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

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MICROENCAPSULAÇÃO DE CULTURAS PROBIÓTICAS POR *SPRAY DRYING* UTILIZANDO DIFERENTES AGENTES ENCAPSULANTES

Santa Maria, RS
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Tese de doutorado apresentada ao Programa de Pós-Graduação em Ciência e Tecnologia dos Alimentos, Área de Concentração em Ciência e Tecnologia dos Alimentos, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do título de **Doutor em Ciência e Tecnologia dos Alimentos.**

Orientador: Prof. Dr. Cristiano Ragagnin de Menezes
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Santa Maria, RS
2018

Nunes, Graciele Lorenzoni
Microencapsulação de culturas probióticas por spray drying utilizando diferentes agentes encapsulantes / Graciele Lorenzoni Nunes.- 2018.
116 p.; 30 cm

Orientador: Cristiano Ragagnin de Menezes
Coorientadora: Cristiane de Bona da Silva
Tese (doutorado) - Universidade Federal de Santa Maria, Centro de Ciências Rurais, Programa de Pós Graduação em Ciência e Tecnologia dos Alimentos, RS, 2018

1. Lactobacillus acidophilus La-5 2. Bifidobacterium Bb-12 3. Spray dryer 4. Matrizes encapsulantes I. Menezes, Cristiano Ragagnin de II. Silva, Cristiane de Bona da III. Título.

Sistema de geração automática de ficha catalográfica da UFSM. Dados fornecidos pelo autor(a). Sob supervisão da Direção da Divisão de Processos Técnicos da Biblioteca Central. Bibliotecária responsável Paula Schoenfeldt Patta CRB 10/1728.

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Santa Maria, RS
2018

RESUMO

MICROENCAPSULAÇÃO DE CULTURAS PROBIÓTICAS POR *SPRAY DRYING* UTILIZANDO DIFERENTES AGENTES ENCAPSULANTES

AUTORA: Graciele Lorenzoni Nunes
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Os produtos funcionais têm desempenhado um papel importante na indústria de alimentos por apresentarem propriedades benéficas a saúde dos consumidores. Neste contexto, encontram-se as bactérias probióticas, que devido aos seus inúmeros benefícios, vêm recebendo destaque e hoje compreendem aproximadamente 65% do mercado mundial de alimentos funcionais. No entanto, apesar da aplicação destes microrganismos na indústria de alimentos, a manutenção da sua viabilidade ainda é bastante discutida e estudada, visto que os probióticos apresentam elevada sensibilidade a fatores ambientais e também na passagem pelo trato gastrointestinal. Desse modo, a microencapsulação apresenta-se como um método promissor para fornecer revestimento adequado para esses microrganismos a fim de que possam se manter viáveis e alcançar seu local de ação em quantidades adequadas. Neste trabalho, diferentes matrizes encapsulantes foram estudadas para o processo de microencapsulação por *spray drying* com o objetivo de conferir maior proteção para *Lactobacillus acidophilus* La-5 e *Bifidobacterium* Bb-12 buscando alcançar altas taxas de sobrevivência e maior eficiência de encapsulação (EE%). Primeiramente, formulações compostas por maltodextrina e goma arábica com *Lactobacillus acidophilus* La-5 (ML) e *Bifidobacterium* BB-12 (MB) foram submetidas a secagem em diferentes condições de temperaturas de entrada de ar no *spray dryer*. A temperatura de entrada de 130 °C foi escolhida por fornecer micropartículas com maior viabilidade, menor atividade de água e umidade para ambos microrganismos microencapsulados. Após, foram produzidas as micropartículas de *Lactobacillus acidophilus* La-5 e *Bifidobacterium* Bb-12 com as diferentes matrizes encapsulantes avaliadas, sendo elas: inulina (MS_2), hi-maize (MS_3) e trealose (MS_4) e a amostra controle (MS_1). As diferentes micropartículas (MS_1, MS_2, MS_3 e MS_4) foram avaliadas com relação a sua resistência a tratamentos térmicos, as condições gastrointestinais simuladas e a diferentes condições de armazenamento. A morfologia e o tamanho médio das diferentes partículas também foram determinados. Para *Lactobacillus acidophilus* La-5 hi-maize (94,26%) e inulina (93,12%) foram as matrizes encapsulantes que apresentaram a maior eficiência de encapsulação. Logo, para *Bifidobacterium* Bb-12 hi-maize (95,24%) e trealose (90,10%) conferiram maior eficiência de encapsulação. O tamanho médio das micropartículas variou de 6.68 a 20.9 μm e a morfologia mostrou que as mesmas se apresentaram em forma esférica e com presença de concavidades. Na avaliação da resistência a tratamentos térmicos, simulação gastrointestinal e nas condições de armazenamento as matrizes encapsulantes hi-maize e trealose foram as que demostram maior potencial de proteção para ambos microrganismos estudados. Por fim, os resultados deste estudo mostraram que a utilização de hi-maize e trealose melhorou a viabilidade e consequentemente a sobrevivência de *Lactobacillus acidophilus* La-5 e *Bifidobacterium* Bb-12 demostrando que estas matrizes encapsulantes apresentaram elevado potencial termoprotetor para as culturas probióticas microencapsuladas em spray dryer.

Palavras-chave: *Lactobacillus acidophilus* La-5. *Bifidobacterium* Bb-12. *Spray dryer*. Matrizes encapsulantes.

ABSTRACT

MICROENCAPSULATION OF PROBIOTIC CULTURE BY *SPRAY DRYING* USING DIFFERENT ENCAPSULANT AGENTS

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Functional products have played an important role in the food industry because they have beneficial health properties for consumers. In this context, there are probiotic bacteria, which, due to their innumerable benefits, have been highlighted and today comprise approximately 65% of the world market for functional foods. However, despite the application of these microorganisms in the food industry, the maintenance of their viability is well discussed and studied, since probiotics are highly sensitive to environmental factors and also to the passage through the gastrointestinal tract. Thus, microencapsulation presents itself as a promising method to provide a suitable coating for such microorganisms, so that they can remain viable and reach their site of action in suitable amounts. In this work, different encapsulation matrices for the spray-drying microencapsulation process were studied in order to provide greater protection for Lactobacillus acidophilus La-5 and Bifidobacterium Bb-12, aiming at high survival rates and higher encapsulation efficiency (EE %). First, formulations composed of maltodextrin and gum arabic with *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* BB-12 (MB) were dried under different inlet air temperature conditions in the spray drier. The inlet temperature of 130 °C was chosen to provide microparticles with higher viability, lower water activity and humidity for both microencapsulated microorganisms. The microparticles of *Lactobacillus acidophilus* La-5 and *Bifidobacterium* Bb-12 with the different encapsulating matrices were produced, being: inulin (MS_2), hi-maize (MS_3) and trehalose (MS_4) and the control sample (MS_1). The different microparticles (MS_1, MS_2, MS_3 and MS_4) were evaluated for their resistance to heat treatments, simulated gastrointestinal conditions and different storage conditions. The morphology and average size of the different particles were also determined. For *Lactobacillus acidophilus* La-5 hi-maize (94.26%) and inulin (93.12%) were the encapsulating matrices that presented the highest encapsulation efficiency. Therefore, for *Bifidobacterium* Bb-12 hi-maize (95.24%) and trehalose (90.10%) confer greater encapsulation efficiency. The average size of the microparticles ranged from 6.68 to 20.9 μm and morphology showed that they were in spherical shape and with concavities. In the evaluation of resistance to heat treatments, gastrointestinal simulation and in the storage conditions, the matrices encapsulantes hi-maize and trehalose were the ones that demonstrate greater potential of protection for both microorganisms studied. Finally, the results of this study showed that the use of hi-maize and trehalose improved the viability and consequently the survival of *Lactobacillus acidophilus* La-5 and *Bifidobacterium* Bb-12 showing that these encapsulating matrices had high thermoprotective potential for microencapsulated probiotic cultures in spray dryer.

Keywords: *Lactobacillus acidophilus* La-5. *Bifidobacterium* Bb-12. *Spray dryer*. Encapsulating matrices.

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SUMÁRIO

1 INTRODUÇÃO

O aumento da conscientização dos consumidores sobre a saúde resultou em uma crescente procura por alimentos que tragam benefícios e que promovam o bem-estar (GRANATO et al., 2010; PERRICONE et al., 2015; PANGHAL et al., 2018). Os probióticos vêm sendo reconhecidos por proporcionarem uma série de benefícios a saúde do consumidor através da manutenção do equilíbrio e da composição do trato gastrointestinal (LOURENS-HATTINGH; VILJOEN, 2001; MARTIN et al., 2015).

No entanto, para promover seus efeitos benéficos ao hospedeiro, estes microrganismos devem sobreviver a passagem pelo trato gastrointestinal, especialmente as condições ácidas do estômago, e serem capazes de atingir o colón em quantidades adequadas para que ocorra sua colonização e proliferação (KOMATSU; BURITI; SAAD, 2008; LI et al., 2011). Devido a estes fatores, e a sensibilidade que as culturas probióticas apresentam ao processamento e também as características de alguns produtos, métodos de microencapsulação têm sido aplicados com a finalidade de proteger os probióticos contra as condições adversas aos quais são expostos (MORTA-ZAVIAN et al., 2007; FÁVARO-TRINDADE; PINHO; ROCHA, 2008; da SILVA; BARREIRA; OLIVEIRA, 2016; TYLKOWSKI et al., 2017).

A microencapsulação é um processo no qual uma barreira é criada sobre um componente ativo, o que vai inibir interações químicas, proteger contra os efeitos de fatores ambientais (temperatura, pH, enzimas e oxigênio) e permitir a liberação controlada do componente ativo em certas condições (SHAHADI; HAN, 1993; DESAI; PARK, 2005; DIAS et al., 2017). Dentre os métodos de microencapsulação disponíveis, a técnica de *spray drying* é uma das mais utilizadas para a encapsulação de ingredientes alimentícios por apresentar algumas vantagens, como custo relativamente baixo, facilidade de operação, altas taxas de produção e possibilidade de aplicação em escala industrial. No entanto, neste processo há necessidade de exposição dos microrganismos probióticos a temperaturas elevadas o que pode prejudicar sua integridade celular, levando a diminuição da sua viabilidade (BURGAIN et al., 2011; GHANDI, et al., 2012; HAFFNER; DIAB; PASC, 2016).

Neste contexto, diferentes estratégias podem ser estudadas para melhorar a sobrevivência dos probióticos, tais como, a avaliação dos parâmetros do *spray dryer* e a seleção de matrizes encapsulantes com potencial de proteção. Diferentes agentes encapsulantes vem sendo utilizados, e o estudo destes, tem se tornado cada mais extensivo e importante para alcançar máxima sobrevivência para culturas probióticas neste processo (FRITZEN-FREIRE et al., 2013; ARSLAN et al., 2015; ECKERT et al., 2017).

Neste trabalho, diferentes condições de temperaturas de entrada de ar no *spray dryer* foram avaliadas a fim de alcançar maior eficiência de encapsulação para *Lactobacillus acidophilus* La-5 e *Bifidobacterium* Bb-12. Após a determinação da melhor condição de temperatura de secagem, micropartículas contendo inulina, hi-maize, trealose e goma arábica (amostra controle) foram produzidas no *spray dryer* afim de avaliar o potencial termoprotetor destas diferentes matrizes encapsulantes para as culturas probióticas.

2 OBJETIVOS

2.1 OBJETIVO GERAL

Desenvolver e caracterizar micropartículas contendo *Lactobacillus acidophilus* La-5 e *Bifidobacterium* Bb-12 através da técnica de *spray dryer* e avaliar o efeito termoprotetor de diferentes matrizes encapsulantes sobre a viabilidade destas culturas probióticas.

2.2 OBJETIVOS ESPECÍFICOS

- Determinar a melhor condição de temperatura de entrada de ar do *spray dryer* (110, 120, 130 ou 140 °C) para alcançar maior viabilidade de *Lactobacillus acidophilus* La-5 e *Bifidobacterium* Bb-12;
- Desenvolver micropartículas de *Lactobacillus acidophilus* La-5 e *Bifidobacterium* Bb-12 utilizando inulina, hi-maize e trealose como matrizes encapsulantes;
- Caracterizar as micropartículas com relação as suas propriedades físico-químicas (morfologia, tamanho de partícula, umidade e atividade de água);
- Avaliar as micropartículas submetidas a diferentes tratamentos térmicos;
- Verificar a resistência das micropartículas submetidas as condições gastrointestinais simuladas;
- Acompanhar a viabilidade das micropartículas durante seu armazenamento em diferentes condições de temperatura.

3 REVISÃO DA LITERATURA

3.1 PROBIÓTICOS

Nas últimas décadas tem ocorrido uma maior conscientização, por parte dos consumidores, sobre o impacto dos alimentos na saúde (GRANATO et al., 2010). Neste contexto, a indústria de alimentos apresentou uma crescente demanda no fornecimento de ingredientes bioativos, com o objetivo de desenvolver os chamados “alimentos funcionais”. Os alimentos funcionais são aqueles que, além dos efeitos nutricionais conhecidos, podem resultar em benefícios clínicos ou de saúde comprovados (SAAD; CRUZ; FARIA, 2011). Segundo a Transparency Market Research (2016) o mercado global de alimentos funcionais cresceu rapidamente, sendo avaliado em mais de R$ 260 bilhões. Além disso, a previsão é de que até 2024 as vendas devem ultrapassar os R$ 380 bilhões.

Dentre os alimentos funcionais, destacam-se os probióticos, que são definidos como microrganismos vivos que quando administrados em quantidades adequadas conferem benefícios a saúde do hospedeiro (FAO/OMS, 2006). Os probióticos vem sendo reconhecidos por proporcionarem uma série de efeitos benéficos à saúde através do seu principal mecanismo de ação, o qual se refere a inibição da colonização do intestino por bactérias patogênicas. Estes microrganismos através da produção de substâncias bactericidas e da disputa por nutrientes, promovem uma alteração no metabolismo microbiano e estimulam o sistema imunológico a partir da sua capacidade de adesão a mucosa intestinal (CORREIA; LIBEREDO; CONSOLI, 2012). Alguns dos benefícios relatados para as bactérias probióticas são especialmente relacionados ao alivio de sintomas gástricos e resposta imune melhorada (BOGSAN et al., 2014; EJTAHED et al., 2011). No entanto, a utilização de probióticos também tem sido associada a terapia do câncer e a pacientes com níveis de colesterol elevados (GOVENDER et al., 2014).

As bactérias associadas a essas funções pertencem principalmente aos gêneros *Lactobacillus* e *Bifidobacterium.* As bactérias do gênero *Lactobacillus* são ácido-láticas, do tipo bastonetes, gram-positivas que não formam esporos. A fonte de energia destes microrganismos é proveniente do metabolismo fermentativo sendo o ácido lático o produto final da degradação de carboidratos, motivo pelo qual sobrevive em ambientes mais ácidos. O pH ótimo de crescimento está na faixa de 5,5 a 6,0 e temperatura ótima entre 35 °C e 40 °C (GOKTEPE et al., 2006). Estas bactérias geralmente são mais resistentes que o gênero *bifidobacterium* em

meio ácido, pois é naturalmente encontrada em produtos fermentados, se adaptando muito bem em produtos lácteos (LEE; SALMINEN, 2009).

Os microrganismos pertencentes ao gênero *Bifidobacterium* são bastonetes gram-positivos não formadores de esporos, não filamentosos, sem motilidade e anaeróbios estritos, apesar de algumas cepas terem a capacidade de tolerar o oxigênio em presença de dióxido de carbono. Possuem morfologia variada, podendo ser uniforme ou bifurcada em forma de "Y" ou "V". O pH e temperatura ótimos para o crescimento de *Bifidobacterium* varia entre 6,0 e 7,0 e 37 e 41 º C respectivamente (SHAH, 2007; RIVERA-ESPINOZA; GALLARDO-NAVARRO, 2010). A resistência desta cultura probiótica em condições gastrointestinais simuladas e a boa capacidade de adesão as células epiteliais intestinais, são algumas das características importantes deste gênero.

A indústria de alimentos tem utilizado extensivamente estas culturas probióticas, especialmente em produtos lácteos, devido a sua estabilidade metabólica, aderência as paredes celulares intestinais, por serem seguras ao consumo humano e também pelo seu efeito positivo a saúde (GOVENDER et al., 2014; MITSUOKA, 2014). No entanto, para que os microrganismos probióticos exerçam os efeitos benéficos desejados devem estar presentes em quantidades apropriadas no alimento e serem ingeridos diariamente (KOMATSU; BURITI; SAAD, 2008). Assim, recomenda-se que uma ingestão diária mínima deve estar situada entre 10^8 e 10^9 células viáveis, o que pode ser alcançado com o consumo diário de pelo menos 100 g de um produto contendo entre $10^6 – 10^7$ células viáveis por grama (BOYLSTON et al., 2004; TRIPATHI; GIRI, 2014).

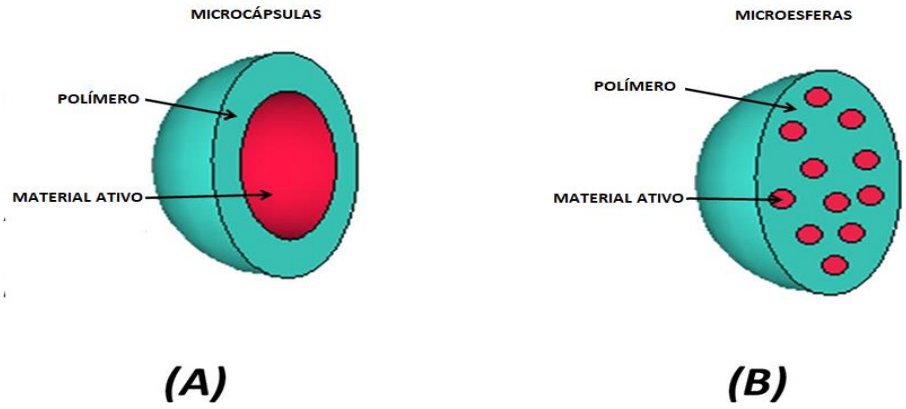
Entretanto, apesar do conhecimento acerca das características dos probióticos, a sua incorporação em alimentos ainda apresenta inúmeros desafios, especialmente em relação a sua estabilidade durante o processamento e armazenamento. Estes microrganismos apresentam sensibilidade a diversos fatores, tais como, presença de oxigênio, pH, temperatura de armazenamento, atividade de água, pressão osmótica e também ao processo digestivo (MORTAZAVIAN et al., 2007). Dessa maneira, durante a produção de alimentos à base de probióticos, as características tecnológicas devem ser avaliadas de modo a evitar interações indesejáveis com a matriz alimentar e para que os probióticos não percam a sua viabilidade e funcionalidade, alcançando o trato gastrointestinal humano e liberando os ingredientes bioativos no sítio alvo (da SILVA et al., 2016). Neste contexto, a microencapsulação apresenta-se como uma alternativa de grande relevância, uma vez que, aborda alguns destes requisitos, tornando-se uma ferramenta útil na proteção dos microrganismos probióticos (COOK et al., 2012; RATHORE et al., 2013; TYLKOWSKI et al., 2017).

3.2 MICROENCAPSULAÇÃO

A microencapsulação é uma técnica conhecida há muitas décadas, no entanto, vem ganhando espaço em aplicações nas mais diversas áreas, tais como farmacêutica, alimentar e cosmética (SUAVE et al., 2006). Seu conceito tem como base a idealização do modelo celular, no qual o núcleo é envolvido por uma membrana semipermeável que o protege do meio externo e, ao mesmo tempo, controla a entrada e saída de substâncias na célula (RÉ, 2006). Assim, a microencapsulação compreende um processo em que ocorre a incorporação de substâncias de interesse (núcleo ou material ativo) em um sistema de revestimento (material de parede, carreador ou agente encapsulante), obtendo-se microcápsulas com um diâmetro que varia entre 1 e 1000 μm (MADENE et al., 2006; OBEIDAT, 2009; TIWARI et al., 2010; BURGAIN et al., 2011; FANG; BHANDARI, 2010).

As micropartículas fisicamente são caracterizadas pela sua forma esférica e por apresentar aspecto sólido. No entanto, o tamanho, a forma e a estrutura das micropartículas dependem dos materiais empregados, ou seja, dos agentes encapsulantes e do método utilizado na produção (FANG; BHANDARI, 2012). Além disso, as micropartículas podem ser classificadas em microcápsulas ou microesferas (Figura 1), de acordo com sua estrutura interna e morfologia (HERRERO-VANRELL; BRAVO-OSUNA; ANDRÉS-GUERRERO, 2014; JYOTHI et al., 2010).

Figura 1 – Diferenças na morfologia interna de microcápsulas (A) e microesferas (B)



Fonte: Adaptado de Herrero-Vanrell, Bravo-Osuna e Andrés-Guerrero (2014).

Na indústria de alimentos a microencapsulação vem apresentando razões significativas que justifiquem sua aplicação, tais como, diminuir qualquer reação indesejada do material do

núcleo com fatores ambientais, como calor, umidade, ar e luz; reduzir ou impedir a perda do material do núcleo para fora do ambiente; facilitar o manuseio; e minimizar ou impedir a liberação do material do núcleo até encontrar o estímulo certo (SHAHIDI; HAN, 1993; DESAI; PARK, 2005; BURGAIN et al., 2011). Além disso, devido ao seu tamanho reduzido, as micropartículas podem melhorar a solubilidade, biodisponobilidade e as características sensorias, como por exemplo, mascarar sabores desagradáveis (CERQUEIRA et al., 2014).

