[4]	8.76^a	8.24^b	7.76^c	7.52^d	7.32^e	6.80^f	6.51^g	5.66^h	5.18^i		
Frozen (-18ºC)											7.54^A
Free	10.29^{Aa}	8.33^{Ab}	6.66^{Cc}	6.47^{Dd}	6.39^{Dd}	6.09^{De}	5.96^{De}	4.93^{Df}	3.89^{Dg}	6.56^D	
ME-0%	8.46^{Ba}	8.36^{Aab}	8.26^{Ab}	7.94^{Bc}	7.59^{Cd}	7.46^{Cd}	7.28^{Ce}	6.96^{Cf}	6.56^{Cg}	7.65^C	
ME-10%	8.47^{Ba}	8.31^{Ab}	8.19^{Abc}	8.12^{Ac}	8.08^{Ac}	7.76^{Bd}	7.50^{Be}	7.29^{Bf}	7.05^{Ag}	7.86^B	
ME-50%	8.32^{Ba}	8.03^{Bb}	7.75^{Bc}	7.73^{Cc}	7.76^{Bc}	7.66^{Bcd}	7.56^{Bde}	7.45^{Ae}	6.89^{Bf}	7.68^C	
ME-100%	8.43^{Ba}	8.31^{Aab}	8.19^{Abc}	8.06^{ABcd}	7.99^{Ad}	7.92^{Ad}	7.92^{Ad}	7.58^{Ae}	7.16^{Af}	7.95^A	
Mean[5]	8.79^a	8.27^b	7.81^c	7.67^d	7.56^e	7.38^f	7.24^g	6.84^h	6.31^i		
Overall mean[2]	8.74^a	8.13^b	7.53^c	7.18^d	6.86^e	6.56^f	6.31^g	5.79^h	5.34^i		

Means followed by different capital letters within a same column and different small letters within a same row differ (P<0.05), respectively, between processing types and times by *Student Newman Keuls* test.

[1] Overall mean: Free= 5.33^e; ME-0%= 7.30^c; ME-10%= 7.49^a; ME-50%= 7.12^d; ME-100%= 7.46^b ($\bar{y}_{ME(0-100\%)Overall} = 7.34$; $\bar{y}_{ME(0-100\%)Ambient} = 6.87$; $\bar{y}_{ME(0-100\%)Chilled} = 7.37$; $\bar{y}_{ME(0-100\%)Frozen} = 7.79$).

[2] $\hat{y}_{Time_{(Overall)}} = 8.5262 - 0.0264x$ $(r^2 = 0.98)$; [3] $\hat{y}_{Time_{(Ambient)}} = 8.2103 - 0.0336x$ $(r^2 = 0.95)$; [4] $\hat{y}_{Time_{(Chilled)}} = 8.7793 - 0.0282x$ $(r^2 = 0.98)$; [5] $\hat{y}_{Time_{(Frozen)}} = 8.5892 - 0.0174x$ $(r^2 = 0.94)$

6 DISCUSSÃO

A morfologia das microcápsulas de *Lactobacillus rhamnsosus* co-microencapsulado com diferentes concentrações de extrato de mirtilo apresentaram forma esférica, irregular e com presença de concavidades. Bustamante et al. (2020) reportaram que estas concavidades são típicas de produtos obtidos por spray drying. Essa característica foi mais evidente para a microcápsula controle (ME-0%), seguida pela microcápsula com 10% de extrato (ME-10%) e com 50% de extrato (ME-50%), enquanto que a microcápsula com 100% de extrato (ME-100%) apresentou uma superfície lisa. Isso demonstra a relação com a concentração de sólidos da solução de alimentação, decorrente da adição do extrato de mirtilo. Por outro lado, as diferentes microcápsulas não apresentaram fissuras ou rupturas na superfície, impossibilitando a entrada de ar, o que resulta em maior proteção para os microrganismos probióticos (FRITZEN-FREIRE et al., 2012; RAJAM; ANANDHARAMAKRISHNAN, 2015).

Em relação ao tamanho de partícula, as microcápsulas apresentaram diâmetros médios variando de 1,7 a 6,3 μm em função da concentração de extrato de mirtilo adicionado às formulações. Segundo Piñón-Balderrama et al. (2020), o sistema de atomização tem grande influência no tamanho das partículas. Soluções contendo maiores concentrações de sólidos apresentam maior viscosidade e, consequentemente, maior tamanho de partícula. Martin et al. (2015) ressalta que, tamanhos de partículas menores são preferíveis, tendo em vista a não apresentação de efeitos sensoriais quando aplicados em alimentos. Contudo, microcápsulas de tamanho maiores geralmente proporcionam maior proteção aos microrganismos contra condições adversas do meio (SANDOVAL-CASTILLA, 2010). Desta forma, o tamanho de partícula ideal é um fator a ser discutido, pois depende da aplicação desejada (ROSAS-FLORES; RAMOS-RAMÍREZ; SALAZARMONTOYA, 2013).