A microencapsulação pode ser alcançada através de diferentes técnicas (Tabela 1), sendo que, para a escolha do método mais adequado alguns fatores devem ser considerados, tais como, as características físicas e químicas do núcleo e materiais de suporte/revestimento, a matriz alimentar em que as micropartículas serão destinadas, o tamanho, textura e forma das micropartículas, bem como o mecanismo de liberação do material a ser encapsulado (BANSODE et al., 2010; KEVITAKE et al., 2018). Além disso, deve-se considerar as vantagens, desvantagens e principais aplicações de cada método (Tabela 1), destacando-se como vantagem, a aplicabilidade de um processo de microencapsulação na indústria de alimentos, incluindo o custo de produção, sua aplicação em diferentes produtos e a facilidade de produção em grande escala (BARROW; NOLAN; JIN, 2007; ERATTE et al., 2018).

Tabela 1 – Principais vantagens, desvantagens e aplicações dos métodos de microencapsulação

(continua)

Método	Principais vantagens	Principais desvantagens	Principais aplicações	Referências
Coacervação simples ou complexa	Técnica versátil, além de maior controle do tamanho das partículas	Aglomeração das partículas, utilização de aldeído no processo	Indústria de alimentos, vitaminas, enzimas, proteínas e medicamentos	(JAMEKHORSHID; SADRAMELI; FARID, 2014)
Evaporação emulsão-solvente	Simplicidade do método, baixo custo	Produção em escala laboratorial	Indústria de fármacos	(LI; ROUAUD; PONCELET, 2008)
Emulsão-solidificação	Pode ser utilizada em escala industrial	Microcápsulas com variação de tamanho e forma, elevado custo	Utilizada na indústria de alimentos, encapsulação de probióticos	(KENT; DOHERTY, 2014)
Spray drying	Baixo custo, equipamento e técnica acessível, produção em escala industrial, solubilização instantânea e estabilidade elevada das cápsulas	Microcápsulas não uniformes, perda de materiais sensíveis ao calor, como aroma e outros compostos voláteis	Amplamente utilizada na indústria de alimentos, encapsulação de probióticos, indústria farmacêutica e química.	(MARTÌN et al., 2015) (SILVA et al., 2014) (KENT; DOHERTY, 2014)

(conclusão)

Método	Principais vantagens	Principais desvantagens	Principais aplicações	Referências
Spray chilling	Envolve temperaturas baixas, econômico, pode utilizar lipídios como material de parede	Baixa capacidade de encapsulação e expulsão do material do núcleo durante o armazenamento	Indústria de alimentos, vitaminas, enzimas, probióticos e medicamentos	(PEDROSO et al., 2012)
Extrusão	Baixo custo, simplicidade do método, não envolve altas temperaturas, pode ser utilizado em sistema aeróbico e anaeróbico	Método mais trabalhoso, necessita de avanços tecnológicos para produção em escala industrial	Amplamente utilizada na indústria de alimentos e encapsulação de probióticos	(FAVAROTRINDADE et al., 2011) (KENT; DOHERTY, 2014)
Gelificação iônica	Uso de baixa temperatura e baixo custo	Alta permeabilidade	Indústria farmacêutica	JAMEKHORSHID; SADRAMELI; FARID, 2014)

Fonte: Vaniski, Corti e Drunkler (2017).

Para a microencapsulação de probióticos diversas técnicas vêm sendo estudadas e utilizadas, tais como *spray drying*, extrusão, emulsificação, coacervação, liofilização, entre outras (SEMYONOV et al., 2010; NAZZARO et al., 2012; HOLKEM et al., 2016; ETCHEPARE et al., 2016). Recentemente Dias et al. (2017) mostraram o número de publicações, nos anos de 2015 e 2016, referente aos estudos de diferentes métodos de microencapsulação para componentes bioativos e probióticos. Estes autores reportaram que o método de *spray dryer* foi o mais utilizado para processos de microencapsulação.

Por fim, pode-se destacar que o método de *spray drying* vem sendo bastante utilizado na microencapsulação de diversos compostos, incluindo os probióticos. Este método apresenta algumas das características desejáveis para o processo de microencapsulação e além disso, tem sido extensivamente estudado para melhorar a estabilidade dos probióticos ao processamento e armazenamento e para proteger estes microrganismos das condições ambientais adversas aos quais são expostos (BUSTOS; BORQUEZ, 2013; JANTZEN et al., 2013).

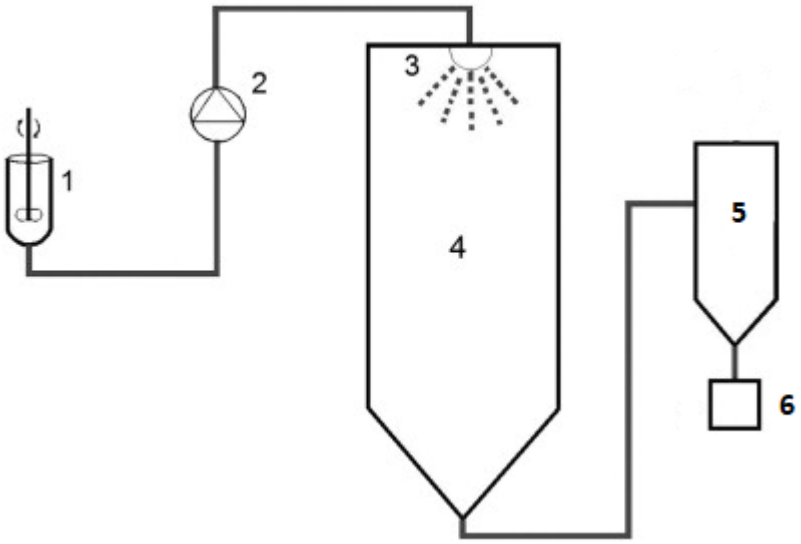
3.3 MÉTODO DE *SPRAY DRYING*

A técnica de *spray drying* é a mais comumente empregada na microencapsulação, sendo também um dos métodos mais antigos, tendo sido originalmente utilizado na década de 30, para preparar os primeiros compostos contendo sabores encapsulados (WILSON; SHAH, 2007). O princípio deste método consiste em uma operação unitária através da qual um produto (solução, emulsão ou suspensão) é transformado do estado fluido para o estado sólido em forma de pó

dentro de uma câmara, onde é feita a dispersão de gotículas do material, que entram em contato com um gás aquecido, em geral, o ar (GHARSALLAOUI et al., 2007). Esta técnica é relativamente rápida e eficiente para produzir micropartículas secas com propriedades desejadas, como, boa fluidez, baixo conteúdo de umidade, forma e distribuição de tamanho uniforme (SOSNIK; SEREMETA, 2015).

O procedimento para a realização deste método, mais detalhadamente, compreende algumas etapas (Figura 2): primeiramente, a substância a ser encapsulada é homogeneamente dispersa ou dissolvida em uma solução aquosa ou dispersão, contendo o agente encapsulante (1). Depois, através de uma bomba de alimentação (2) o sistema conduzido até o bico atomizador (3) onde é atomizado ao entrar em contato com uma corrente de ar quente que vai promover a evaporação do solvente na câmara de secagem (4), obtendo-se a rápida solidificação das gotículas que depois serão enviadas para o ciclone (5) e recolhidas no tubo coletor na base do ciclone (6).

Figura 2 – Representação esquemática do processo de microencapsulação por *spray dryer*



Legenda: (1) solução de alimentação; (2) bomba de alimentação; (3) processo de atomização; (4) câmara de secagem; (5) ciclone; (6) tubo coletor.

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

O produto seco obtido por *spray dryer* apresenta algumas vantagens, especialmente relacionadas ao baixo teor de umidade e atividade de água, o que vai resultar em um produto com maior estabilidade (SHISHIR et al., 2016; PATIL; CHAUHAN; SINGH, 2014; KHA

et al., 2010). Assim, a secagem é um processo amplamente utilizado para garantir uma vida útil estável e prolongada, e consequentemente reduzindo os custos de transporte (SCHUCK et al., 2016). A microencapsulação por *spray dryer* para microrganismos probióticos apresenta algumas limitações relacionadas as altas temperaturas empregadas neste processo (ANEKELLA; ORSAT, 2013). Santivarangkna et al. (2008) reportaram que o dano as bactérias durante a secagem em *spray dryer* além do efeito térmico, está também relacionado a perda de água ligada na superfície celular. No entanto, apesar destes obstáculos, estudos vem demostrando boa sobrevivência de células bacterianas e boa eficiência de encapsulação utilizando este método (RAJAN; ANADHARISKAM, 2015; ARSLAN et al., 2015; VERRUCK et al., 2017). Além disso, Sunny-Roberts e Knorr (2009) reportaram que uma alta taxa de sobrevivência de microrganismos probióticos pode ser alcançada através do *spray dryer* especialmente quando o processo é realizado em condições ideais e com agentes encapsulastes adequados.

Neste sentido, o estudo adequado das variáveis deste processo, tais como, temperatura de entrada e saída de ar do sistema, o fluxo de ar ou fluído de arraste, a distribuição da temperatura e umidade, o tempo de permanência e temperatura da câmara, as características do agente encapsulante (tamanho de moléculas, solubilidade) e do material ativo (polaridade, pressão de vapor, tamanho de molécula) são de grande importância, pois vão determinar a sobrevivência dos microrganismos probióticos, bem como, a eficiência de encapsulação (RÉ, 1998; KISSEL et al., 2006). Wang et al. (2002) reportaram que a temperatura de secagem, em combinação com o tempo de secagem, é o fator chave para a viabilidade probiótica final dos pós. Outro fator de grande importância é o conteúdo de sólido no meio de secagem, sendo que a literatura reporta um valor entre 20 e 30% (m/v) para garantir alta viabilidade de probióticos (LIAN et al., 2002). Por fim, uma estratégia que vem sendo muito discutida para melhorar a secagem em *spray dryer* consiste na adição de componentes específicos que apresentem propriedades protetoras aos microrganismos (HUANG et al., 2017).

3.4 AGENTES ENCAPSULANTES

No processo de microencapsulação têm sido utilizados diversos agentes encapsulantes, que são responsáveis pelo revestimento da substância de interesse, dando também forma as micropartículas. Estes revestimentos podem ser de diferentes origens, desde natural, sintético e semissintético, incluindo materiais poliméricos, hidrófilos e hidrofóbicos ou ainda uma associação de ambos (AZEREDO, 2005; ASSUNÇÃO, 2014).

Dentre os materiais mais utilizados na microencapsulação destacam-se os carboidratos (amidos, dextrinas e sacarose), celuloses (carboximetilcelulose, acetilcelulose, metilcelulose, etilcelulose e nitrocelulose), lipídios (parafina, cera, ácido esteárico, triesterina, monoglicerídeo, óleos, gordura hidrogenada e diglicerídeos), proteínas (glúten, caseína, isolado proteico de soro de leite, gelatina e albumina), gomas (alginato de sódio, carragena e goma arábica) entre outros (SUAVE, 2006; TRIPATHI; GIRI, 2014; DORDEVIC et al., 2015).

A etapa de escolha e seleção dos agentes encapsulantes é dependente de uma série de fatores, dentre eles, a sua não reatividade com o material a ser encapsulado, as suas propriedades físico-químicas, tais como, massa molar, solubilidade e porosidade, e por fim, o método utilizado para a produção das micropartículas e também a sua aplicação final desejada (SUAVE et al., 2006; WEINBRECK; BODNÁR; MARCO, 2010). Além destes fatores, algumas características desejáveis para os materiais de revestimento são descritas, entre elas, baixa viscosidade em concentrações elevadas, ser fácil de manusear durante o processo de microencapsulação, apresentar baixa higroscopicidade, boa capacidade de incorporar o material a encapsular para impedir a perda deste, proteger o material encapsulado de circunstâncias adversas, como oxigênio, luz e pH e apresentar sabor agradável quando administrado por via oral (VENKATESAN et al., 2009).

Maltodextrinas e goma arábica são alguns dos agentes encapsulantes mais estudados e aplicados em processos de microencapsulação. A grande utilização da maltodextrinas como agente encapsulante deve-se a fatores como: custo relativamente baixo, apresentam baixa higroscopicidade, são insípidas, praticamente sem sabor doce e são excelentes contribuintes para o corpo e volume de sistemas alimentícios (SHAHIDI; HAN, 1993; REINECCIUS, 2001). A goma arábica devido a sua boa capacidade de emulsão e viscosidade reduzida em soluções aquosas também é bastante utilizada na microencapsulação por *spray drying*. Além disso, é reconhecida por proporcionar uma proteção contra oxidação e retenção de compostos, propriedades emulsificantes e boa solubilidade (RIGHETTO; NETTO, 2005).

Neste contexto, apesar da disponibilidade de diferentes materiais encapsulantes e do conhecimento de algumas das características desejáveis a eles, uma das principais áreas de pesquisa atual no campo da microencapsulação está voltada para a identificação de elementos estruturais apropriados e métodos de produção para elaboração de partículas de grau alimentício (CARMO; FERNANDES; BORGES, 2015).

A microencapsulação de probióticos pelo método de *spray drying* é um processo de grande relevância, porém, requer especial atenção por empregar em seu processo temperaturas

relativamente altas, o que pode prejudicar a sobrevivência destes microrganismos. Dessa forma, agentes encapsulantes que possam proteger os microrganismos tornam-se um recurso importante a ser utilizado. Alguns agentes encapsulantes já foram anteriormente relatados por apresentarem características positivas relacionadas a melhor proteção de compostos neste processo, entre eles destacam-se a trealose (SUNNYROBERTS; KNORR, 2009), leite desengordurado (SELMER-OLSEN et al., 1999), amido granular (CRITTENDEN et al., 2001) e combinações com prebióticos (DESMOND et al., 2002). Assim, destaca-se a importância de estudar e avaliar diferentes compostos com relação as suas propriedades termoprotetoras, pois estes como agentes encapsulantes refletem diretamente nas propriedades morfológicas e funcionais (tamanho, morfologia, textura, porosidade), bem como, influenciam na proteção e entrega dos microrganismos probióticos (PRISCO; MURIELLO, 2016).

3.4.1 Inulina

Os prebióticos, como a inulina, são definidos como ingredientes seletivamente fermentados que conferem benefícios a saúde do hospedeiro por ser capaz de promover mudanças especificas na composição e na atividade da microbiota gastrointestinal, através da estimulação a proliferação ou atividade de bactérias benéficas no cólon (GIBSON et al., 2004; ROBERFROID, 2007). Diferentes prebióticos vêm sendo estudados, tais como, galacto-oligossacarídeos (GOS), isomalto-oligossacarídeos (IMO), xilo-oligossacarídeos (XOS), oligossacarídeos da soja (SOS), lactulose e polidextrose. No entanto, os mais empregados em alimentos são a inulina e os fruto-oligossacarídeos (FOS) (GIBSON et al., 2004; WANG, 2009; SAAD et al., 2013).

A inulina é um carboidrato de reserva, naturalmente presente em diversos vegetais fazendo parte do grupo de polissacarídeos chamados frutanos, sendo que, sua extração ocorre principalmente a partir de raízes da chicória (BIEDRZYCKA; BIELECKA, 2004; KAWAI et al., 2011). Este polissacarídeo tem atraído muita atenção pelas indústrias farmacêuticas e de alimentos pelos seus vários benefícios, incluindo fibras dietéticas não digeridas no trato gastrointestinal e pela natureza prebiótica (KIM, 2002; BURITI et al., 2007). Além de ser utilizada como prebiótico, também confere propriedades tecnológicas, como função edulcorante para uso na indústria alimentícia, além de ser um ingrediente capaz de substituir gorduras e fibras alimentícias (SAAD, 2006). A inulina, bem como outros prebióticos, vem demonstrando resultados significativos com relação à melhora da viabilidade e altas taxas de sobrevivência durante o armazenamento na microencapsulação em *spray dryer* (FRITZEN-

FREIRE et al., 2012; OKURO et al., 2013). Recentemente, Peredo et al. (2016) avaliaram a influência dos prebióticos amido de batata, *Plantago psyllium* e inulina juntamente com alginato sobre a viabilidade de *L. casei* e *L. plantarum*. Estes autores reportaram maior viabilidade durante o armazenamento e proteção nas condições gastrointestinais quando *Plantago psyllium* e inulina foram utilizados em co-encapsulação com alginato. Além dos efeitos positivos da inulina como material encapsulante, a associação de prebióticos com probióticos pode resultar em um efeito simbiótico positivo. Shamekhi et al. (2013) reportaram que *B. lactis* encapsulada com prebiotícos e alginato de cálcio foi eficiente na preparação de um pó simbiótico para uso pediátrico.

3.4.2 Hi-maize

Hi-maize é um amido resistente derivado do milho com alto teor de amilose, composto por aproximadamente 56% de amido resistente (fibra dietética) e 40% de amido digestível. Este pode ser facilmente adicionado a preparações padrão substituindo parcialmente a farinha regular, diminuindo o teor de calorias e aumento o perfil nutricional dos alimentos. Amido resistente é definido como a quantidade total de amido e seus produtos de degradação resistentes a digestão no intestino delgado de pessoas saudáveis, assim, apresenta efeitos e benefícios comparados aos dos prebióticos (FIGUEROA-GONZALEZ et al., 2011). Além disso, o amido resistente é uma superfície ideal para a aderência das células probióticas aos grânulos de amido, podendo aumentar assim, a entrega probiótica em um estado viável e metabolicamente ativo no intestino (ANAL; SINGH, 2007; KRASAEKOOPT et al., 2003; VIVEK, 2013). Dessa maneira, amidos vem sendo reconhecidos em processos de microencapsulação por apresentarem boas propriedades emulsionantes e estabilizadoras e também por exibirem capacidade de formação de película (BAI; SHI, 2011). Além disso, as soluções aquosas de amido são praticamente incolores e assim podem se tornar interessantes quando se deseja não modificar o sabor natural e cor do produto a ser encapsulado (TESCH et al., 2002).

Recentemente, diferentes amidos têm sido utilizados para diferentes técnicas de microencapsulação e para diferentes compostos a serem encapsulados (YING et al., 2016; CHEOW; KIEW; HADINOTO, 2016; DOMIAN; BRYNDA-KOPYTOWSKA; SWIRYDOW, 2015). Hi-maize, recentemente, foi utilizado por Pancasemsuk et al. (2016) e Etchepare et al. (2016) para encapsular *Lactobacillus casei* 01 e *Lactobacillus acidophilus* La-14 por emulsão e extrusão, respectivamente. Estes autores reportaram grande potencial de utilização deste amido na proteção de probióticos frente as condições adversas a que são

expostos. Li et al. (2016) reportaram que a microencapsulação de *Lactobacillus plantarum* 299 v em amidos de milho nativos ou parcialmente hidrolisados após modificação enzimática resultaram em maior sobrevivência dos microrganismos probióticos após a exposição ao ácido (pH= 2,0, 1 h), sais biliares (3% m/v, 4 h), e ao tratamento térmico (60 °C por 15 minutos). Estes autores concluíram que materiais de encapsulação a base de amidos podem aumentar a eficiência de encapsulação e garantir ainda, que o número de bactérias viáveis permaneça acima da dosagem mínima recomendada.

3.4.3 Trealose

A trealose é um dissacarídeo da glicose que apresenta eficácia na proteção de células bacterianas durante o congelamento e secagem, e assim, sua utilização já vem sendo reconhecida por promover maior proteção as células durante processos de estresse, como altas temperaturas, choque osmótico e desidratação (SUNNY-ROBERTS; KNORR, 2009). Alder e Lee (1999) e Yoshii et al. (2005) reportaram que a trealose é bastante eficaz na preservação de estruturas e funções dos sistemas biológicos durante a desidratação. Este efeito de proteção, pode estar relacionado a sua temperatura de transição vítrea elevada, o que faz com que a mesma permaneça neste estado em uma ampla faixa de temperaturas e umidade (CERIMEDO et al., 2008; DRUSCH; MANNINO, 2009). A temperatura de transição vítrea é uma temperatura em que, o estado do produto muda do estado vítreo (amorfo) para "emborrachado" (pegajoso) por processo de sorção térmica ou plastificante. Dessa maneira, uma baixa temperatura de transição vítrea pode causar alterações físicas durante a secagem, como por exemplo, aderência, aglomeração e amassamento. Além disso, outras mudanças podem ocorrer na cor e aroma, composição química e no rendimento do processo, o qual pode ser reduzido (SHISHIR; CHEN, 2017).

A trealose, devido suas características positivas, vem sendo utilizada como agente encapsulante para diferentes substâncias ativas e técnicas de encapsulação. Estudos realizados com a sua utilização reportaram sucesso e boa eficiência deste agente encapsulante no processo (YOSHII et al., 2008; DOMIAN; BRYNDA-KOPYTOWSKA; SWIRYDOW, 2015; LIM et al., 2016; LIM; ROOS, 2016). Semyonov et al. (2010) utilizaram trealose para alcançar maior proteção e melhor viabilidade celular de microrganismos probióticos durante o congelamento, liofilização e armazenamento de bactérias secas. Viveck (2013) reportou que a adição de trealose juntamente com amido e fibra solúvel melhora a viabilidade de culturas probióticas durante o armazenamento. Recentemente, Agudelo et al. (2017) reportaram que a adição de

sacarose ou trealose em uma matriz encapsulante de proteína de soro de leite e maltodextrina foi uma estratégia eficiente para prolongar a vida útil de *Lactobacillus rhamnosus* encapsulados em *spray dryer*.

4 ARTIGO 1 – ENCAPSULATION OF *Lactobacillus acidophilus* LA-5 AND B*ifidobacterium* BB-12 BY SPRAY DRYING AND EVALUATION OF ITS RESISTANCE IN SIMULATED GASTROINTESTINAL CONDITION

Esse artigo foi aceito para publicação no periódico Ciência Rural, ISSN 0103-8478, Área de avaliação em Ciência de Alimentos, Classificação B2.

Encapsulation of *Lactobacillus acidophilus* La-5 and *Bifidobacterium* Bb-12 by spray drying and evaluation of its resistance in simulated gastrointestinal conditions, thermal treatments and storage conditions

Encapsulação de *Lactobacillus acidophilus* e *Bifidobacterium* Bb-12 por *spray drying* e avaliação da sua resistência em condições gastrointestinais simuladas, tratamentos térmicos e em condições de armazenamento

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ABSTRACT

Lactobacillus acidophilus La-5 (ML) and *Bifidobacterium* Bb-12 (MB) microparticles were produced at different temperatures by spray dryer. The influence of the different temperatures on the viability, encapsulation efficiency, water activity and moisture were evaluated. The microparticles that presented more viability were submitted to thermal resistance, gastrointestinal simulation, storage stability, morphology and particle size analyses. The drying temperature of 130°C showed higher encapsulation efficiency, 84.61 and 79.73% for *Lactobacillus acidophilus* (ML) and *Bifidobacterium* Bb-12 (MB) microparticles, respectively. In the evaluation of thermal resistance and gastrointestinal simulation, the microparticles of *Lactobacillus acidophilus* La-5 (ML) presented higher survival than *Bifidobacterium* Bb-12 (MB) under these conditions. In storage viability only the *Lactobacilus acidophilus* La-5 (ML) microparticles remained viable at all evaluated temperatures during the 120 days. The particle sizes found were 4.85 for *Lactobacillus acidophilus* La-5 (ML) and 8.75

for *Bifidobacterium* Bb-12 (MB), being in agreement with the desired values for products obtained by spray dryer. Finally, the *Lactobacillus acidophilus* La-5 (ML) microparticles were shown to be more resistant under the conditions evaluated in this study.

Keywords: spray dryer, viability, probiotics.

RESUMO

Micropartículas de *Lactobacillus acidophilus* La-5 (ML) e *Bifidobacterium* Bb-12 (MB) foram produzidas em diferentes temperaturas de secagem no spray dryer. A influência das diferentes temperaturas sobre a viabilidade, eficiência de encapsulação, atividade de água e umidade foram avaliadas. As micropartículas que apresentaram maior viabilidade foram submetidas a análises de resistência térmica, simulação gastrointestinal, estabilidade ao armazenamento, morfologia e tamanho de partícula. A temperatura de secagem de 130 ° C mostrou maior eficiência de encapsulação, 84.61 e 79.73 % para micropartículas de *Lactobacillus acidophilus* (ML) e *Bifidobacterium* Bb-12 (MB), respectivamente. Na avaliação da resistência térmica e simulação gastrointestinal as micropartículas de *Lactobacillus acidophilus* La-5 (ML) apresentaram maior sobrevivência que *Bifidobacterium* Bb-12 (MB) nestas condições. Na viabilidade ao armazenamento somente as micropartículas *Lactobacillus acidophilus* La-5 (ML) mantiveram-se viáveis em todas as temperaturas avaliadas durante os 120 dias. Os tamanhos de partícula encontrados foram de 4.85 para *Lactobacillus acidophilus* La-5 (ML) e 8.75 para *Bifidobacterium* Bb-12 (MB), estando de acordo aos valores desejáveis para produtos obtidos por spray dryer. Por fim, as micropartículas de *Lactobacillus acidophilus* La-5 (ML) demostraram ser mais resistentes frente as condições avaliadas neste estudo.

Palavras-chave: spray dryer, viabilidade, probióticos.

INTRODUCTION

Probiotics are live microorganisms, which when consumed in adequate amounts, confer a health benefit for the host. In this context, in recent years there has been a great deal of interest in its use and application (FAO/OMS, 2001; DAS & GOYAL, 2015; FIJAN, 2014). *Bifidobacterium lactis* and *Lactobacillus acidophilus* are the probiotic bacteria most widely studied and frequently used in food (FELICIO et al., 2016; HOMAYOUNI et al., 2008). According to ANAL & SINGH (2007), the ability of probiotic microorganisms to survive and develop in the host will directly influence their probiotic effects. Therefore, the microorganism that is metabolically stable in the product and survive the passage through the gastrointestinal tract reaching the intestine with high viability will be able to develop its beneficial effects. The application of microencapsulation processes has been studied as an alternative to maintain high viability of these microorganisms (FUNG, YUEN & LIONG, 2011; KIM; NAG, HAN & SINGH, 2011). Among different microencapsulation techniques, spray drying is commonly used for its advantages such as low operating costs, high production rates, low moisture content in the final product and possibility of application on an industrial scale (CORCORAN et al., 2004). However, this process requires high temperatures, which may affect the survival of probiotic microorganisms (BOZA, BARBIN & SCAMPARINI, 2004). In this sense, the optimization of the spray-drying conditions as well as the composition of the encapsulation solution are parameters of great importance in order to achieve high survival of the probiotic microorganisms during this process (CORCORAN et al., 2004; FRITZEN-FREIRE et al., 2012; RAJAM et al., 2012; SIMPSON et al., 2005).

In this context, the aim of this study was to evaluate the influence of different temperatures by spray drying on the viability, encapsulation efficiency, water activity and moisture of microparticles containing *Lactobacillus acidophilus* La-5 and *Bifidobacterium* Bb-12. Subsequently, the microparticles that presented the highest viability and best physical-

chemical characteristics were evaluated in relation to their thermal resistance, gastrointestinal simulation and storage stability. In addition, the morphology and particle size were also evaluated.

Materials and Methods

To produce microparticles, the following compounds were used: Gum arabic (CNI, São Paulo, Brazil); Maltodextrin (Ingredion, São Paulo, Brazil); Tween 80 (Vetec, Rio de Janeiro, Brazil); Glycerol (Vetec, Rio de Janeiro, Brazil), and probiotic culture *Lactobacillus acidophilus* La-5 and *Bifidobacterium* Bb-12 obtained by Chr. Hansen from Brazil (Valinhos, São Paulo).

Inoculum

The *Lactobacillus acidophilus* La-5 probiotic culture (Chr. Hansen, São Paulo, Brazil) was activated in MRS broth (Himedia Curitiba, Parana, Brazil) and incubated for 15 h at 37°C. The *Bifidobacterium* Bb-12 culture was rehydrated in reconstituted milk (Molico, Nestlé, São Paulo, Brazil) at a concentration of 12% and incubated for 5 hours at 37°C. Then, it was centrifuged at 4670 g for 15 min and washed in NaCl solution (0.85%). The cells were then suspended in saline to obtain a solution containing about 12 and 10 log CFU/g^{-1}.

Production of microparticles by spray drying

The feed solutions were prepared with gum arabic (8 g), maltodextrin (2 g), glycerol (1.9 mL), tween 80 (0.1 mL) containing *Lactobacillus acidophilus* La-5 (SL) and *Bifidobacterium* Bb-12 (SB) to a final concentration of 12% m/v. The microencapsulation process was performed in a lab spray dryer (MSD 1.0 Labmaq, Sao Paulo, Brazil). Initially, the feed solutions (SL and SB) were submitted to different drying temperatures, 110, 120, 130 and 140°C. Next, the microparticles produced at the inlet temperature of 130°C were chosen to be further evaluated. The different feed solutions, kept stirring, were introduced into the drying

chamber using a peristaltic pump with feed rate of 0.48 L / h, drying air flow rate of 40 L / min, and air pressure of 0.6 MPa. The microparticles (ML and MB) were collected at the base of the cyclone, transferred to sterile vials, and stored in a desiccator.

Viable cell count

Serial dilutions for *Lactobacillus acidophilus* La-5 and *Bifidobacterium* Bb-12 were transferred to sterile Petri plates containing MRS agar (Himedia Curitiba, Paraná, Brazil), in triplicate. The MRS agar used for *Bifidobacterium* Bb-12 was added lithium chloride (0.1%) and L-cysteine (0.05%), according to manufacturer recommendations (Chr Hansen, 1999). Plates were incubated at 37°C for 72 h in anaerobic jars with an anaerobic generator (Oxoid, São Paulo, Brazil).

The dilution of the microparticles comprised weighing 1 g of microparticles followed by the addition of 9 mL sterile phosphate buffer solution (pH 7.5), following the methodology described by SHEU, MARSHALL, & HEYMANN (1993). Results were shown as log colony forming units per gram (log CFU/g^{-1}).

Efficiency of encapsulation (EE)

The efficiency of encapsulation (EE) is the survival rate of the microorganisms during the microencapsulation process, calculated according to Eq. (1), as proposed by MARTIN et al. (2013):

$$EE\% = (N / N_o) \times 100 \quad (1)$$

Where N is the number of viable cells (log CFU/g^{-1}) released from the microparticles and N_0 is the number of viable cells (log CFU/g^{-1}) free in the feed solution before the spray-drying process. The viable cell count was performed as described in Section "*Viable cell count".*

Moisture and Water Activity (Aw)

The moisture content of the microparticles was determined in an oven at 105°C until constant weight, according to the methodology proposed by AOAC (2005). The water activity was measured at 25°C using Aqualab 4TE equipment (Decagon Devices, Pullman, WA, USA) after prior stabilization of the samples for 15 min.

Microparticle morphology and size

The morphology of the microcaparticles was evaluated using an optical microscope (Carl Zeiss Axio Scope.A1, Oberkochen, Germany) equipped with an Axio Cam MRc digital camera (Carl Zeiss) and scanning electronic microscope (SEM; JEOL JM6360, Tokyo, Japan). The distribution of microparticle size was measured using a Mastersizer 3000 (Malvern, Germany), with water as the dispersion medium.

Resistance to heat treatment

Thermal resistance was assessed as proposed by ZHANG, LIN & ZHONG (2015), with some adaptations. Microparticles and free culture (1 g) were transferred to 9 ml of peptone water in test tubes. The contents were then subjected to thermal conditions of 72°C for 15 seconds and 63°C for 30 minutes, after which the tubes were immediately cooled by immersion on ice for 10 min. Finally, aliquots were collected and probiotic cultures were counted according to Section "*Viable cell count".*

Assessment of the survival of encapsulated Lactobacillus acidophilus La-05 and Bifidobacterium Bb-12 exposed to simulated gastrointestinal conditions

The method proposed by MADUREIRA et al. (2011), with some adaptations, was used to submit the microparticles to simulated gastrointestinal conditions. The viability of the bacteria was determined in media simulating the different sections of the gastrointestinal tract, such as esophagus/stomach (addition of pepsin, pH adjusted to 2.0 for 90 min), duodenum

(addition of pancreatin and bile salts, pH adjusted to 5.0 for 20 min), and ileum (pH adjusted to 7.5 for 90 min). Analysis was conducted on a TE 421 Shaker (Tecnal, Piracicaba, SP, Brazil) at a temperature of 37°C, simulating the temperature of the human body. Finally, aliquots were removed after 90 min (esophagus or stomach), 110 min (duodenum), or 200 min (ileum) to determine the survival of free and microencapsulated *Lactobacillus acidophilus* La-5. Probiotic cultures were counted in MRS medium as described in Section "*Viable cell count".*

Viability of microparticles during storage at different temperatures

Viability of the microencapsulated microorganisms was determined by enumeration in MRS agar, as described in Section 2.3. The microparticles were examined after storage for 0, 15, 30, 45, 60, 75, 90, 105, and 120 days at 25°C, −18°C and 7°C.

Statistical Analysis

The data were submitted to analysis of variance (ANOVA) using Statistica version 7.0 software (2004; Statsoft Inc., Tulsa, OK, USA), followed by Tukey's means comparison test at a level of 5% significance of treatments showing possible significant differences. All experiments were performed in triplicate; data are expressed as means ± standard deviations.

RESULTS AND DISCUSSION

Viability, encapsulation efficiency, water activity and moisture of microparticles produced t different drying temperatures

The viability of the microparticles containing *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) produced at the different drying temperatures can be seen in Table 1. The air inlet temperature of 140°C had a significant effect ($p < 0.05$) in relation to the other conditions evaluated, presenting the lowest results for viability for both microparticles studied (ML and MB). The temperature of 130°C showed the greatest viability for the *Lactobacillus acidophilus* La-5 (ML) and Bifidobacterium Bb-12 (MB) microparticles, but there was no

significant difference ($p < 0.05$) among temperatures of 110, 120 and 130°C. Similar results were reported by BUSTAMANTE et al. (2016) when encapsulating *Lactobacillus acidophilus* with mucilage extracted from the chia seed at two different spray dryer temperatures (110°C and 140°C). These authors reported lower survival of encapsulated *Lactobacillus acidophilus* at 140°C. ARSLAN et al. (2015) reported that an increase in the air inlet temperature of the spray dryer resulted in decreased viability and lower survival rates of *Saccharomyces cerevisiae var. boulardi*. The increase in the inlet temperature of the spray dryer consequently causes an increase in the outlet temperature. PISPAN, HEWITT & STAPLEY (2013) explained that an increase in the outlet temperature directly increases the temperature at which the microparticles are exposed. On the other hand, a reduction in the outlet temperature results in a longer drying time. Thus, viability losses during the spray drying process can arise from dehydration and high temperatures. These two mechanisms occurring at the same time cause a negative effect on the survival of probiotic microorganisms (PEIGHAMBARDOUST, TAFTI & HESARI, 2011; RIVEROS, FERRER & BORQUEZ, 2009). The encapsulation efficiency (Table 1) ranged from 81.17 to 84.61% and 74.31 to 79.73% for the microparticles containing *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB), respectively. Therefore, it is possible to observe that *Lactobacillus acidophilus* La-5 presented greater resistance on the spray-drying conditions compared to *Bifidobacterium* Bb-12. OLIVEIRA (2006) reported similar results when encapsulating *Bifidobacterium lactis* (B01) and *Lactobacillus acidophilus* (LAC4) by complex coacervation and then, submitting it to the spray-drying process. The current study showed greater resistance to the drying conditions used for the *Lactobacillus acidophilus* (LAC4) culture compared to *Bifidobacterium lactis* (B01). The highest encapsulation efficiency for both microparticles produced, ML (84.61%) and MB (79.73%) was observed at 130°C.

In the evaluation of the effect of different inlet temperatures on the water activity (Table 1) of *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) microparticles,

we can observe that the temperature of 140°C had a significant influence ($p < 0.05$), presenting the lowest water activity content for both studied microparticles. Nonetheless, the water activity found in the different evaluated temperatures for the *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 microparticles ranged from 0.195 to 0.289. Thus, these results are as expected for microparticles dried by spray dryer (0.150 to 0.300) to ensure their microbiological stability (CORCORAN et al., 2004; ARSLAN et al., 2015).

The microparticles moisture content ranged from 4.60% to 5.71% (Table 1) and the lowest moisture contents were observed as the inlet temperature of the spray dryer was raised. FERRARI, RIBEIRO & AGUIRRE (2012) reported that higher temperatures imply a higher rate of heat transfer to the microparticles, resulting in a higher water evaporation and consequently, low moisture contents are obtained. The results obtained in the present work are in accordance with those reported by other authors who recommend that the moisture content should be around 4-5% to guarantee better storage stability (CHAVEZ & LEDEBOER, 2007). In this sense, studies have shown that a lower inlet temperature and, consequently output, results in increased post-encapsulation viability; however, this condition may imply greater moisture and water activity, which adversely affects the prolonged storage of the powders (PEIGHAMBARDOUST, TAFTI & HESARI, 2011; VESTERLUND, SALMINEN & SALMIMEN, 2012). Thus, the relevance of the study of different drying temperatures, not only on the viability of the microorganisms, but also their influence on the physical characteristics of the microparticles is highlighted. MORGAN et al. (2006) reported that spray-drying temperatures are of great importance for the viability of bacteria and need to be optimized individually for every new application. In this context, *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) microparticles produced at 130°C were chosen to be evaluated in this study.

Morphology and size of microparticles

Scanning electron microscopy of *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) microparticles can be observed in Figure 1. The microparticles produced presented a rounded shape containing concavities. The same was observed by FAVARO-TRINDADE & GROSSO (2002) and FRITZEN-FREIRE et al. (2012). These authors reported that these concavities are typical of spray-dried products. Moreover, it is possible to observe that the microparticles of *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) presented high porosity (Figure 1a) and ruptures in their structure (Figure 1b). This fact may be related to the low solids concentration (12% m/v) used in the formulations. Similar results were shown by PINTO et al. (2015) in the production of microparticles containing *Bifidobacterium* Bb-12 and using a concentration of 10% w/v in combinations with liquid whey, whey retentate, inulin and polydextrose.

The particle size observed for the microparticles of Lactobacillus acidophilus La-5 (ML) and *Bifidobacterium* Bb-12 (MB) was 4.85 and 8.75 μm, respectively. RAJAM & ANANDHARAMAKRISHNAN (2015) reported particle sizes from 6.68 to 23.89 μm for microparticles containing *Lactobacillus plantarum* (MTCC 5422) using oligofructose, whey protein isolate and denatured whey protein isolate at a concentration of 20% (w/v). ARSLAN et al. (2015) verified particle sizes that ranged between 8.56 and 21.38 μm by encapsulating *Saccharomyces cerevisiae* var. boulardii using gelatin, gum Arabic, maltodextrin, modified starch, whey protein concentrate and pea protein isolate as encapsulating agents. The smaller particle sizes shown in the present work may relate to the different film-forming and gelling properties of the materials used in the microencapsulation process. In addition, according to KUROZAWA, PARK & HUBINGER (2009), higher concentrations of encapsulating agents in the feed solution promote an increase in particle size. However, it is worth mentioning that microparticles obtained by spray dryer presented a desirable size, once that smaller particles are preferred to ensure homogeneity and quality when applied to food (BURGAIN et al., 2011).

Resistance of microparticles to heat treatment

The microparticles of *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) were evaluated to the heat treatments of 63°C / 30 min and 72°C / 15 s (Table 2). The microparticles of *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) presented reductions of 1.91 and 1.93, at 63°C / 30 min and 1.36 and 1.42 at 72°C / 15 s, respectively. Thus, *Lactobacillus acidophilus* La-5 (ML) microparticles presented higher resistance to the thermal treatments studied. FAVARO-TRINDADE & GROSSO (2002) and LIAN, HSIAO & CHOU (2002) have shown in previous studies that different strains of microorganisms can vary their ability to resist high temperatures. Bifidobacteria are known to be more susceptible to high temperatures than lactobacilus (DOLEYRES & LACROIX, 2005). However, it is noteworthy that for both thermal treatments studied, all viable cell counts of *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) microparticles were superior than 6 log CFU/g^{-1}.

Regarding the different applied thermal treatments, the higher temperature and the shorter time (72°C / 15 s) resulted in higher survival for the *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) microparticles. These results are in accordance with those found by ZHANG, LIN & QIXIN (2015) and NUNES et al. (2018), who reported better survival of *Lactobacillus salivarius* NRR B-30514 and *Lactobacillus acidophilus* La-5 encapsulated by the emulsion and spray drying methods, respectively, when subjected to heat treatment at 72°C / 15 s in relative to 63°C / 30 min.

Exposure of microparticles to simulated gastrointestinal conditions

Table 3 shows viable cell counts of *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) microparticles exposed to simulated gastrointestinal conditions. After 90 min incubation in the presence of a pepsin solution and pH adjusted to 2.0 (simulated esophagus/stomach), there was significantly decreased ($p < 0.05$) of *Lactobacillus acidophilus*

La-5 (ML) and *Bifidobacterium* Bb-12 (MB) microparticles compared to the initial count of 3.58 and 2.85 log cycles, respectively. Subsequently, when the microparticles are in contact with bile salts and pH 5.0 (section of the gastrointestinal tract comprising the duodenum), it was observed an increased number of viable cells (Table 4). HOLKEM et al. (2016), NUNES et al. (2018) and RAJAM et al. (2012) attributed the increase in the number of viable cells from the esophagus/stomach for the duodenum due to a greater rupture of the microparticles when submitted to a higher pH (5.0). In the last section of the simulated gastrointestinal tract, the ileum (pH 7.5), the microparticles of *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) continued to show a significant increase ($p < 0.05$) in the number of viable cells (Table 3). As the pH was rising, the number of bacterial cells was increasing. The acid conditions of the stomach cause a dormant state in the bacterial cells, as the pH goes up they regain their growth (MOUMITA et al., 2017). The microparticles of *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) after exposure to simulated gastrointestinal tract conditions presented reductions of 1.72 and 2.04 log cycles, respectively. Therefore, the *Lactobacillus acidophilus* La-5 (ML) microparticles were more resistant to simulated gastrointestinal conditions than *Bifidobacterium* Bb-12 (MB). These results differ from those reported by PEDROSO et al. (2012) who found greater gastrointestinal survival for *Bifidobacterium lactis* compared to *Lactobacillus acidophilus*. According to GOMES & MALCATA (1999) and KOLL et al. (2008), there is a variation in the ability of *Bifidobacterium* and Lactobacillus acidophilus to resist acid and bile conditions. These authors further reported that these properties are specific to strains and species.