Quanto aos parâmetros de cor, o parâmetro L* (luminosidade) seguiu um comportamento decrescente para as microcápsulas com menor concentração de extrato (ME-10%) para as de maior concentração (ME-100%), indicando que quanto menor foi a adição de extrato de mirtilo, mais clara a cor dos pós obtidos. Com relação aos parâmetros a* e b*, as microcápsulas apresentaram valor positivo para a* e negativo para b*, indicando uma tendência às colorações vermelho e azul, respectivamente. O parâmetro C* indica o grau de saturação da amostra, o qual foi

mais forte no processamento ME-100% e menos forte em ME-10%, sugerindo maior intensidade da cor nas microcápsulas obtidas com 100% de extrato. O ângulo Hue (h*) refere-se a cor percebida. Todas as microcápsulas apresentaram valores de h* > 330º, sendo descritas, portanto, como amostras avermelhadas. Tal comportamento era esperado, uma vez que o extrato de mirtilo é rico em antocianinas, composto responsável pela coloração azul escuro dos frutos. Em virtude disso, o mirtilo pode ser uma fonte de pigmentos naturais, podendo ser aplicada na produção de novos produtos alimentícios (SUN et al., 2019).

Dentre os compostos fenólicos presentes no mirtilo, a classe predominante são as antocianinas. No estudo, o extrato de mirtilo obtido por MHG apresentou uma concentração de 528,4 ± 15,3 mg/L de delfinidina-3-glicosídeo. Ao co-microencapsular o extrato de mirtilo em diferentes proporções para obtenção de microcápsulas de *L. rhamnosus* secas por spray, observou-se que as microcápsulas obtidas com 100% de extrato (ME-100%) foram as microcápsulas que apresentaram maior concentração de antocianinas, com 168,88 ± 4,79 mg/100 g de delfinidina-3-glicosídeo, seguida das microcápsulas com 50% de extrato (ME-50%) com 55,35 ± 2,89 mg/100 g de delfinidina-3-glicosídeo, e por fim as microcápsulas com 10% de extrato de mirtilo (ME-10%) com 15,12 ± 0,75 mg/100 g de delfinidina-3-glicosídeo. Nota-se que quanto maior a proporção de extrato de mirtilo adicionado, maior foi a concentração de antocianinas nas microcápsulas probióticas obtidas por spray drying. Isso se dá pelo fato que nas microcápsulas com 100% de extrato, o extrato foi adicionado bruto, já nas microcápsulas com 50% e 10% de extrato, além de ter sido adicionado uma concentração menor de extrato, o mesmo foi adicionado de água para completar o volume da solução de alimentação (100 mL), o que evidenciou ainda mais na concentração de antocianinas das microcápsulas. Comportamento semelhante foi obtido para o resultado de antocianinas monoméricas totais, com 198,7 ± 3,4 mg/100 g de antocianinas para ME-100%, 118,22 ± 0,76 para ME-50% e 27,9 ± 0,6 mg/100 g de antocianinas para ME-10%. Já o extrato bruto apresentou 631,93 ± 33,99 mg/L de antocianinas. Desta forma, nota-se que a microcápsula mais indicada para uso ou aplicação em novos produtos, seria a microcápsula com 100% de extrato de mirtilo, já que foi a microcápsula que apresentou maior concentração de antocianinas, sendo esta a mais benéfica fitoquimicamente. Além disso, a obtenção de microcápsulas com extrato de mirtilo por spray drying é capaz de proteger os compostos antociânicos do mirtilo que são considerados termo-sensíveis (MAHDAVI et al., 2016).

Na avaliação da sobrevivência dos probióticos em condições gastrointestinais simuladas pode-se perceber que ocorreu uma redução maior que 8 log UFC / g dos *L. rhamnosus* livres nas condições simuladas do íleo (pH 6,5) quando comparada à contagem inicial antes da simulação. Isso demonstra que, em condições ácidas e na presença de enzimas gástricas, a exposição das células livres resultou em uma perda significativa na viabilidade das bactérias probióticas. Quanto a viabilidade das microcápsulas secas, a exposição das células co-microencapsuladas em condições ácidas (simulação do esôfago / estômago) obteve-se resultados de cerca de 4 a 5 log UFC / g enquanto que a contagem inicial antes da simulação foi de aproximadamente 8 log UFC / g, indicando que não houve o rompimento total das microcápsulas. Após as condições simuladas do íleo (pH 6,5), as microcápsulas apresentaram uma redução de 1,33 a 2,71 log UFC / g comparada com a contagem inicial antes da simulação (p < 0,05). Isso se deve ao fato que, o processo de co-microencapsulação conferiu proteção as bactérias probióticas na simulação gastrointestinal. Além disso, foi demonstrado que a adição do extrato de mirtilo às microcápsulas não interferiu na viabilidade dos *L. rhamnosus*. A co-microencapsulação de *L. rhamnosus* com extrato de mirtilo ofereceu um meio eficaz de entrega de células bacterianas viáveis a nível intestinal, em condições adequadas, e ajudou a manter a sua sobrevivência durante a passagem pelo transito gastrointestinal simulado.