Stability of microparticles during storage at different temperatures

Table 5 shows the viability of *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) microparticles at room temperature (25°C), below freezing (-18°C) and under refrigeration (7°C). Room temperature (25°C) was the most damaging to the

viability of *Lactobacillus acidhopilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) microparticles, promoting reductions after 120 days' storage between 3.82 and 3.51 logs CFU/g^{-1}. HUANG et al. (2017) microencapsulated by spray drying *Lactobacillus casei* BL23 and *Propionibacterium freudenreichii* TG P20 and verified that storage at room temperature (25°C) resulted in greater viability loss. KOTULA (2008) reported that storage of probiotic powders above refrigeration temperatures increases rates of bacterial metabolism, which can lead to the accumulation of toxic residues and lead to a reduction in viability.

For the freezing and refrigeration temperatures, the microparticles presented losses of 2.81 and 2.36 log cycles for *Lactobacillus acidophilus* La-5 (ML) and 2.42 and 2.17 for *Bifidobacterium* Bb-12 (MB), respectively. Thus, the refrigeration temperature promoted the greatest viability during storage for 120 days for both studied microparticles. OLIVEIRA et al. (2007) showed that *L. acidophilus* exhibited greater viability at a storage temperature of 7°C, thus reporting similar results. Among the microparticles of *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB), it is possible to observe that for the different evaluated temperatures, *Bifidobacterium* Bb-12 (MB) presented the smallest reductions during the 120 days of storage. However, considering the minimum level of 10^6 logs CFU/g^{-1} (TALWALKAR et al., 2004), *Bifidobacterium* Bb-12 (MB) microparticles had a shelf life of only 60 days at 25°C and 105 days at -18°C while the *Lactobacillus acidophilus* La-5 (ML) microparticles remained viable throughout the storage period at all studied temperatures. Similar results were reported by PEDROSO et al. (2012) who microencapsulated *Bifidobacterium lactis* and *Lactobacillus acidophilus* using spray-chilling. However, BUSTAMANTE et al. (2017) found greater viability for *Bifidobacterium infantis* in comparison to *Lactobacillus plantarum* incorporated in instant juice powder stored at 4°C for 45 days. According to MARTIN et al. (2015) different probiotic strains present distinct abilities to resist environmental conditions such as oxygen, pH, light and temperature. In addition, the conditions of the microencapsulation

process are of great importance for the microorganisms to remain viable during their storage (OLIVEIRA et al., 2007).

CONCLUSION

The inlet temperature of 130°C in the spray dryer promoted the highest viability and encapsulation efficiency for the *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) microparticles, submitted to different drying temperatures. The *Lactobacillus acidophilus* La-5 (ML) microparticles showed greater viability when exposed to thermal treatments and gastrointestinal simulation. In the storage viability for 120 days, the refrigeration temperature (7°C) was the one that maintained the highest viability for both produced microparticles. However, only the microparticles of *Lactobacillus acidophilus* La-5 (ML) maintained their counts higher than 6 log CFU/g^{-1} at all temperatures that were studied (25, -18 and 7°C). *Bifidobacterium* Bb-12 (MB) microparticles had a 60 days shelf life at 25°C and 105 days at -18°C, thus demonstrating that they could be applied to food products with shorter shelf life. Among the studied microparticles, *Lactobacillus acidophilus* La-5 (ML) showed greater viability and resistance under the conditions evaluated in this work.

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Table 1 – Viability, encapsulation efficiency, water activity and moisture of microparticles containg *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) produced at different inlet temperatures in the spray dryer.

ML (*Lactobacillus acidophilus* La-5)	**Initial viability log CFU/g**	**Temperatures inlet**	**Post-encapsulation viability log CFU/g**	**Encapsulation efficiency (EE%)**	**Water activity**	**Moisture (%)**
	12.22 ± 0.20^{a}	110 °C	10.18 ± 0.05^{b}	83.30 ± 0.28^{a}	0.289 ± 0.06^{a}	5.71 ± 0.10^{a}
		120 °C	10.22 ± 0.05^{b}	83.63 ± 0.33^{a}	0.275 ± 0.02^{a}	5.41 ± 0.33^{a}
		130 °C	10.34 ± 0.10^{b}	84.61 ± 0.51^{a}	0.270 ± 0.05^{a}	5.26 ± 0.20^{ab}
		140 °C	9.92 ± 0.07^{c}	81.17 ± 0.39^{b}	0.237 ± 0.11^{b}	4.60 ± 0.33^{b}
MB (*Bifidobacterium* Bb-12)						
	10.51 ± 0.02^{a}	110 °C	8.19 ± 0.07^{b}	77.92 ± 0.39^{a}	0.228 ± 0.07^{a}	5.34 ± 0.06^{a}
		120 °C	8.25 ± 0.11^{b}	78.49 ± 0.55^{a}	0.213 ± 0.05^{a}	4.96 ± 0.07^{b}
		130 °C	8.38 ± 0.12^{b}	79.73 ± 0.60^{a}	0.208 ± 0.09^{a}	4.83 ± 0.04^{c}
		140 °C	7.81 ± 0.15^{c}	74.31 ± 0.77^{a}	0.195 ± 0.08^{a}	4.61 ± 0.04^{d}

ML: microparticles produced with 8 g of gum arabic, 2 g of maltodextrin, 1.9 mL of glycerol, 0.1 mL of tween 80 and *Lactobacillus acidophilus* La-5; MB: microparticles produced with 8 g of gum arabic, 2 g of maltodextrin, 1.9 mL of glycerol, 0.1 mL of tween 80 and *Bifidobacterium* Bb-12.

Means followed by the same letter, lowercase in the column, do not differ statistically from each other by the Tukey test at 5% significance. Means found in triplicate.

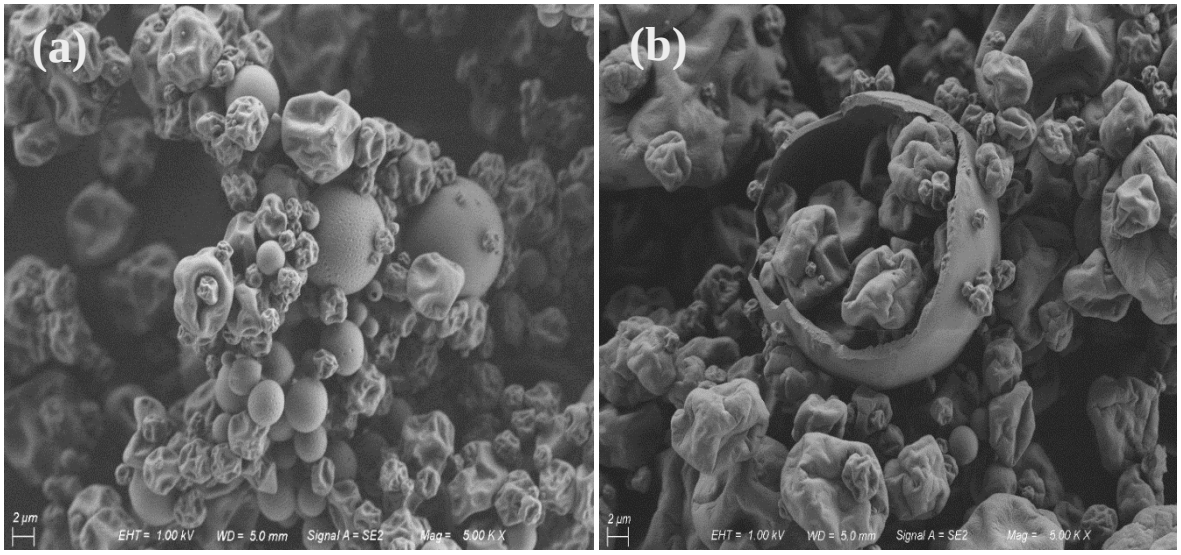

Figure 1. Micrographs of the microparticles produced with 8 g of gum arabic, 2 g of maltodextrin, 1.9 mL of glycerol, 0.1 mL of tween 80 and *Lactobacillus acidophilus* La-5 (ML) e microparticles produced with 8 g of gum arabic, 2 g of maltodextrin, 1.9 mL of glycerol, 0.1 mL of tween 80 and *Bifidobacterium* Bb-12 (MB).

Table 2 – Effect of heat treatments on the viability of microparticles containg *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) produced at inlet temperature of 130 °C in the spray dryer.

Heat treatments	**ML (*Lactobacillus acidophilus* La-5)**	**MB (*Bifidobacterium* Bb-12)**
Initial count log CFU/g	10.34 ± 0.10^{aA}	8.38 ± 0.12^{aB}
63 °C/ 30 min	8.43 ± 0.03^{cA}	6.45 ± 0.10^{cB}
72 °C/ 15 s	8.98 ± 0.15^{bA}	6.96 ± 0.17^{bB}

ML: microparticles produced with 8 g of gum arabic, 2 g of maltodextrin, 1.9 mL of glycerol, 0.1 mL of tween 80 and *Lactobacillus acidophilus* La-5; MB: microparticles produced with 8 g of gum arabic, 2 g of maltodextrin, 1.9 mL of glycerol, 0.1 mL of tween 80 and *Bifidobacterium* Bb-12.

Means followed by the same letter, lowercase in the column and upper case in the row, do not differ statistically from each other by the Tukey test at 5% significance. Means found in triplicate.

Table 3 – Viability of microparticles containing *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) produced at inlet temperature of 130 °C in the spray dryer against simulated gastrointestinal conditions.

	ML (*Lactobacillus acidophilus* La-5)	**MB (*Bifidobacterium* Bb-12)**
Initial count	10.34 ± 0.10^{aA}	8.38 ± 0.12^{aB}
Esophagus/ stomach 90 min/pH 2,0	6.76 ± 0.07^{dA}	5.53 ± 0.06^{cB}
Duodenum 20 min/ pH 5,0	7.06 ± 0.09^{cA}	5.69 ± 0.05^{cB}
Ileum 90 min/ pH 7,5	8.62 ± 0.08^{bA}	6.34 ± 0.06^{bB}

ML: microparticles produced with 8 g of gum arabic, 2 g of maltodextrin, 1.9 mL of glycerol, 0.1 mL of tween 80 and *Lactobacillus acidophilus* La-5; MB: microparticles produced with 8 g of gum arabic, 2 g of maltodextrin, 1.9 mL of glycerol, 0.1 mL of tween 80 and *Bifidobacterium* Bb-12.

Means followed by the same letter, lowercase in the column and upper case in the row, do not differ statistically from each other by the Tukey test at 5% significance. Means found in triplicate.

Table 4 – Effect of room temperature (25 °C), freezing (−18 °C), and refrigeration (7 °C) on the viability of microparticles containing *Lactobacillus acidophilus* La-5 (ML) and *Bifidobacterium* Bb-12 (MB) produced at inlet temperature of 130 °C in the spray dryer during storage for 120 days.

Temperature	**Room (25 °C)**	
Treatments Time (Days)	**ML (*Lactobacillus acidophilus* La-5)**	**MB (*Bifidobacterium* Bb-12)**
0	9.92 ± 0.10^{aA}	8.25 ± 014^{aB}
15	8.94 ± 0.02^{bA}	7.79 ± 0.02^{bB}
30	8.68 ± 0.08^{cA}	7.23 ± 0.08^{cB}
45	8.16 ± 0.15^{dA}	6.82 ± 0.15^{dB}
60	7.86 ± 0.05^{eA}	6.37 ± 0.06^{eB}
75	7.15 ± 0.09^{fA}	5.99 ± 0.10^{efB}
90	6.63 ± 0.06^{gA}	5.75 ± 0.12^{fB}
105	6.41 ± 0.08^{gA}	5.34 ± 0.13^{gB}
120	6.10 ± 0.10^{hA}	4.74 ± 0.13^{hB}
Temperature	**Freezing (-18 °C)**	
Treatments Time (Days)	**ML (*Lactobacillus acidophilus* La-5)**	**MB (*Bifidobacterium* Bb-12)**
0	9.92 ± 0.10^{aA}	8.25 ± 014^{aB}
15	8.96 ± 0.08^{bA}	7.92 ± 0.03^{bB}
30	8.68 ± 0.07^{cA}	7.59 ± 0.02^{cB}
45	8.26 ± 0.03^{dA}	7.23 ± 0.08^{dB}
60	7.95 ± 0.06^{eA}	6.93 ± 0.10^{eB}
75	7.65 ± 0.04^{fA}	6.77 ± 0.04^{eB}
90	7.43 ± 0.10^{fA}	6.43 ± 0.09^{fB}
105	7.23 ± 0.11^{gA}	6.12 ± 0.12^{gB}
120	7.11 ± 0.10^{hA}	5.83 ± 0.12^{gB}
Temperature	**Refrigeration (7 °C)**	
Treatments Time (Days)	**ML (*Lactobacillus acidophilus* La-5)**	**MB (*Bifidobacterium* Bb-12)**
0	9.92 ± 0.10^{aA}	8.25 ± 014^{aB}
15	9.35 ± 0.14^{bA}	8.10 ± 0.08^{aB}
30	9.01 ± 0.03^{cA}	7.80 ± 0.09^{bB}
45	8.91 ± 0.04^{cA}	7.56 ± 0.09^{bB}
60	8.67 ± 0.05^{dA}	7.18 ± 0.14^{cB}
75	8.38 ± 0.09^{eA}	6.85 ± 0.03^{dB}
90	8.12 ± 0.10^{fA}	6.58 ± 0.06^{eB}
105	7.89 ± 0.12^{fA}	6.22 ± 0.08^{fB}
120	7.56 ± 0.11^{gA}	6.08 ± 0.08^{fB}

ML: microparticles produced with 8 g of gum arabic, 2 g of maltodextrin, 1.9 mL of glycerol, 0.1 mL of tween 80 and *Lactobacillus acidophilus* La-5; MB: microparticles produced with 8 g of gum arabic, 2 g of maltodextrin, 1.9 mL of glycerol, 0.1 mL of tween 80 and *Bifidobacterium* Bb-12.

Means followed by the same letter, lowercase in the column and upper case in the row, do not differ statistically from each other by the Tukey test at 5% significance. Means found in triplicate.

5 ARTIGO 2 – INULIN, HI-MAIZE, AND TREHALOSE AS THERMAL PROTECTANTS FOR INCREASING VIABILITY OF L*actobacillus acidophilus* ENCAPSULATED BY SPRAY DRYING

Esse artigo foi publicado no periódico LWT – Food Science and Technology, ISSN 1096-1127, Área de avaliação em Ciência dos Alimentos, Classificação A1.

LWT - Food Science and Technology 89 (2018) 128–133

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Inulin, hi-maize, and trehalose as thermal protectants for increasing viability of *Lactobacillus acidophilus* encapsulated by spray drying

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ARTICLE INFO

Keywords:
Spray dryer
Thermal protectants
Viability
Probiotics

ABSTRACT

Microparticles containing inulin, hi-maize, and trehalose were produced through spray drying to encapsulate *Lactobacillus acidophilus* La-5. Afterwards, the encapsulation efficiency, thermal resistance, gastrointestinal simulation, storage stability, and the microparticles' size and morphology were analyzed to evaluate the protective effect against the thermal conditions of the spray dryer of the different encapsulating matrices. Inulin and hi-maize encapsulating matrices showed the greatest encapsulation efficiency of 93.12% and 94.26%, respectively. Concerning thermal resistance, the trehalose encapsulating matrix provided the greatest protection for this microorganism. The microparticles produced with hi-maize showed the greatest viability in simulated gastrointestinal conditions thus providing higher protection for *Lactobacillus acidophilus* La-5. Regarding storage stability, microparticles containing trehalose showed the fewest viability losses during 120 days of storage. However, notably, at the end of 120 days of storage at room temperature (25 °C), microparticles containing inulin, hi-maize, and trehalose all kept their counts above the recommended level (> 6 log CFU/g^{-1}). Concerning the physical characteristics of the microparticles, particle sizes were as expected for products obtained by spray drying. Scanning electron microscopy showed no ruptures or cracks on the surfaces of the microparticles, a desirable characteristic for high protection.

1. Introduction

The food industry has great influence on the diet and lifestyle of the population, which is increasingly looking for products aimed at health and well-being. Among these, we highlight probiotics, living microorganisms that provide many beneficial effects to health by maintaining the balance and composition of the gastrointestinal tract. However, probiotic microorganisms are sensitive to heat, humidity, acidic environments, and the presence of oxygen, among other factors, all of which may reduce viability (Puuponem-Pimia et al., 2002; Mortazavian, Razavi, Ehsani, & Sohrabvandi, 2007). Thus, researchers have searched for tools to ensure their viability during processing, storage, and passage through the gastrointestinal tract (Betoret, Betoret, Vidal, & Fito, 2011; Tripathi & Giri, 2014). Several types of protection for microorganisms have been studied, among them microencapsulation and the addition of thermoprotective encapsulating matrices. Microencapsulation by spray drying, a technique known for many decades, is widely used in the pharmaceutical and food industries. Relatively low costs and industrial-scale production make this technique greatly relevant for the production of dry probiotic products (Schuck, Dolivet, Méjean, Hervé, & Jeantet, 2013). However, spray drying has the challenge of submitting products to high temperatures, which can lead to cell damage (Ananta, Volkert, & Knorr, 2005). Thus, strategies to achieve maximum viability aim to optimize the process, seeking to apply encapsulating matrices that aid in the protection and improvement of cell resistance (Desmond, Roos, O'Callaqhan, Fitzquerald, & Stanton, 2002; Fu & Chen, 2011; Liu et al., 2015). The application of thermal protectants is an important protective feature; accordingly, promising encapsulating agents have been identified, such as trehalose (Sunny-Roberts & Knorr, 2009), defatted milk (Selmer-Oslen, Sorhaug, Birkelend, & Pehrson, 1999), granular starch (Crittenden et al., 2001), and combinations of prebiotics (Desmond, Roos, O'Callaghan, Fitzqerald, & Stanton, 2002).

Prebiotics are nondigestible food components that positively affect

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http://dx.doi.org/10.1016/j.lwt.2017.10.032
Received 24 May 2017; Received in revised form 14 October 2017; Accepted 16 October 2017
Available online 17 October 2017
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their host by selectively stimulating the growth of beneficial colonic bacteria. Among prebiotics, inulin has been used widely to protect probiotic bacteria through microencapsulation during spray drying (Corcoran, Roos, Fitzgerald, & Stanton, 2004; Fritzen-Freire et al., 2012; Pinto et al., 2012). In addition, there is growing interest in the use of symbiotics (Tripathi & Giri, 2014). Trehalose is a non-reducing disaccharide shown to have promising physicochemical characteristics for microencapsulation (Cerimedo, Cerdeira Candal, & Herrera, 2008). Starches are another important component for microencapsulation, as these have been shown to exhibit film-forming ability, which may further protect encapsulated substances (Pegg & Shahidi, 2007). Finally, these encapsulating matrices have been offered as an alternative to gum arabic, one of the most-used compounds for microencapsulating food components using spray drying (Krishnan, Bhosale, & Singhal, 2005). Thus, inulin, hi-maize and trehalose have been carefully selected and will be evaluated in order to know their properties for future food applications. Studies have demostrated the importance of application of probiotic microparticles in different food matrices (Pinto, Fritzen-Freire, Munoz, Barreto, & Prudêncio, 2012; Antunes et al., 2013; Paim, Costa, Walter, & Tonon, 2016).

In this context, further studies are needed to assess potential thermal protectants, broaden knowledge about these compounds, and explore their use in spray-drying microencapsulation processes. Thus, this work is intended to assess the thermal protective effectiveness of different encapsulating matrices, measuring encapsulation efficiency, thermal resistance, simulated gastrointestinal survival, and storage stability, as well as assessing the size and morphology of the microparticles.

2. Materials and methods

To produce microparticles, the following compounds were used: Gum arabic (CNI, São Paulo, Brazil); Maltodextrin (Ingredion, São Paulo, Brazil); Inulin (Metachen, São Paulo, Brazil); Hi-maize (Ingredion, São Paulo, Brazil); Trehalose (Hayashibara, São Paulo, Brazil); Tween 80 (Vetec, Rio de Janeiro, Brazil); Glicerol (Vetec, Rio de Janeiro, Brazil), and probiotic culture *Lactobacillus acidophilus* La-5 obtained by Chr. Hansen from Brazil (Valinhos, São Paulo).

2.1. Inoculum

The probiotic culture of *Lactobacillus acidophilus* La-5 (Chr. Hansen, São Paulo, Brazil) was activated in MRS broth (Himedia Curitiba, Parana, Brazil) and incubated for 15 h at 37 °C. Afterwards, it was centrifuged at 4670 g for 15 min and washed in NaCl solution (0.85%). The cells were then suspended in saline to obtain a solution containing about 12 log CFU/g^{-1}.

2.2. Production of microparticles by spray drying

Different feed solutions were prepared containing *Lactobacillus acidophilus* to a final concentration of 20% m/v, namely SA_1 (gum arabic), SA_2 (Inulin), SA_3 (Hi-maize), and SA_4 (trehalose), as described in Table 1. In addition, glycerol and tween 80 were added in all formulations to improve the viability of probiotic microorganisms and promote greater homogenization of feed solutions, respectively (Sultana et al., 2000).

The microencapsulation process was performed in a lab spray dryer (MSD 1.0 Labmaq, Sao Paulo, Brazil) with inlet operating temperature of 130 °C and outlet temperature of 76 °C ± 5 °C. The different feed solutions, kept stirring, were introduced into the drying chamber using a peristaltic pump with feed rate of 0.48 L/h, drying air flow rate of 40 L/min, and air pressure of 0.6 MPa. The microparticles were collected at the base of the cyclone, transferred to sterile vials, and stored in a desiccator.

Table 1
Composition of feed solutions produced with different encapsulating matrices (S_1, S_2, S_3 and S_4) containing *Lactobacillus acidophilus* La-5.

Encapsulating matrices	S_1	S_2	S_3	S_4
Gum arabic (g)	8	8	8	8
Glycerol (mL)	1.9	1.9	1.9	1.9
Tween (ml)	0.1	0.1	0.1	0.1
Maltodextrin (g)	2.0	2.0	2.0	2.0
Gum arabic (g)	8	–	–	–
Inulin (g)	–	8	–	–
H-imaze (g)	–	–	8	–
Trehalose (g)	–	–	–	8
Final concentration (m/v):	20%			

S_1: initial solution with 8 g of gum arabic; S_2: initial solution with 8 g of inulin; S_3: initial solution with 8 g of hi-maize and S_4: initial solution with 8 g of trehalose.

2.3. Viable cell count

Serial dilutions were transferred to sterile Petri plates containing MRS agar (Himedia Curitiba, Paraná, Brazil), in triplicate. Plates were incubated at 37 °C for 72 h in anaerobic jars with an anaerobic generator (Oxoid, São Paulo, Brazil). The dilution of the microparticles comprised weighing 1 g of microparticles followed by the addition of 9 mL sterile phosphate buffer solution (pH 7.5), following the methodology described by Sheu, Marshall, and Heymann (1993). Results were shown as log colony forming units per gram (log CFU/g^{-1}).

2.4. Efficiency of encapsulation (EE)

The efficiency of encapsulation (EE) is the survival rate of the microorganisms during the microencapsulation process, calculated according to Eq. (1), as proposed by Martin, Lara-Villoslada, Ruiz, and Morales (2013):

$$EE\% = (N / N_0) \times 100 \quad (1)$$

Where N is the number of viable cells (log CFU/g^{-1}) released from the microparticles and N_0 is the number of viable cells (log CFU/g^{-1}) free in the feed solution before the spray-drying process. The viable cell count was performed as described in Section 2.3.

2.5. Microparticle morphology and size

The morphology of the microcaparticles was evaluated using an optical microscope (Carl Zeiss Axio Scope. A1, Oberkochen, Germany) equipped with an Axio Cam MRc digital camera (Carl Zeiss) and scanning electronic microscope (SEM; JEOL JM6360, Tokyo, Japan). The distribution of microparticle size was measured using a Mastersizer 3000 (Malvern, Germany), with water as the dispersion medium.

2.6. Resistance to heat treatment

Thermal resistance was assessed as proposed by Zhang, Lin, and Zhong (2015), with some adaptations. Microparticles and free culture (1 g) were transferred to 9 ml of peptone water in test tubes. The contents were then subjected to thermal conditions of 72 °C for 15 s and 63 °C for 30 min, after which the tubes were immediately cooled by immersion on ice for 10 min. Finally, aliquots were collected and probiotic cultures were counted according to Section 2.3.

2.7. Assessment of the survival of free and encapsulated Lactobacillus acidophilus La-05 exposed to simulated gastrointestinal conditions

The method proposed by Madureira, Amorim, Gomes, Pintado, and Malcata (2011), with some adaptations, was used to submit the microparticles to simulated gastrointestinal conditions. The viability of

the bacteria was determined continuously in media simulating the different sections of the gastrointestinal tract, such as esophagus/stomach (addition of pepsin, pH adjusted to 2.0 for 90 min), duodenum (addition of pancreatin and bile salts, pH adjusted to 5.0 for 20 min), and ileum (pH adjusted to 7.5 for 90 min). Analysis was conducted on a TE 421 Shaker (Tecnal, Piracicaba, SP, Brazil) at a temperature of 37 °C, simulating the temperature of the human body. Finally, aliquots were removed after 90 min (esophagus or stomach), 110 min (duodenum), or 200 min (ileum) to determine the survival of free and microencapsulated *Lactobacillus acidophilus* La-5. Probiotic cultures were counted in MRS medium as described in Section 2.3.

2.8. Stability of microparticles during storage at different temperatures

Viability of the microencapsulated microorganisms was determined by enumeration in MRS agar, as described in Section 2.3. The microparticles were examined after storage for 0, 15, 30, 45, 60, 75, 90, 105, and 120 days at 25 °C, −18 °C and 7 °C.

2.9. Statistical analysis

The data were submitted to analysis of variance (ANOVA) using Statistica version 7.0 software (2004; Statsoft Inc., Tulsa, OK, USA), followed by Tukey's means comparison test at a level of 5% significance of treatments showing possible significant differences. All experiments were performed in triplicate; data are expressed as means ± standard deviations.

3. Results and discussion

3.1. Viability and efficiency of encapsulation (EE%) of microparticles produced with different encapsulating agents

Table 2 shows the viability of *Lactobacillus acidophilus* La-5 after encapsulation and the efficiency of encapsulation (EE%) of microparticles produced with different encapsulating matrices. Viability for all different of produced microparticles was greater than the recommended minimum of 6.0 log CFU/g^{-1} (FAO/WHO, 2001). Among the encapsulating matrices studied, resistant starch (hi-maize) and inulin yielded the best viability of *Lactobacillus acidophilus*; however, no significant difference ($p > 0.05$) was noted between them. Because of this enhanced viability, these two encapsulating matrices provided the greatest survival rates, of 94.26% and 93.12%, respectively, followed by trehalose (90.34%) and gum arabic (89.68%). Ying et al. (2016) studied several combinations of spray-drying encapsulating agents (glucose, protein, corn starch, and oil) to enhance the protection of *Lactobacillus rhamnosus* GG, finding that a combination of encapsulating agents in the presence of resistant starch was more efficient in protecting this microorganism. In general, starches have been widely used as materials for encapsulation (Domian, Brynda-Kopytowska, Cenkier, & Swirydow, 2015; Krishnan et al., 2005). This fact may be associated with the helical structure of amylose that is able to encompass molecules forming stable complexes with them (Pegg & Shahidi, 2007). In the present work, inulin promoted good encapsulation efficiency, making it a potential protective agent. Rajam and Anandharamakrishnan (2015) reported that adding galacto-oligosaccharides and fructooligossaccharides to maltodextrin systems and isolate milk protein increased *Lactobacillus plantarum* viability after spray drying. Bustamante, Oomah, Rubilar, and Shene (2017) used chia seed, linseed mucilage, and soluble protein of chia seed as encapsulating agents for *Lactobacillus plantarum*, reaching 88.25% efficiency with linseed mucilage. Arslan, Erbas, Tontul, and Topuz (2015) used different wall materials (gelatin, whey protein concentrate, maltodextrin, modified starch-isolated pea protein, and acacia) to microencapsulate *Saccharomyces cerevisiae* var. *Boulardi.*, reporting efficiency between 84.69% and 91.81%. The high survival rates obtained in the present work can be related to both the encapsulating matrices used and the concentration of wall material (20% m/v). According to Huang et al. (2017), a solids concentration of around 20–30% in the feed solution is one of the best conditions ensuring high viability.

Table 2
Viability, encapsulation efficiency and particle size of the microparticles containing *Lactobacillus acidophilus* La-5 produced with different encapsulating matrices.

	Post-encapsulation viability CFU/g	Encapsulation efficiency (EE%)	Particles size (μm)
Free culture	12.22 ± 0.20^{a}	–	–
MS_1	10.96 ± 0.07^{c}	89.68 ± 0.56^{b}	19.30 ± 0.91^{a}
MS_2	11.38 ± 0.11^{b}	93.12 ± 0.93^{a}	6.68 ± 0.40^{d}
MS_3	11.50 ± 0.09^{b}	94.26 ± 0.74^{a}	11.40 ± 0.47^{c}
MS_4	11.04 ± 0.07^{c}	90.34 ± 0.53^{b}	15.30 ± 0.66^{b}

MS_1: MS_1: initial solution with 8 g of gum arabic; MS_2: initial solution with 8 g of inulin; MS_3: initial solution with 8 g of hi-maize and MS_4: initial solution with 8 g of trehalose. Means followed by the same letter, lowercase in the column, do not differ statistically from each other by the Tukey test at 5% significance. Means found in triplicate.

3.2. Morphology and size of microparticles

Micrographs of the microparticles produced with different encapsulating matrices containing *Lactobacillus acidophilus* La-5 are shown in Fig. 1. The different produced microparticles have a spherical shape, with some concavities, as was also observed by Fritzen-Freire et al. (2012), who reported that these concavities are typical of products obtained by spray drying. However, the produced microparticles showed no visual cracks or fractures on their surfaces, suggesting that air permeability is minimal or non-existent and thus guaranteeing greater protection of probiotic microorganisms (Rajam & Anandharamakrishnan, 2015; Fritzen-Freire et al., 2012). The microparticles produced with trehalose presented a regular shape and a slightly smooth surface. Domian et al. (2015) reported similar characteristics when encapsulating oil with corn starch (OSA) and trehalose for spray drying, with trehalose reportedly stiffening the surface of the microparticles. On the other hand, when producing microparticles with corn starch alone (OSA), these authors observed a rounded external surface with many characteristic concavities. The same was observed in the present work for microparticles containing resistant starch maize.

Table 2 shows the particle sizes, which varied from 6.68 to 19.30 μm for different microparticles containing *Lactobacillus acidophilus* La-5. Rajam and Anandharamakrishnan (2015) reported particle sizes of 6.68–23.89 μm for microparticles containing *Lactobacillus plantarum* (MTCC 5422), verifying the larger particle sizes and reporting that they may be related to the different film-forming and gelling properties of the wall materials used in microencapsulation. In general, microparticles obtained by spray drying exhibit desirable smaller particle size, which ensures homogeneity and quality when applied to foods (Burgain, Gaiani, Linder & Scher, 2011).

3.3. Resistance of microparticles to heat treatment

To investigate the protective effect of different encapsulating matrices on the viability of *Lactobacillus acidophilus* La-5 the microparticles were submitted to different thermal treatments (Table 3). The microparticles produced with different encapsulating matrices were more resistant to thermal treatments (63 °C/30 min and 72 °C/15 s) compared to free-culture *Lactobacillus acidophilus* La-5. Among the different microparticles produced, MS_4 containing trehalose provided the best protection under both thermal treatments. Recent studies have shown great potential for trehalose use. Domian et al. (2015) used maize starch and trehalose to encapsulate spray-dried oil emulsions, reporting encapsulation efficiency greater than 90%. Recently, Lim and Roos (2016) and Lim, Zuzana Burdikova, Sheehan, & Roos (2016) used trehalose-maltodextrin systems, and trehalose-isolated whey protein

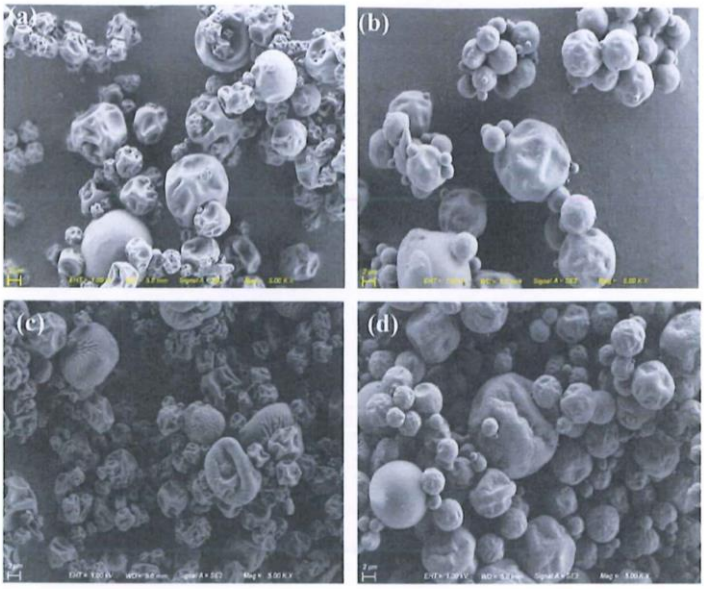

Fig. 1. Micrographs of microcapsules of *Lactobacillus acidophillus* produced with: (a) MS_1: initial solution with 8 g of gum arabic; (b) MS_2: initial solution with 8 g of inulin; (c) MS_3: initial solution with 8 g of hi-maize and (d) MS_4: initial solution with 8 g of trehalose.

Table 3
Effect of heat treatments on the viability of free and microencapsulated *Lactobacillus acidophilus* La-5 with different encapsulating matrices.

	Initial count CFU/g	63 °C/30 min	72 °C/15 s
Free culture	12.22 ± 0.20^{a}	7.69 ± 0.08^{dB}	8.39 ± 0.07^{dA}
MS_1	10.96 ± 0.07^{d}	7.91 ± 0.08^{cB}	8.88 ± 0.05^{cA}
MS_2	11.38 ± 0.11^{b}	8.94 ± 0.30^{bB}	9.39 ± 0.07^{bA}
MS_3	11.50 ± 0.09^{b}	9.13 ± 0.07^{bB}	9.46 ± 0.10^{bA}
MS_4	11.04 ± 0.07^{c}	9.43 ± 0.04^{aB}	10.33 ± 0.01^{aA}

MS_1: initial solution with 8 g of gum arabic; MS_2: initial solution with 8 g of inulin; MS_3: initial solution with 8 g of hi-maize and MS_4: initial solution with 8 g of trehalose.
Means followed by the same letter, lowercase in the column and upper case in the row, do not differ statistically from each other by Tukey test at 5% significance. Means found in triplicate.

(WPI) to encapsulate and promote greater stability of spray-dried carotenoids, reportedly achieving high-quality powders when using trehalose as an encapsulating agent. With respect to different heat treatments, the higher temperature and shorter exposure time (72 °C/15 s) had greater *Lactobacillus acidophilus* La-5 survival, whether exposed in its free form or as microencapsulates, agreeing with findings by Zhang et al. (2015). They reported better survival of *Lactobaciclus salivarius* NRR B-30514 encapsulated by the emulsion method when subjected to heat treatment at 72 °C/15 s relative to 63 °C/30 min.

3.4. Exposure of microparticles to simulated gastrointestinal conditions

Table 4 shows viable cell counts of free and microencapsulated *Lactobacillus acidophilus* La-5 exposed to simulated gastrointestinal conditions. After 90 min incubation in the presence of a pepsin solution and pH adjusted to 2.0 (simulated esophagus/stomach), there was significantly decreased ($p < 0.05$) free *Lactobacillus acidophilus* La-5 compared to the initial count of 5.73 log cycles. The microparticles of *Lactobacillus acidophilus* La-5 produced with the different encapsulating matrices also showed significant decreases ($p < 0.05$) in the simulated stomach/esophagus with respect to the initial counts. However, this difference does not necessarily imply a loss of viability, since the microparticles should not have ruptured at a pH value 2.0; consequently, the probiotic microorganisms were not released (Holken et al., 2016).

In the section of the gastrointestinal tract comprising the duodenum (bile salts, pancreatin, and pH 5.0), microparticles and free cultures both showed an increased number of viable cells (Table 4). However, this increase was greater for the microparticles, which therefore suffered greater disruption in these conditions. The lower increase in viable cell counts observed in the free cultures may be related to the fact that they were already subjected to damage in the acidic conditions of the simulated esophagus/stomach (Holken et al., 2016). Rajam, Parthasarathi, Joseph & Anandharamakrishnan (2012) encapsulated *L. plantarum* with isolated whey protein and alginate by spray drying and observed that viability was enhanced when the cultures were added to a pH 5.0 with bile salts.

In the last section of the simulated gastrointestinal tract, the ileum (pH 7.5), free-cultured *Lactobacillus acidophilus* La-5 showed a loss of 3.59 log cycles, while microparticles ranged from a loss of 2.76 to 1.01 log cycles in relation to the initial counts. Thus, the microparticles studied here protected the microorganism compared to losses in free culture. These results agree with those of Gebara et al. (2013), who reported increased resistance of microencapsulated probiotics when exposed to simulated gastrointestinal conditions compared to free-cell survival. Among the microparticles produced with different matrices, the encapsulant presenting the highest protection for *Lactobacillus acidophilus* La-5 was MS_3, containing hi-maize. Similar results were reported by Pankasemsuk, Apichartsrangkanon, & Techarang (2016), who encapsulated *Lactobacillus casei* with 2% alginate together with 0.5–2% starch (hi-maize) by an emulsion technique. Their results showed that alginate capsules incorporating 1% corn starch promoted the highest number of viable cells in both gastric and biliary fluids. Etchepare et al. (2016) encapsulated *Lactobacillus acidophilus* by extrusion using sodium alginate alone and in combination with resistant starch (hi-maize), finding that capsules made with hi-maize were most resistant to simulated gastrointestinal conditions.

Table 4
Viability of microparticles containing *Lactobacillus acidophilus* La-5 produced with different encapsulating matrices against simulated gastrointestinal conditions.

	Free Culture	MS_1	MS_2	MS_3	MS_4
Initial count	12.22 ± 0.20^{aA}	10.96 ± 0.07^{aC}	11.38 ± 0.11^{aB}	11.50 ± 0.09^{aB}	11.04 ± 0.07^{aC}
Esophagus/stomach 90 min/pH 2.0	6.49 ± 0.16^{dA}	5.83 ± 0.08^{dAB}	3.44 ± 0.16^{dD}	3.72 ± 0.18^{dD}	4.85 ± 0.09^{dC}
Duodenum 20 min/pH 5.0	7.16 ± 0.06^{cB}	6.24 ± 0.03^{cDE}	6.57 ± 0.67^{cC}	6.42 ± 0.10^{cCD}	8.49 ± 0.09^{cA}
Ileum 90 min/pH 7.5	8.63 ± 0.03^{bC}	8.20 ± 0.04^{bD}	10.16 ± 0.08^{bA}	10.49 ± 0.12^{bA}	9.65 ± 0.20^{bB}

MS_1: initial solution with 8 g of gum arabic; MS_2: initial solution inulin 8 g; MS_3: initial solution with hi-maize 8 g and MS_4: 8 g initial solution with trehalose.
Means followed by the same letter, lowercase in the column and upper case in the row, do not differ statistically from each other by Tukey test at 5% significance. Means found in triplicate.

3.5. Stability of microparticles during storage at different temperatures

Table 5 shows the viability of microencapsulated *Lactobacillus acidhopilus* La-5 stored at room temperature (25 °C), below freezing (−18 °C) and under refrigeration (7 °C). Of the three, room-temperature storage (25 °C) was the most damaging to the viability of *Lactobacillus acidhopilus* La-5 in different microparticles, promoting reductions after 120 days' storage between 4.55 and 3.49 log CFU/g^{-1}. Huang et al. (2017) microencapsulated by spray drying *Lactobacillus casei* BL23 and *Propionibacterium freudenreichii* TG P20 using sweet whey, reporting that storage at 25 °C resulted in greater viability loss, about 5 log CFU/g^{-1} after six months of storage. After 120 days of storage, the microparticles containing inulin, hi-maize and trehalose, respectively, remained viability greater than recommended. However, there was no significant difference ($p < 0.05$) between the encapsulating matrices of hi-maize and trehalose after 120 days of storage.

For freezing temperatures and refrigeration, losses ranged from 2.93 to 1.99 and 2.23 to 1.09 log CFU/g^{-1}, respectively. Thus, freezing temperatures (18 °C) during storage reduced viability and consequently stability of the studied microparticles. This fact may be related to cell deaths after freezing due to the formation of ice crystals, which structurally damage cell membranes and change cells' physiological states (Conrad, Miller, Cienlenski, & Pablo, 2000). Regarding refrigeration,

Table 5
Effect of room temperature (25 °C), freezing (−18 °C), and refrigeration (7 °C) on the feasibility of microparticles containing *Lactobacillus acidophilus* La-5 produced with different encapsulating matrices during storage for 120 days.