A proteção dos diferentes tipos de processamento na avaliação da resistência térmica das microcápsulas demonstrou que o microencapsulado ME-100% promoveu a menor perda de viabilidade para *L. rhamnosus*, com 0,47, 0,83 e 1,63 ciclos logarítmicos quando submetidos ao tratamento a 63°C por 30, 60 e 120 min, respectivamente. Já o tratamento a 72 °C por 15 s e 5 min, foram observadas perdas de 0,71 e 1,47 ciclos logarítmicos, respectivamente. O processamento microencapsulado ME-50% foi o que apresentou menor viabilidade em todas as temperaturas. Ademais, entre os tratamentos térmicos estudados, 63 °C / 30 min e 72 °C / 15 s promoveram maior sobrevivência para todos os tipos de processamentos. Verruck et al. (2017) destacam a importância de estudos frente a sobrevivência de microrganismos probióticos a diferentes tratamentos térmicos, pois no processamento de alimentos, a capacidade da bactéria em tolerar altas temperaturas é um fator chave para a aplicação destes nos alimentos.

Durante o armazenamento, o processo de microencapsulação mostrou-se eficiente na proteção dos probióticos, aumentando o tempo de viabilidade dos

Lactobacillus rhamnosus quando comparado à bactéria na forma livre. Além disso, observou-se que, o armazenamento congelado (-18 °C) apresentou a maior viabilidade probiótica média, com 7,54 log UFC / g, enquanto que a temperatura de refrigeração (7 °C) e ambiente (25 °C) apresentaram viabilidade de 7,08 e 6,19 log UFC / g, respectivamente. Na temperatura ambiente as bactérias livres mantiveram-se viáveis por cerca de 30 dias de armazenamento, enquanto que os processamentos microencapsulados foram viáveis por mais de 90 dias. A maior viabilidade foi observada com o microencapsulado ME-100%, com 5,88 log UFC / g em 120 dias de armazenamento. Em condição de armazenamento à temperatura de refrigeração observou-se um aumento na viabilidade média dos *Lactobacillus rhamnosus* livres, alcançando mais de 60 dias de armazenamento viáveis. Os microencapsulados ME-0% e ME-10% não diferiram significativamente ao final dos 120 dias de armazenamento, alcançando contagens superiores a 6 log UFC / g. Já os processos microencapsulados ME-50% e ME-100%, ao final de 120 dias, apresentaram a menor e a maior viabilidade probiótica, com contagens de 5,96 e 6,65 log UFC / g, respectivamente. Na temperatura de congelamento, a contagem de *Lactobacillus rhamnosus* permaneceu acima de 6 log UFC / g para todos os processamentos microencapsulados após 120 dias de armazenamento, com melhores resultados para os microencapsulados ME-10% e ME-100%, os quais alcançaram viabilidade superior a 7 log UFC / g, não diferindo estatisticamente. As células livres mantiveram-se viáveis por aproximadamente 75 dias.

7 CONCLUSÃO

A co-microencapsulação de extrato de mirtilo apresenta efeito positivo na sobrevivência de *Lactobacillus rhamnosus*. A adição de extrato de mirtilo em microcápsulas melhora a proteção das bactérias probióticas de *L. rhamnosus* durante a passagem pelo fluído gástrico simulado, melhorando a taxa de liberação no intestino. No final da simulação, microcápsulas com 100% de extrato apresentaram viabilidade máxima, com uma perda de apenas 1,33 log UFC / g, enquanto que 50% de extrato apresentou a menor taxa de sobrevivência. Também oferece maior proteção aos probióticos quando submetidos a diferentes tratamentos térmicos. Os *L. rhamnosus* microencapsulados foram mais resistentes ao calor quando adicionados de 100% de extrato às microcápsulas, com contagem superior a 6 log UFC / g após 120 min de aquecimento a 63 ºC. Além disso, a adição de extrato de mirtilo às microcápsulas contendo *L. rhamnosus* melhora a estabilidade ao armazenamento dos probióticos, uma vez que foi possível alcançar uma estabilidade superior a 120 dias à -18 °C. Portanto, o uso de extrato de mirtilo é uma alternativa promissora na proteção de *L. rhmanosus* microencapsulados, pois além da proteção contribui fitoquimicamente, fornecendo microcápsulas ricas em compostos bioativos.

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