Treatments Time (Days)	MS_1	MS_2	MS_3	MS4
Temperature	Room (25 °C)			
0	10.01 ± 0.14^{aB}	10.83 ± 017^{aA}	10.98 ± 0.13^{aA}	10.30 ± 0.26^{aB}
15	9.01 ± 0.10^{bB}	9.88 ± 0.08^{bA}	9.96 ± 0.03^{bA}	9.76 ± 0.10^{bA}
30	8.64 ± 0.08^{cC}	9.51 ± 0.09^{bA}	9.57 ± 0.05^{bA}	9.26 ± 0.04^{cB}
45	8.38 ± 0.08^{cC}	9.10 ± 0.15^{cA}	8.91 ± 0.09^{dAB}	8.72 ± 0.04^{dB}
60	7.87 ± 0.10^{dB}	8.65 ± 0.29^{dA}	8.59 ± 0.05^{eA}	7.98 ± 0.07^{eB}
75	7.41 ± 0.12^{eC}	7.93 ± 0.15^{eB}	8.23 ± 0.02^{fA}	7.75 ± 0.05^{fB}
90	6.89 ± 0.07^{fB}	7.44 ± 0.10^{fA}	7.65 ± 0.15^{gA}	7.49 ± 0.09^{gA}
105	6.30 ± 0.06^{gC}	6.75 ± 0.09^{gB}	7.12 ± 0.11^{hA}	7.01 ± 0.12^{hA}
120	5.46 ± 0.19^{hC}	6.03 ± 0.09^{hB}	6.67 ± 0.09^{iA}	6.81 ± 0.10^{hA}
Temperature	**Freezing (-18 °C)**			
0	10.01 ± 0.14^{aB}	10.83 ± 017^{aA}	10.98 ± 0.13^{aA}	10.30 ± 0.26^{aB}
15	9.25 ± 0.14^{bC}	10.12 ± 0.14^{bB}	10.62 ± 0.08^{bA}	9.96 ± 0.10^{aB}
30	8.93 ± 0.09^{cD}	9.88 ± 0.14^{bB}	10.34 ± 0.04^{cA}	9.58 ± 0.04^{bC}
45	8.69 ± 0.05^{cD}	9.53 ± 0.03^{cB}	10.04 ± 0.12^{dA}	9.31 ± 0.08^{bcC}
60	8.13 ± 0.07^{dC}	9.19 ± 0.07^{dB}	9.69 ± 0.09^{eA}	9.11 ± 0.09^{cdB}
75	7.87 ± 0.09^{deC}	8.95 ± 0.11^{deB}	9.41 ± 0.08^{fA}	8.93 ± 0.04^{deB}
90	7.61 ± 0.18^{eC}	8.68 ± 0.04^{efB}	9.02 ± 0.12^{gA}	8.77 ± 0.03^{efAB}
105	7.31 ± 0.10^{fC}	8.44 ± 0.06^{fB}	8.84 ± 0.09^{ghA}	8.54 ± 0.14^{fgB}
120	7.08 ± 0.08^{fD}	7.97 ± 0.12^{gC}	8.40 ± 0.07^{hA}	8.31 ± 0.08^{gA}
Temperature	**Refrigeration (7 °C)**			
0	10.01 ± 0.14^{aB}	10.83 ± 017^{aA}	10.98 ± 0.13^{aA}	10.30 ± 0.26^{aB}
15	9.72 ± 0.09^{bC}	10.48 ± 0.11^{bA}	10.64 ± 0.05^{bA}	10.12 ± 0.13^{abB}
30	9.27 ± 0.06^{cC}	10.17 ± 0.13^{bcAB}	10.36 ± 0.03^{cA}	10.04 ± 0.08^{abB}
45	8.93 ± 0.06^{dB}	9.99 ± 0.14^{cdA}	10.01 ± 0.11^{dA}	9.93 ± 0.05^{bcA}
60	8.65 ± 0.12^{eC}	9.68 ± 0.05^{deB}	9.88 ± 0.08^{deA}	9.82 ± 0.04^{bcdAB}
75	8.37 ± 0.11^{fC}	9.35 ± 0.10^{efB}	9.74 ± 0.02^{efA}	9.78 ± 0.10^{bcdA}
90	8.16 ± 0.06^{fgC}	9.06 ± 0.09^{fgB}	9.67 ± 0.12^{efA}	9.63 ± 0.15^{cdA}
105	7.91 ± 0.06^{ghC}	8.98 ± 0.13^{gB}	9.54 ± 0.09^{fgA}	9.49 ± 0.10^{deA}
120	7.78 ± 0.12^{hC}	8.79 ± 0.09^{gB}	9.33 ± 0.11^{gA}	9.21 ± 0.09^{eA}

MS_1: initial solution with 8 g of gum arabic; MS_2: initial solution inulin 8 g; MS_3: initial solution with hi-maize 8 g and MS_4: 8 g initial solution with trehalose.
Means followed by the same letter, lowercase in the column and upper case in the row, do not differ statistically from each other by Tukey test at 5% significance. Means found in triplicate.

however, studies have reported that lower temperatures improve the rates of cell viability due to the reduction of possible chemical reactions that are detrimental to microorganisms (Corcoran et al., 2004). Among the other produced microparticles, hi-maize and trehalose presented greater viability; however, there was no significant difference ($p < 0.05$) between these after 120 days of storage. Trehalose, however, showed the greatest stability, with reductions of only 1.99 and 1.09 log CFU/g^{-1} after storage at freezing and under refrigeration, respectively. This fact may be related to the elevated vitreous transition temperature trehalose presents (Cerimedo, Cerdeira, Candal, & Herrera, 2008). Elevated glass transition temperatures have been previously related to greater stability for probiotic bacteria (Rokka & Rantamaki, 2010). Zhang et al. (2015) reported that *Lactobacillus salivarius* NRRL B-30514 after spray-drying feed solutions in the presence of trehalose showed better survivability during storage than powders produced with only reconstituted skimmed milk.

Hi-maize was also a good protective agent for *Lactobacillus acidophilus* La-5 during storage. Etchepare et al. (2016) microencapsulated *Lactobacillus acidophilus* L-14 by the extrusion method using alginate alone and in combination with the hi-maize prebiotic, reporting similar results; microparticles produced in the presence of hi-maize had greater stability after 135 days' storage.

4. Conclusion

The higher encapsulation efficiency was provided using resistant starch (hi-maize) and inulin. Concerning thermal resistance and gastrointestinal simulation, trehalose and hi-maize, respectively, were the encapsulating matrices with the higher protective capacity. The stability after 120 days of storage was improved using trehalose, mainly under refrigeration. Thus, the production of microparticles using resistant starch (hi-maize) and trehalose through the spray-drying method presented the best thermal protective potential. Finally, the use of such thermal protectants in spray drying offers a promising tool to increase the viability of probiotic microorganisms.

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6 MANUSCRITO 3 – INCREASED VIABILITY OF *bifidobacterium* BB-12 ENCAPSULATE BY SPRAY DRYING USING INULIN, HI-MAIZE, AND TREHALOSE

Esse artigo foi submetido ao periódico Food Control, ISSN 0956-7135, Área de avaliação em Ciência dos Alimentos, Classificação A1.

Increased viability of *Bifidobacterium* Bb-12 encapsulated by spray drying using inulin, hi-maize and trehalose

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Abstract

The present study was carried out to evaluate the use of encapsulating matrices of inulin, hi-maize and tealose as thermal protectants to promote greater viability of probiotic *Bifidobacterium* Bb-12 cells produced by spray drying. The different microparticles produced were assessed for encapsulation efficiency, thermal resistance, gastrointestinal simulation, storage stability and size, and morphology. Hi-maize and trehalose showed the highest encapsulation efficiency, 95.24% and 90.10%, respectively. Regarding thermal resistance and gastrointestinal simulation conditions, trehalose and hi-maize provided the greatest protective effect for *Bifidobacterium* Bb-12 under such conditions. During storage, trehalose showed the

lowest viability losses throughout the 120 days. Particle size ranged from 7.68 to 20.9 μm and scanning electron microscopy showed that the microparticles did not have ruptures or cracks in their surface. Thus, the production of microparticles using trehalose and hi-maize as a thermal protectant proved to be a very important tool to increase the viability of *Bifidobacterium* Bb-12.

Keywords: Spray dryer, encapsulating agents, microparticle, probiotic, viability, potential protection.

1. Introduction

The food industry has been seeking the development of products that offer benefits to the consumer as the world's population has become increasingly aware regarding their health. Probiotic microorganisms have received global importance due to their numerous beneficial effects on human health (Das & Goyal, 2015; Fijan, 2014). However, for these microorganisms to exert their beneficial effects, it is essential that they remain viable during their use (FAO/WHO, 2001).

Probiotic microorganisms present sensitivity to a variety of factors, for instance the heating in heat treatments, mechanical processing, storage at room temperature and the presence of oxygen (Mortazavian, Razavi, Ehsani, & Sohrabvandi, 2007; Fu & Chen, 2011). In this context, in the last decades, appropriate procedures and materials for the protection of probiotics have been extensively investigated in order to maintain high viability for these microorganisms (Arslan, Erbas, Tontul & Topuz, 2015; Pinto et al., 2015; Muhammad, Ramzam, Huo, Tian & Bian, 2017).

Microencapsulation by spray drying is known and widely used in the food industry. Industrial scale production and its relatively low costs make this technique a relevant alternative

for the obtaining dried probiotic products (Schuck, Dolivet, Méjean, Hervé & Jeantet, 2013). However, high temperatures used in this process may cause lesions in the microorganisms' cells, consequently resulting in the loss of viability (Golowczyc et al., 2011; Martín, Lara-Villoslada, Ruiz & Morales, 2015). El-Salam & El-Shibiny (2015) reported that the composition of the encapsulating material could improve the survival of probiotics during drying and also after storage. Thus, the study and the selection of encapsulating matrices to be used in microencapsulation by spray drying is an important tool to achieve high encapsulation efficiency, which is one of the most important objectives of this technology in order to ensure that a greater number of viable bacteria reach the colon. (Cook, Tzortzis, Charalampopoulos, & Khutoryanskiy, 2011). In the microencapsulation of probiotics, it is possible to use different encapsulating matrices alone or in combinations, some examples are gelatin, whey protein concentrate, modifiedi starch, maltodextrin, pea protein isolate, gum arabic and prebiotics (Arslan, Erbas, Tontul & Topuz, 2015; Pinto et al., 2015; Muhammad, Ramzam, Huo, Tian & Biam, 2017). Prebiotics, such as inulin, have been receiving attention in the microencapsulation of probiotics because of their symbiotic effect, which is described as positive, since when probiotics reach the colon, prebiotics can be used to assist in their survival and colonization and, therefore, benefit the host (Tripathi & Giri, 2014). The hi-maize, a resistant starch, which has the ability to resist the degradation action of the intestinal enzymes, undergoing colon fermentation, and thus presenting prebiotic and chemopreventive activity (Fuentes-Zaragoza et al., 2011). Pegg & Shahidi (2007) demonstrated that starches present ability to form films, which may result in enhanced protection for encapsulated compounds. Furthermore, Homayouni et al. (2013) reported that resistant starches have good characteristics for use in the food industry due to their mild taste, white color and low water retention capacity, being an important component for the formulation of several functional foods. Trehalose is a non-reducing disaccharide that has been presenting important physico-chemical characteristics for

its use in microencapsulation processes. Its high glass transition temperature and the formation of an amorphous state in the wall matrix of the dried microparticles are some of these characteristics (Bhandari & Hartel, 2005; Drusch et al., 2006; Cerimedo, Cerdeira Candal, & Herrera, 2008). Based on the above explanations, it is evident the importance of selection and study concerning the encapsulating matrices, making it possible to know how their physicochemical and functional properties may be associated with loss/improvement of probiotic survival during spray drying (Muhammad, Ramzam, Huo, Tian & Biam, 2017).

In this context, this work is intended to assess the thermal protective effectiveness of inulin, hi-maize and trehalose as encapsulating matrices for *bifidobacterium* Bb-12, measuring encapsulation efficiency, thermal resistance, simulated gastrointestinal survival, and storage stability, as well as assessing the size and morphology of the microparticles.

2. Materials and methods

To produce microparticles, the following compounds were used: Gum arabic (CNI, São Paulo, Brazil); Maltodextrin (Ingredion, São Paulo, Brazil); Inulin (Metachen, São Paulo, Brazil); Hi-maize (Ingredion, São Paulo, Brazil); Trehalose (Hayashibara, São Paulo, Brazil); Tween 80 (Vetec, Rio de Janeiro, Brazil); Glycerol (Vetec, Rio de Janeiro, Brazil) and probiotic culture *Bifidobacterium* Bb-12 obtained by Chr. Hansen from Brazil (Valinhos, São Paulo).

2.1 Inoculum

The probiotic culture of *Bifidobacterium* Bb-12 (Chr. Hansen, São Paulo, Brazil) was activated in reconstituted milk (Molico, Nestlé, São Paulo, Brazil) and incubated for 15 h at 37 °C. Afterwards, it was centrifuged at 4670 g for 15 min and washed in NaCl solution (0.85%). The cells were then suspended in saline to obtain a solution containing about 10 log CFU/g^{-1}.

2.2 Production of microparticles by spray drying

Different feed solutions were prepared containing *Bifidobacterium* Bb-12 to a final concentration of 20% m/v, namely SA_1 (gum arabic) which is the control sample, SA_2 (Inulin), SA_3 (Hi-maize), and SA_4 (trehalose), as described in Table 1. Glycerol and tween 80 were added in all formulations to improve the viability of probiotic microorganisms and promote greater homogenization of feed solutions, respectively (Sultana et al., 2000).

The microencapsulation process was performed in a lab spray dryer (MSD 1.0 Labmaq, São Paulo, Brazil) with inlet operating temperature of 130 °C and outlet temperature of 76 °C ± 5 °C. The different feed solutions, kept stirring, were introduced into the drying chamber using a peristaltic pump with feed rate of 0.48 L / h, drying air flow rate of 40 L / min, and air pressure of 0.6 MPa. The microparticles were collected at the base of the cyclone, transferred to sterile vials, and stored in a desiccator.

2.3 Viable cell count

Serial dilutions were transferred to sterile Petri plates, in triplicate, containing MRS agar (Himedia Curitiba, Paraná, Brazil) containing lithium chloride (0.1%) and L-cysteine (0.05%), according to manufacturer recommendations (Chr Hansen, 1999). Plates were incubated at 37 °C for 72 h in anaerobic jars with an anaerobic generator (Oxoid, São Paulo, Brazil). The dilution of the microparticles comprised weighing 1 g of microparticles followed by the addition of 9 mL sterile phosphate buffer solution (pH 7.5), according to the methodology described by Sheu, Marshall, & Heymann (1993). Results were shown as log colony forming units per gram (log CFU/g^{-1}).

2.4 Encapsulation efficiency (EE)

The encapsulation efficiency (EE) is the survival rate of the microorganisms during the microencapsulation process, calculated according to Eq. (1), as proposed by Martin, Lara-Villoslada, Ruiz, & Morales (2013):

$$EE\% = (N / N_o) \times 100 \quad (1)$$

Where N is the number of viable cells (log CFU/g^{-1}) released from the microparticles and N_0 is the number of viable cells (log CFU/g^{-1}) free in the feed solution before the spray drying process. The viable cell count was performed as described in Section 2.3.

2.5 Microparticle morphology, size and distribuition

The morphology of the microparticles was evaluated using an optical microscope (Carl Zeiss Axio Scope.A1, Oberkochen, Germany) equipped with an AxioCam MRc digital camera (Carl Zeiss) and scanning electron microscope (SEM; JEOL JM6360, Tokyo, Japan). The distribution of microparticle size was measured using a Mastersizer 3000 (Malvern, Germany), with water as the dispersion medium.

2.6 Resistance to heat treatment

Thermal resistance was assessed as proposed by Zhang, Lin, & Zhong (2015), with some adaptations. Microparticles and free culture (1 g) were transferred to 9 ml of peptone water in test tubes. The contents were then subjected to thermal conditions of 72 °C for 15 seconds and 63 °C for 30 minutes, afterwards the tubes were immediately cooled by immersion on ice for 10 min. Finally, aliquots were collected and probiotic cultures were counted according to Section 2.3.

2.7 Viability of free and encapsulated *Bifidobacterium* Bb-12 exposed to simulated gastrointestinal conditions

The method proposed by Madureira et al. (2011) was used, with some adaptations, to submit the microparticles to simulated gastrointestinal conditions. The viability of the bacteria was determined continuously in media simulating the different sections of the gastrointestinal tract, such as esophagus / stomach (pepsin addition, pH adjusted to 2.0 for 90 min), duodenum (pancreatin and bile salts addition, pH adjusted to 5.0 for 20 min), and ileum (pH adjusted to 7.5 for 90 min). The analysis was conducted on a TE 421 Shaker (Tecnal, Piracicaba, SP, Brazil) at 37 °C, simulating the human body temperature. Finally, aliquots were removed after 90 min (esophagus or stomach), 110 min (duodenum), or 200 min (ileum) to determine the survival of free and microencapsulated *Bifidobacterium* Bb-12. Probiotic cultures were counted in MRS medium as described in Section 2.3.

2.8 Viability of microparticles during storage at different temperatures

Viability of the microencapsulated microorganisms was determined by enumeration in MRS agar, as described in Section 2.3. The microparticles were examined after storage for 0, 15, 30, 45, 60, 75, 90, 105, and 120 days at 25 °C, −18 °C and 7 °C.

2.9 Statistical Analysis

The data were submitted to analysis of variance (ANOVA) using Statistica version 7.0 software (2004; Statsoft Inc., Tulsa, OK, USA), followed by Tukey's means comparison test at a level of 5% significance of treatments showing possible significant differences. All analyzes were performed in triplicate; data are expressed as means ± standard deviations.

3. Results and discussion

3.1 Viability and encapsulation efficiency (EE%) of microparticles produced with different encapsulating agents

The viability of *Bifidobacterium* Bb-12 post-encapsulation and the encapsulation efficiency (EE%) of the microparticles produced with the different encapsulating matrices are shown in Table 2. Initially it is possible to observe that the used encapsulating matrices presented a significant difference ($p<0.05$) on the viability of *Bifidobacterium* Bb-12 in the different microparticles produced. Different from Paim, Costa, Walter & Tonon (2016) who, by encapsulating jussara juice (*Euterpe edulis* M.) with *bifidobacterium animalis* using maltodextrin alone and combined with inulin and/or oligofructose, found very similar viable cell counts, which demonstrates that prebiotic addition had no influence on the survival of the microorganisms during drying. Recently, Verruck et al. (2017) encapsulated *Bifidobacterium* Bb-12 using goat milk together with inulin and oligofructose both alone and in combination, and found no significant difference in encapsulation efficiency. Moreover, different from the present study, these authors found high encapsulation efficiency using these encapsulating matrices. In the present study, the best survival rates of *Bifidobacterium* Bb-12 and higher encapsulation efficiency (EE%) were observed for the encapsulating matrices hi-maize and trehalose, 95.24 and 90.10%, respectively (Table 2). Starches have been widely used as encapsulation materials (Krishnan, Bhosale & Singhal, 2005; Domian, Brynda-Kopytowska, Cenkier & Swirydow, 2015). Recently, Ying et al. (2016) studied the different associations of encapsulating agents in the spray dryer (glucose, protein, cornstarch and oil) to improve the protection of *Lactobacillus rhamnosus* GG. They have found that the combination of encapsulating agents in the presence of resistant starch was more efficient in protecting this microorganism. Martin et al. (2013) reported that a combination of alginate with cornstarch was

10 times more efficient in the encapsulation of *L. fermentum* in comparsion to the use of alginate alone. Pegg & Shahidi (2007) stated that the helical structure of amylose present in the starch composition is capable of encompassing molecules forming stable complexes with them; therefore, its use in microencapsulation processes is emphasized.

The use of trehalose as an encapsulating matrix has also showed a positive result on the viability of *Bifidobacterium* Bb-12 post-encapsulation. Domian et al. (2015) used cornstarch and trehalose to encapsulate oil emulsions in spray dryer and reported the presence of trehalose resulted in an encapsulation efficiency greater than 90%. Recently, Lim e Roos (2016) e Lim, Burdikova, Sheehan & Roos (2016) used trehalose-maltodextrin and trehalose-whey protein isolate (WPI) systems in spray dryer, respectively, to encapsulate and promote greater stability of carotenoids. These authors reported success and high quality of the powders using trehalose as an encapsulating agent. Lastly, it can be observed that all the produced microparticles presented higher encapsulation efficiency than the control sample (MS_1), which was produced with the gum arabic encapsulating matrix (80.11%). Similar results were reported by Arslan, Erbas, Tontul & Topuz (2015), who by using gum Arabic to encapsulate *Saccharomyces cerevisiae* var. *boulardii* in spray dryer observed encapsulation efficiency of 84.69%. In addition, these authors demonstrated that gum arabic resulted in the lowest encapsulation efficiency found in relation to the other encapsulating matrices studied.

3.2 Morphology, size and distribution of microparticles

Micrographs of the microparticles produced with different encapsulating matrices containing *Bifidobacterium* Bb-12 are shown in Figure 1. The microparticles produced with inulin, hi-maize, trehalose and gum arabic presented a spherical shape, with some concavities. The same behavior was observed by Fritzen-Freire et al. (2012) and Pinto et al. (2015) who reported that these concavities are typical of products obtained by spray drying. According to

Teixeira, Andrade, Farina & Rocha-Leão (2004), the concavities presented by the micrparticles are formed by their shrinkage during the initial stages of drying. However, the microparticles showed no visual cracks or fractures on their surfaces. Rajan & Anandharamakrishnan (2015) and Fritzen-Freire et al. (2012) stated that the absence of ruptures or cracking is important for the permeability to be minimal or non-existent, thus ensuring greater protection for probiotic microorganisms. The microparticles produced with trehalose presented a regular shape and a slightly smooth surface. Similar results were shown by Domian, Brynda-Kopytowska, Cenkier, & Swirydow (2015) e Zhang, Lin & zhong (2016), by using trehalose in their feed solutions to encapsulate oil and probiotic *Lactobacillus salivarius* NRRL B-30514, respectively. Paramita et al. (2010) reported that the addition of disaccharides, such as trehalose, in drying media increases the solids content, which results in obtaining particles with smooth surfaces.

The size of the microcapsules ranged between 7.68 and 20.9 μm (Table 2). Pinto et al. (2015) reported similar results by encapsulating *Bifidobacterium* Bb-12 in the spray dryer, using liquid whey, whey retentate, inulin and polydextrose alone or in combination. These authors stated particle sizes ranging from 10.55 to 12.77 μm. Martín et al. (2015) argued that smaller particle sizes are preferred in order to show no sensory effects when applied in food. Overall, particle sizes for products produced by spray dryer should vary from 10 to 100 μm; therefore, the values found in the present work are in agreement with those expected for particles obtained by spray drying (Fang & Bhandari, 2010).

3.3 Resistance of microparticles to heat treatments

In order to investigate the protective effect of the different encapsulating matrices on the viability of *Bifidobacterium* Bb-12, the microparticles were submitted to different thermal treatment conditions (Table 3). The microparticles were more resistant to the thermal treatment conditions employed (63 °C / 30 min and 72 °C / 15 s) in relation to the free culture. Fritzen-

Freire, Prudêncio, Pinto, Munõz & Amboni (2013) observed similar results by submitting free and microencapsulated *Bifidobacterium* Bb-12 to thermal treatments of 55, 65 and 75 °C, for 0, 1 and 10 min. Verruck et al. (2017) also stated that the free culture of *bifidobacterium* BB-12 was more affected by the thermal treatments of 55, 65 and 75 °C, for 5, 10 and 15 min, in relation to this same microencapsulated culture. Golowczyc et al. (2011) verified that when higher temperatures are used, damage in the bacterial cell occurs, harming its integrity, which includes DNA, RNA, cytoplasmic membrane and cell wall.

For the different microparticles produced, losses varied from 1.41 to 0.92 and 0.62 to 0.48 log cycles for the thermal treatment conditions of 63 °C/ 30 min and 72 °C/ 15 s, respectively. However, it is worth noticing that for both thermal treatments studied, all viable cell counts, including free cells, were superior than 6 log CFU/g^{-1}. Pinto et al. (2015), by submitting microparticles produced with sweet whey alone and in combination with inulin and polydextrose containing *Bifidobacterium* Bb-12 to the thermal treatment at 60 °C for 15 min, found losses superior than 3.0 log cycles. In addition, these authors also verified that in these conditions some of the microparticles did not present probiotic counts anymore. Verruck et al. (2017) microencapsulated *Bifidobacterium* Bb-12 with goat milk, inulin and olifofructose alone and in combinations and found losses greater than 2 logs for a thermal treatment performed at 65 °C for 15 min. Among the different microparticles, the ones produced with trehalose and hi-maize had the lowest viability losses, respectively 0.92 and 0.96 logs at 63 °C/ 30 min and 0.48 and 0.51 logs at 72 °C/ 15 s. Recent studies have shown great potential use of trehalose and resistant starches, such as hi-maize, in microencapsulation processes (Domian, Brynda-Kopytowska, Cenkier & Swirydow, 2015; Lim & Roos, 2016; Lim, Zuzana Burdikova, Sheehan & Roos, 2016; Pankasemsuk, Apichartsrangkoon, Worametrachanon & Techarang, 2016; Etchepare et al., 2016). Therefore, the present study also emphasizes the use of these matrices for the probiotic microorganisms' protection against the conditions of thermal

treatments. Verruck et al. (2017) highlighted the importance of studies that verify the survival capacity of microencapsulated probiotic microorganisms in different matrix compositions. The bacteria's ability to tolerate the different temperatures used in food processing is a matter of great importance for the future of the food industry. The different thermal treatments studied demonstrated that the highest temperature and the lowest time (72 °C/ 15 s) resulted in a higher survival for *Bifidobacterium* Bb-12, both in its free and microencapsulated form. Zhang, Lin, & Zhong (2015) reported similar results by encapsulating *Lactobaciclus salivarius* NRR B-30514 in emulsions with multiple lipid-protein-pectin layers.

3.4 Viability of microparticles submitted to simulated gastrointestinal conditions

Table 4 shows the viable cell counts of free and microencapsulated *Bifidobacterium* Bb-12 exposed to simulated gastrointestinal conditions. It is possible to observe that after 90 min incubation with the presence of a pepsin solution and pH adjusted to 2.0 (esophagus/stomach simulation) there was a significant ($p<0.05$) decrease of free *Bifidobacterium* Bb-12 in relation to the initial count of 5.48 log cycles. The different microparticles of *Bifidobacterium* Bb-12 also presented a significant decrease ($p<0.05$) in relation to the initial count in the stomach/esophagus section. Holken et al. (2016) showed corresponding results when encapsulating *Bifidobacterium* Bb-12 by emulsification/internal gelation. These authors reported that the viability decrease in the microparticles may be connected to the fact that they did not suffer disruption under these conditions. In the gastrointestinal tract section, that comprises the duodenum (bile salts, pancreatin and pH 5.0); the microparticles increased the number of viable cells (Table 4). The same behavior was observed by Rajam, Karthik, Parthasarathi, Joseph & Anandharamakrishnan (2012) and Nunes et al. (2018) for microparticles containing *Lactobacillus plantarum* and *Lactobacillus acidophilus*, respectively, produced in spray dryer. According to Picot & Lacroix (2004), the increase in the viable cell

counts under these conditions could not be attributed to cell multiplication and, most likely, it resulted from a recovery of the sub-damaged cells. Furthermore, a greater rupture of the microparticles may have occurred, which would explain the increase in viable cells under these conditions (Holken et al., 2016).

In the last section of the gastrointestinal tract corresponding to ileum (pH 7.5), the free culture and the different microparticles of *Bifidobacterium* Bb-12 showed a significant increase ($p < 0.05$) in the viable cell count. Verruck et al. (2017) presented similar results for free culture and microparticles of *Bifidobacterium* Bb-12 produced with a combination of goat milk, inulin and oligofructose. Moumita et al. (2017) reported that some bacterial strains enter latent state after contact with the acidic conditions of the stomach and return to their growth when they reach the highest pH of the intestine (pH 6.0). Maciel et al. (2014) found that microparticles containing *Lactobacillus acidophilus* la-5 under simulated gastrointestinal conditions increased the number of viable cells when the pH was raised from 2 to 7. At the end of the passage through simulated gastrointestinal conditions, it is possible to observe a loss of 4.22 log cycles for the free culture, whereas for the microparticles produced with the different encapsulating matrices, the losses varied from 1.73 to 1.18 log cycles. Thus, it is possible to observe that the microparticles studied in this work provided greater protection for *Bifidobacterium* Bb-12 in comparison to their free cells. Among the different microparticles evaluated, those produced with trehalose and hi-maize were the ones that promoted greater protection for *Bifidobacterium* Bb-12, presenting the lowest viable cell loss, 1.18 and 1.26 log cycles, respectively, during the evaluation of the simulated gastrointestinal conditions. Trehalose has recently been studied in microencapsulation processes, which demonstrated greater encapsulation efficiency and particle stability by using this encapsulating matrix (Domian, Brynda-Kopytowska, Cenkier & Swirydow, 2015; Lim & Roos, 2016; Lim, Zuzana Burdikova, Sheehan & Roos, 2016). Liao et al. (2017) verified that microparticles produced with trehalose and skim milk presented high

protective effect on *Lactobacillus casei* under simulated gastric conditions. Relevant results were also found for the matrix encapsulant hi-maize. Recently, Muhammad, Ramzam, Huo, Tian & Biam (2017) found better survival of *Lactobacillus plantarum* in gastric fluids when encapsulated with potato resistant starch (PRS). Nunes et al. (2018) and Etchepare et al. (2016) reported high protection for microencapsulated *Lactobacillus acidophilus* using hi-maize by spray drying and extrusion techniques, respectively, under simulated gastrointestinal conditions.

3.5 Viability of the microparticles under different storage conditions

The effect of room temperature (25 °C), refrigeration (7 °C) and freezing (-18 °C) on the viability of microencapsulated *Bifidobacterium* Bb-12 during storage can be seen in Table 5. Among the different temperatures evaluated in the 120 days of storage, refrigeration (7 °C) resulted in the lowest reductions found, ranging from 1.56 to 0.97 logs CFU/g^{-1}. Then, followed by freezing temperature (-18 °C) from 1.88 to 1.34 logs CFU/g^{-1} and; finally, room temperature (25 °C) of 2.58 and 1.92 logs CFU/g^{-1} for the microparticles produced with the different encapsulating matrices. Therefore, it can be observed that the refrigeration temperature promoted the greatest viability for the different microparticles studied over the 120 days of storage. In contrast to the present study, Bernucci et al. (2017) evaluated the stability for 90 days of three different microparticles containing *Bifidobacterium logum* 5^{1A}, which were produced by emulsification technique using calcium alginate and chitosan (CaAIC) and calcium alginate with resistant starch (CaAISr) and also the ones produced with skim milk and maltodextrin in spray drying (SD). These authors concluded that the viability of *Bifidobacterium logum* 5^{1A} in the the spray dried microparticles and calcium alginate and chitosan did not decrease significantly stored at -20 °C/ 90 days. Teixeira, Castro, Malcata & Kirby (1995) and Corcoran, Roos, Fitzqerald & Stanton (2004) reported that lower temperatures

improve the cell viability rates since it reduces chemical reactions that may occur and are harmful to microorganisms.

Among different microparticles produced, MS_4 containing trehalose presented the highest viability by the end of the 120 days of storage at all evaluated temperatures. Chavéz & Ledeboer (2007) showed that disaccharides, such as trehalose, can protect bacteria by displacing water molecules near the cell membrane. Moreover, trehalose presents high glass transition temperature, which has been related to enhanced stability for probiotic bacteria (Leslie, Israeli, Ligthtart, Crowe & Crowe; 1995; Rokka & Rantamäki, 2010). Zhang, Lin & Zhong (2016) have verified that microparticles containing *Lactobacillus salivarius* NRRL B-30514 produced with trehalose and reconstituted skim milk showed better survival capacity during storage than microparticles produced with only reconstituted skim milk. Nonetheless, it is worth noting that all the encapsulating matrices studied; inulin, hi-maize, trehalose and gum arabic (control sample), maintained their viable cell counts at levels higher than the ones recommended at all temperatures (> 6 log CFU/g), which is considered essential for these microorganisms to exert their beneficial effects on health (Tripathi & Giri, 2014).

Therefore, our results are relevant once it becomes necessary to develop drying products containing probiotic microorganisms that remain viable at room temperature to achieve greater stability for bacterial cells over time without the requirement of a cold chain (Eckert et al., 2017).

4. Conclusion

The investigation the protective effect of the different encapsulating matrices used in the *Bifidobacterium* Bb-12 microencapsulation by spray drying, it was reported that resistant starch (hi-maize) and trehalose provided the highest encapsulation efficiency in this process. In the thermal resistance evaluation and gastrointestinal simulation, the microparticles MS_3 and

MS_4 produced with hi-maize and trehalose showed the greatest protection potential for *Bifidobacterium* Bb-12 under these conditions. In the storage viability for 120 days, the refrigeration temperature (7 °C) was the one that maintained the highest viability for all microparticles produced. However, it is worth mentioning that all microparticles produced with different encapsulating matrices maintained counts superior to 6 log CFU/g^{-1} at all temperatures studied (25, -18 e 7 °C). Trehalose was the encapsulating matrix that promoted greater viability for *Bifidobacterium* Bb-12 in the different storage conditions evaluated. Thus, it was verified that the resistant starch (hi-maize) not only presents important functional properties but also it is an efficient encapsulating matrix for the protection of *Bifidobacterium* Bb-12. The use of trehalose has also shown to be a very important tool in the microencapsulation process by spray drying and later in the microparticles storage.

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Table 1 - Composition of feed solutions produced with different encapsulating matrices (S_1, S_2, S_3 and S_4) containing *Bifidobacterium* Bb-12.

Encapsulating matrices	**S_1**	**S_2**	**S_3**	**S_4**
Gum arabic (g)	8	8	8	8
Glycerol (mL)	1.9	1.9	1.9	1.9
Tween (ml)	0.1	0.1	0.1	0.1
Maltodextrin (g)	2.0	2.0	2.0	2.0
Gum arabic (g)	8	-	-	-
Inulin (g)	-	8	-	-
H-imaze (g)	-	-	8	-
Trehalose (g)	-	-	-	8
Final concentration (m / v):	20%			

S_1: initial solution with 8 g of gum arabic; S_2: initial solution with 8 g of inulin; S_3: initial solution with 8g of hi-maize and S_4: initial solution with 8g of trehalose.

Table 2 - Viability, encapsulation efficiency and particle size of the microparticles containing *Bifidobacterium* Bb-12 produced with different encapsulating matrices.

	Post-encapsulation viability log CFU/g	Encapsulation efficiency (EE%)	Particles size (µm)
Free culture	10.51 ± 0.02^{a}		
MS_1	8.42 ± 0.10^{e}	80.11 ± 0.97^{d}	20.9 ± 0.95^{a}
MS_2	8.93 ± 0.03^{d}	84.96 ± 0.29^{c}	8.47 ± 0.33^{c}
MS_3	10.03 ± 0.04^{b}	95.24 ± 0.61^{a}	9.96 ± 0.41^{b}
MS_4	9.50 ± 0.29^{c}	90.10 ± 0.80^{b}	7.68 ± 0.28^{d}

MS_1: initial solution with 8 g of gum arabic; MS_2: initial solution with 8 g of inulin; MS_3: initial solution with 8g of hi-maize and MS_4: initial solution with 8g of trehalose.

Means followed by the same letter, lowercase in the column, do not differ statistically from each other by the Tukey test at 5% significance. Means found in triplicate.

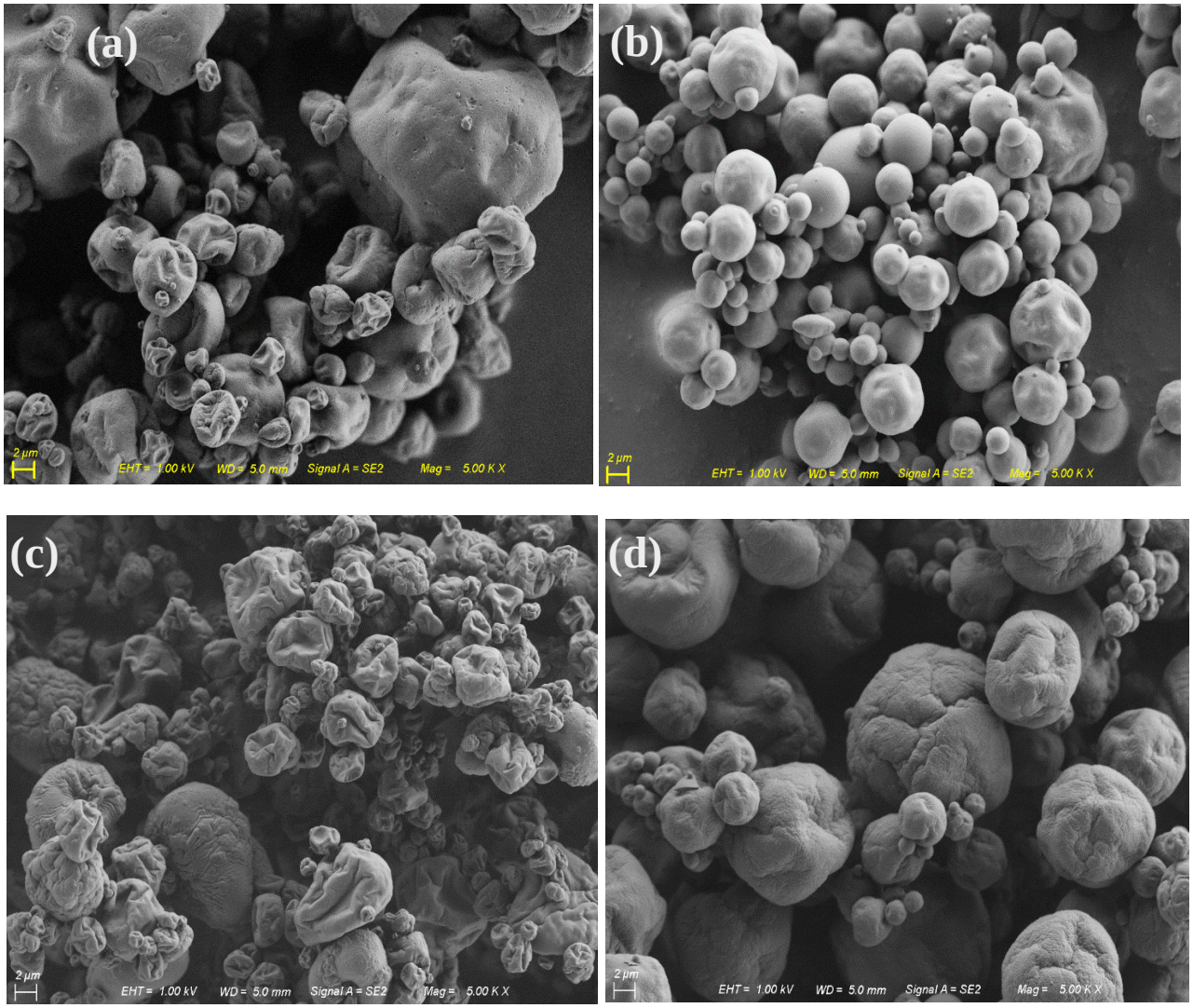

Figure 1. Micrographs of microparticles of *Bifidobacterium* Bb-12 produced with: (a) MS_1: initial solution with 8 g of gum arabic; (b) MS_2: initial solution with 8 g of inulin; (c) MS_3: initial solution with 8g of hi-maize and (d) MS_4: initial solution with 8g of trehalose.

Table 3 - Effect of heat treatments on the viability of free and microencapsulated *Bifidobacterium* Bb-12 with different encapsulating matrices.

	Initial count Log CFU/g	**63 °C/ 30 min**	**72 °C/ 15 s**
Free culture	10.51 ± 0.02^{a}	6.53 ± 0.14^{eA}	6.98 ± 0.21^{dA}
MS_1	8.42 ± 0.10^{e}	7.01 ± 0.11^{dB}	7.80 ± 0.16^{cA}
MS_2	8.93 ± 0.03^{d}	7.95 ± 0.08^{cA}	8.35 ± 0.23^{cbA}
MS_3	10.01 ± 0.04^{b}	9.05 ± 0.06^{aB}	9.50 ± 0.04^{aA}
MS_4	9.47 ± 0.29^{c}	8.55 ± 0.06^{bB}	8.99 ± 0.08^{abA}

MS_1: initial solution with 8 g of gum arabic; MS_2: initial solution with 8 g of inulin; MS_3: initial solution with 8g of hi-maize and MS_4: initial solution with 8g of trehalose.

Means followed by the same letter, lowercase in the column and upper case in the row, do not differ statistically from each other by Tukey test at 5% significance. Means found in triplicate.

Table 4 - Viability of free and microencapsulated *Bifidobacterium* Bb-12 with different matrices agaisnt simulated gastrointestinal conditions.

	Free culture	**MS_1**	**MS_2**	**MS_3**	**MS_4**
Initial count	10.51 ± 0.02^{aA}	8.42 ± 0.10^{aE}	8.93 ± 0.03^{aD}	10.01 ± 0.04^{aB}	9.47 ± 0.29^{aC}
Esophagus / stomach 90 min / pH 2.0	5.03 ± 0.05^{dA}	4.21 ± 0.15^{dC}	4.12 ± 0.07^{dC}	$4.46 \pm 0,04^{dB}$	3.87 ± 0.03^{dD}
Duodenum 20 min / pH 5.0	5.40 ± 0.03^{cE}	6.33 ± 0.14^{cD}	6.98 ± 0.05^{cC}	7.53 ± 0.07^{cB}	7.90 ± 0.08^{cA}
Ileum 90 min / pH 7.5	6.29 ± 0.05^{bE}	7.07 ± 0.08^{bD}	7.20 ± 0.04^{bC}	8.75 ± 0.08^{bB}	8.29 ± 0.10^{bA}

MS_1: initial solution with 8 g of gum arabic; $MS_{2:}$ initial solution inulin 8g; $MS_{3:}$ initial solution with hi-maize 8g and $MS_{4:}$ 8g initial solution with trehalose.

Means followed by the same letter, lowercase in the column and upper case in the row, do not differ statistically from each other by Tukey test at 5% significance. Means found in triplicate.

Table 5 – Effect of room temperature (25 °C), freezing (−18 °C), and refrigeration (7 °C) on the viability of microparticles containing *Bifidobacterium* Bb-12 produced with different encapsulating matrices during storage for 120 days.

Temperature	**Room (25 °C)**			
Treatments Time (Days)	**MS_1**	**MS_2**	**MS_3**	**MS_4**
0	8.44 ± 0.05^{aC}	8.72 ± 0.25^{aC}	9.86 ± 0.04^{aA}	9.39 ± 0.06^{aB}
15	8.02 ± 0.13^{bC}	8.16 ± 0.11^{bC}	9.29 ± 0.09^{bA}	8.99 ± 0.10^{bB}
30	$7.83 \pm 0.19b^{cB}$	7.96 ± 0.01^{bcB}	8.95 ± 0.02^{cA}	8.64 ± 0.09^{cA}
45	7.58 ± 0.16^{cdB}	7.67 ± 0.08^{cdB}	8.52 ± 0.10^{dA}	8.25 ± 0.04^{dA}
60	7.56 ± 0.07^{cdB}	7.69 ± 0.09^{cdB}	8.55 ± 0.14^{dA}	8.26 ± 0.18^{dA}
75	7.28 ± 0.09^{deB}	7.39 ± 0.09^{deB}	8.12 ± 0.12^{eA}	8.05 ± 0.04^{deA}
90	7.01 ± 0.04^{efB}	7.13 ± 0.08^{efB}	7.84 ± 0.03^{fA}	7.88 ± 0.10^{efA}
105	6.75 ± 0.07^{fB}	6.89 ± 0.04^{fgB}	7.51 ± 0.08^{gA}	7.64 ± 0.12^{fgA}
120	6.41 ± 0.06^{gC}	6.63 ± 0.06^{gB}	7.28 ± 0.03^{gA}	7.47 ± 0.11^{gA}
Temperature	**Freezing (-18 °C)**			
Treatments Time (Days)	**MS_1**	**MS_2**	**MS_3**	**MS_4**
0	8.44 ± 0.05^{aC}	8.72 ± 0.25^{aC}	9.86 ± 0.04^{aA}	9.39 ± 0.06^{aB}
15	8.09 ± 0.08^{bD}	8.38 ± 0.15^{abC}	9.44 ± 0.09^{bA}	9.08 ± 0.04^{bB}
30	7.88 ± 0.10^{bcB}	8.10 ± 0.14^{bcB}	9.15 ± 0.02^{cA}	8.90 ± 0.10^{bA}
45	7.69 ± 0.13^{cdC}	7.83 ± 0.14^{cdC}	8.90 ± 0.09^{cdA}	8.52 ± 0.04^{cB}
60	7.81 ± 0.08^{cC}	7.85 ± 0.073^{cdC}	8.96 ± 0.13^{cA}	8.64 ± 0.06^{cB}
75	7.50 ± 0.06^{dD}	7.73 ± 0.08^{cdC}	8.71 ± 0.10^{dA}	8.49 ± 0.06^{cB}
90	7.23 ± 0.07^{eC}	7.53 ± 0.09^{deB}	8.37 ± 0.07^{eA}	8.26 ± 0.14^{dA}
105	6.98 ± 0.10^{eC}	7.24 ± 0.13^{efB}	8.16 ± 0.06^{efA}	8.17 ± 0.02^{dA}
120	6.56 ± 0.13^{fC}	7.08 ± 0.04^{fB}	8.01 ± 0.13^{fA}	8.05 ± 0.09^{dA}
Temperature	**Refrigeration (7 °C)**			
Treatments Time (Days)	**MS_1**	**MS_2**	**MS_3**	**MS_4**
0	8.44 ± 0.05^{aC}	8.72 ± 0.25^{aC}	9.86 ± 0.04^{aA}	9.39 ± 0.06^{aB}
15	8.26 ± 0.04^{aD}	8.56 ± 0.04^{abC}	9.63 ± 0.04^{aA}	9.16 ± 0.04^{abB}
30	7.96 ± 0.08^{bD}	8.23 ± 0.14^{bcC}	9.34 ± 0.09^{bA}	9.03 ± 0.06^{bcB}
45	7.74 ± 0.07^{bcC}	8.04 ± 0.02^{cB}	9.09 ± 0.14^{bcA}	8.81 ± 0.15^{cdeA}
60	7.91 ± 0.09^{bB}	8.12 ± 0.12^{cdB}	9.14 ± 0.13^{bcA}	8.92 ± 0.06^{bcdA}
75	7.80 ± 0.10^{bC}	8.00 ± 0.09^{cdeC}	9.12 ± 0.06^{bcA}	$8.74 \pm 0.14_{deB}$
90	7.46 ± 0.12^{cdD}	7.83 ± 0.12^{deC}	9.03 ± 0.06^{cA}	8.67 ± 0.05^{efB}
105	7.21 ± 0.16^{deC}	7.65 ± 0.08^{efB}	8.86 ± 0.12^{cA}	8.56 ± 0.09^{efA}
120	6.94 ± 0.14^{eC}	7.34 ± 0.07^{fB}	8.30 ± 0.14^{dA}	8.42 ± 0.09^{fA}

MS_1: initial solution with 8 g of gum arabic; MS_2: initial solution inulin 8g; MS_3: initial solution with hi-maize 8g and MS_4: 8g initial solution with trehalose.

Means followed by the same letter, lowercase in the column and upper case in the row, do not differ statistically from each other by Tukey test at 5% significance. Means found in triplicate.

7 DISCUSSÃO

Inicialmente, diferentes temperaturas de entrada de ar no *spray dryer* foram estudadas para alcançar maior eficiência de encapsulação e as melhores características físico-químicas para as micropartículas. Após, inulina, hi-maize e trealose foram adicionadas a composição das micropartículas e avaliou-se seu potencial termoprotetor. Os resultados estão apresentados e discutidos no artigo 1, publicado no periódico *LWT - Food Science and Technology*, intitulado "Inulin, hi-maize and trehalose as thermal protectants for increasing viability of *Lactobacillus acidophilus* encapsulated by spray drying"; no manuscrito 2, submetido ao periódico *Food Research International*, intitulado "Increased viability of *Bifidobacterium* Bb-12 encapsulate by spray drying using inulin, hi-maize and trehalose" e no manuscrito 3, submetido ao periódico Ciência Rural, intitulado "Encapsulation of *Lactobacillus acidophilus* La-5 and *Bifidobacterium* Bb-12 by spray drying and evaluation of its resistance in simulated gastrointestinal conditions, thermal treatments and storage condition".

Na avaliação da inulina, hi-maize e trealose como matrizes encapsulantes de proteção na microencapsulação em *spray dryer*, podemos destacar que a maior eficiência de encapsulação foi encontrada utilizando hi-maize, 94,26% para *Lactobacillus acidophilus* La-5 e 95,24% para *Bifidobacterium* Bb-12. De maneira geral, amidos vêm sendo bastante utilizados como materias de encapsulação (KRISHNAN; BHOSALE; SINGHAL, 2005; DOMIAN et al., 2015) especialmente pela amilose, uma estrutura helicoidal presente na sua composição, a qual é capaz de englobar moléculas formando complexos estáveis (PEGG; SHAHIDI, 2007). Arslan et al. (2015) utilizaram diferentes agentes encapsulantes (gelatina, concentrado de proteína de soro de leite, maltodextrina, proteína de ervilha, amido modificado e goma arábica) para encapsular *Saccharomyces cerevisiae* var. *Boulardi*. e encontraram uma eficiência de encapsulação que variou de 84,69% e 91,81%.

A proteção das diferentes matrizes encapsulantes na avaliação da resistência térmica das micropartículas demostrou que a trealose promoveu a menor perda de viabilidade para *Lactobacillus acidophilus* La-5, 1,61 e 0,71 ciclos logarítmicos, e *Bifidobacterium* Bb-12, 0,92 e 0,48 ciclos logarítmicos, quando submetidos aos tratamentos de 63 °C/ 30 min e 72 °C/ 15 s, respectivamente. Assim, nestas condições pode-se verificar que *Bifidobacterium* Bb-12 se mostrou mais resistente que *Lactobacillus acidophilus* La-5. Estudos recentes, tem demostrado o potencial de utilização da trealose, reportando encontrar alta eficiência de encapsulação e maior estabilidade *(*DOMIAN et al., 2015; LIM; ROOS, 2016; LIM et al., 2016). Entre os tratamentos térmicos estudados 72 °C/ 15 s promoveu maior sobrevivência para ambos

microrganismos probióticos. Resultados semelhantes foram reportados por Zhang, Lin e Zhong (2015). Estes autores relataram melhor sobrevivência de *Lactobaciclus salivarius* NRR B-30514 encapsulado pelo método da emulsão quando submetido a tratamento térmico a 72 ° C / 15 s em relação a 63 ° C/ 30 min. Por fim, vale destacar que para ambos tratamentos térmicos, todas as micropartículas produzidas com as diferentes matrizes encapsulantes (inulina, hi-maize, trealose e amostra controle) mantiveram suas contagens de células viáveis superiores a 6 log UFC/g^{-1}. Verruck et al. (2017) destacaram a importância de estudos que verifiquem a capacidade de sobrevivência de microrganismos probióticos encapsulados em diferentes matrizes encapsulantes frente a tratamentos térmicos, pois, a capacidade da bactéria em tolerar as diferentes temperaturas empregadas no processamento de alimentos é um ponto de grande relevância para o futuro da indústria de alimentos.

Nas condições do trato gastrointestinal simulado (esôfago/ estômago, 90 min, pH=2,0; duodeno, 20 min, pH=5,0; íleo, 90 min, pH=7,5) verificou-se que entre as diferentes matrizes encapsulantes avaliadas, a trealose promoveu maior proteção para *Bifidobacterium* Bb-12, mostrando ao final da simulação do trato gastrointestinal uma perda na viabilidade de 1,18 ciclos logarítmicos. Logo, para *Lactobacillus acidophilus* La-5, hi-maize foi a matriz encapsulante que proporcionou maior proteção, demonstrando uma perda de 1,01 ciclos logarítmicos. Muhammad et al. (2017) encontraram melhor sobrevivência de *Lactobacillus plantarum* em fluidos gástricos quando encapsulados com amido resistente de batata (PRS). Liao et al. (2017) verificaram que micropartículas produzidas com trealose e leite desnatado apresentaram elevado efeito protetor sobre *Lactobacillus casei* em condições gástricas simuladas.

O acompanhamento da viabilidade de *Lactobacillus acidophilus* La-5 e *Bifidobacterium* Bb-12 encapsulados durante o armazenamento por 120 dias nas temperaturas ambiente (25 °C), congelamento (-18 °C) e refrigeração (7 °C) mostrou que a temperatura de refrigeração foi que manteve a viabilidade mais elevada para ambos microrganismos estudados. Teixeira et al. (1995) e Corcoran et al. (2004) reportaram que temperaturas mais baixas melhoram as taxas de viabilidade celular em razão da redução de reações químicas que podem acontecer e que são prejudiciais aos microrganismos.

Ao avaliar as diferentes matrizes encapsulantes, pode-se observar que hi-maize e trealose apresentaram maior viabilidade ao final dos 120 dias de armazenamento em todas as temperaturas estudadas, porém, não houve diferença significativa ($p<0,05$) entre eles. Ao verificar as perdas de viabilidade durante os 120 dias de armazenamento, podemos observar

que a trealose foi a matriz encapsulante que proporcionou maior proteção para os microrganismos probióticos, pois apresentou as menores reduções de viabilidade, que variaram de 1,09 a 3,49 ciclos logarítmicos para *Lactobacillus acidophilus* La-5 e entre 0,97 a 1,92 ciclos logarítmicos para *Bifidobacterium* Bb-12 nas diferentes temperaturas estudadas. Chavéz e Ledeboer (2007) mostraram que os dissacarídeos, como a trealose, podem proteger as bactérias ao deslocar as moléculas de água próximas da membrana celular. Além disso, a trealose apresenta elevada temperatura de transição vítrea, a qual vem sendo relacionada a melhor estabilidade para bactérias probióticas (LESLIE et al., 1995; ROKKA; RANTAMAKI, 2010).

A temperatura ambiente foi a mais prejudicial para *Lactobacillus acidophilus* La-5 e *Bifidobacterium* Bb-12, no entanto, vale destacar que as micropartículas produzidas com os agentes encapsulantes inulina, hi-maize e trealose mantiveram suas contagens acima de 6 log UFC/g^{-1}, o qual é recomendado para que estes microrganismos exerçam seus efeitos positivos à saúde do hospedeiro. Além disso, Eckert et al. (2017) reportaram que se torna necessário desenvolver produtos com baixo teor de umidade que contenham microrganismos probióticos e que estes, permaneçam viáveis a temperatura ambiente para alcançar maior estabilidade para as células bacterianas ao longo do tempo sem a exigência de uma cadeia de frio.

Para as micropartículas de *Lactobacillus acidophilus* La-5 (ML) e *Bifidobacterium* Bb-12 (MB) produzidas em diferentes temperaturas de entrada de ar no *spray dryer* a maior eficiência de encapsulação pode ser observada na temperatura de 130 °C, 84,61% para *Lactobacillus acidophilus* La-5 e 79,73% para *Bifidobacterium* Bb-12. As características físico-químicas das micropartículas ML e MB produzidas em diferentes temperaturas de entrada de ar no *spray dryer* mostraram valores de atividade de água e umidade de 0,195 a 0,289 e 4,60% a 5,71%, respectivamente. Estes resultados estão de acordo com o esperado para micropartículas secas por *spray dryer* para que seja assegurada sua estabilidade microbiológica (CORCORAN et al., 2004; ARSLAN et al., 2015; CHAVEZ; LEDEBOER, 2007).

Na avaliação da resistência térmica das micropartículas de *Lactobacillus acidophilus* La-5 (ML) e *Bifidobacterium* Bb-12 (MB) foram encontradas reduções de 1,91 e 1,93, para 63 °C/ 30 min e 1,36 e 1,42 para 72 °C/ 15 s, respectivamente. Dessa maneira, as micropartículas de *Lactobacillus acidophilus* La-5 (ML) apresentaram maior resistência aos tratamentos térmicos estudados. Favaro-Trindade e Grosso (2002) e Lian, Hsiao e Chou (2002) demostraram em estudos prévios que diferentes estirpes de microrganismos podem variar sua capacidade para resistir a temperaturas elevadas. Bifidobactérias são conhecidas por serem mais susceptíveis a altas temperaturas do que os lactobacilos (DOLEYRES; LACROIX, 2005). A

maior temperatura e o menor tempo (72 °C/ 15 s) resultaram em maior sobrevivência para as micropartículas de *Lactobacillus acidophilus* La-5 (ML) e *Bifidobacterium* Bb-12 (MB).

Nas condições gastrointestinais simuladas (esôfago/estômago; duodeno e íleo) as micropartículas de *Lactobacillus acidophilus* La-5 (ML) e *Bifidobacterium* Bb-12 (MB) apresentaram reduções de 1,72 e 2,04 ciclos logarítmicos, respectivamente. Assim, as micropartículas de *Lactobacillus acidophilus* La-5 (ML) foram mais resistentes as condições gastrointestinais simuladas do que *Bifidobacterium* Bb-12 (MB). Esses resultados diferem dos reportados por Pedroso et al. (2012) que encontraram maior sobrevivência gastrointestinal para *Bifidobacterium lactis* em comparação a *Lactobacillus acidophilus*. Segundo Gomes e Malcata (1999) e Koll et al. (2008) existe uma variação na capacidade de *Bifidobacterium* e *Lactobacillus acidophilus* resistir as condições de ácido e a bile. Estes autores reportaram ainda que essas propriedades são específicas de estirpes e espécies.

Nas diferentes condições de armazenamento por 120 dias, a temperatura ambiente (25 °C), congelamento (-18 °C) e refrigeração (7 °C) as micropartículas apresentaram perdas de 3,82, 2,81 e 2,36 ciclos logarítmicos para *Lactobacillus acidophilus* La-5 (ML) e 3,51, 2,42 e 2,17 para *Bifidobacterium* Bb-12 (MB), respectivamente. Assim, a temperatura de refrigeração promoveu a maior viabilidade para ambas micropartículas estudadas. Resultados semelhantes foram reportados por Oliveira et al. (2007) que mostraram que *L. acidophilus* exibiu maior viabilidade a uma temperatura de armazenamento de 7 °C. Entre as micropartículas de *Lactobacillus acidophilus* La-5 (ML) e *Bifidobacterium* Bb-12 (MB) podemos observar que para as diferentes temperaturas avaliadas, *Bifidobacterium* Bb-12 (MB) apresentou as menores reduções durante os 120 dias de armazenamento. No entanto, considerando o nível mínimo de 10^6 UFC/g^{-1} as micropartículas de *Bifidobacterium* Bb-12 (MB) apresentaram vida útil de apenas 60 dias a 25° C e 105 dias a -18° C enquanto que as micropartículas de *Lactobacillus acidophilus* La-5 (ML) mantiveram-se viáveis durante todo o período de armazenamento em todas as temperaturas estudadas. Segundo Martin et al. (2015) diferentes cepas probióticas apresentam capacidades distintas de resistir as condições ambientais, como, oxigênio, pH, luz e temperatura. Além disso, as condições do processo de microencapsulação são determinantes de grande importância para que os microrganismos permaneçam viáveis durante seu armazenamento (OLIVEIRA et al., 2007).

Com relação ao tamanho de partícula, podemos observar que o mesmo variou de 7,68 a 20,9 μm para *Bifidobacterium* Bb-12 e de 4,85 a 19,30 μm para *Lactobacillus acidophilus* La-5 para as micropartículas produzidas com as diferentes matrizes encapsulantes (inulina, hi-maize, trealose, ML e MB). Fang e Bhandari (2010) reportaram que tamanhos de partícula para produtos produzidos por *spray dryer* devem variar de 10 a 100 μm, assim, os

valores encontrados no presente trabalho estão de acordo aos esperados para partículas obtidas por *spray dryer.* Além disso, vale ressaltar que partículas de tamanhos menores são preferidas afim de não apresentarem efeitos sensoriais quando aplicadas em alimentos (MARTIN et al., 2015).

A morfologia das micropartículas de *Bifidobacterium* Bb-12 e *Lactobacillus acidophilus* La-5 produzidas com as matrizes encapsulantes, inulina, hi-maize, trealose e a amostra controle (goma arábica) mostram-se em forma esférica e com presença de concavidades. Fritzen-Freire et al. (2012) reportaram que estas concavidades são típicas de produtos obtidos por *spray dryer*. No entanto, vale destacar, que as diferentes micropartículas não apresentaram fissuras ou rupturas em sua superfície sugerindo que a permeabilidade do ar é mínima ou inexistente, o que vai resultar em maior proteção para os microrganismos probióticos (RAJAM; ANANDHARAMAKRISHNAN, 2015; FRITZEN-FREIRE et al., 2012). As micropartículas de *Bifidobacterium* Bb-12 e *Lactobacillus* La-5 produzidas com trealose apresentaram forma regular e uma superfície ligeiramente lisa. Domian et al. (2015) mostraram caracteristicas semelhantes ao encapsularem óleo com amido de milho (OSA) e trealose em *spray dryer*. Estes autores reportaram que a trealose provocou um enrijecimento na superfície das micropartículas. Para as micropartículas de *Lactobacillus acidophilus* La-5 (ML) e *Bifidobacterium* Bb-12 (MB) também foram observadas forma esférica e presença de concavidades. No entanto, as micropartículas de *Lactobacillus acidophilus* La-5 (ML) apresentaram grande porosidade em sua superfície, enquanto que as micropartículas de *Bifidobacterium* Bb-12 (MB) mostraram rupturas visíveis em sua superfície. Este fato pode estar relacionado com a menor concentração de sólidos utilizada, 12% m/v, para a produção destas micropartículas. As micropartículas contendo as diferentes matrizes encapsulantes (inulina, hi-maize, trealose e amostra controle) foram produzidas a uma concentração de sólidos de 20% m/v. Lian et al. (2002) reportaram que o conteúdo de sólidos no meio de secagem deve ficar entre 20 e 30% para garantir alta viabilidade para microrganismos probióticos.

8 CONCLUSÃO

O estudo das diferentes condições de temperanturas de entrada de ar no *spray dryer* mostraram que a temperatura de entrada de 130 ºC e saída de 76 ± 5 ºC foi a condição que resultou em maior sobrevicência e eficiência de encapsulação para *Lactobacillus acidophilus* La-5 e *Bifidobacterium* Bb-12. Além disso, nesta condição as micropartículas apresentaram valores de atividade de água e umidade dentro do recomendado para garantir maior estabilidade microbiológica.

Ao estudar as diferentes matrizes encapsulantes, hi-maize promoveu maior eficiência de encapsulação para ambos microrganismos estudados. Na avaliação da resistência aos tratamemtos térmicos a trealose forneceu maior proteção para *Lactobacillus acidophilus* La-5 e *Bifidobacterium* Bb-12 em ambas condições estudadas, 63 °C/ 30 min e 72 °C/ 15 s. Nas condições gastrointestinais simuladas o hi-maize promoveu maior proteção para *Lactobacillus acidophilus* La-5 enquanto que para *Bifidobacterium* Bb-12 trealose foi a matriz encapsulante com maior capacidade de proteção. No armazenamento por 120 dias em diferentes temperaturas a trealose promoveu as menores perdas de viabilidade para ambos microrganismos, sendo assim, foi a matriz encapsulante com maior poder de proteção nestas condições.

Dessa forma, estes resultados evidenciam o potencial termoprotetor do hi-maize e trealose e apontam que a utilização destas matrizes encapsulantes na produção de microparticulas por *spray dryer* é uma estratégia efetiva para alcançar maior viabilidade e sobrevivência para *Lactobacillus acidophilus* La-5 e *Bifidobacterium* Bb-12. Por fim, destaca-se que os microrganismos probióticos vem ganhando cada vez mais espaço no mercado e este fato está fortemente relacionado aos avanços científicos nas áreas de nutrição, ciência e tecnologia de alimentos e na microbiologia alimentar. Assim, os resultados deste estudo corroboram com o conhecimento científico e impulsionam o desenvolvimento de produtos funcionais a base de probióticos.

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