DAYANA PEREIRA DE ANDRADE

MICROENCAPSULATION OF *LACTOBACILLUS* STRAINS

LAVRAS – MG
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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós Graduação em Microbiologia Agrícola, para obtenção do título de Doutor.

Prof. Dr. Disney Ribeiro Dias
Orientador

Profa. Dra. Rosane Freitas Schwan
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Coorientadoras

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RESUMO GERAL

A técnica de microencapsulação tem sido uma alternativa para a estabilização de bactérias probióticas, pois a matriz encapsulante fornece uma barreira física contra vários fatores que afetam a viabilidade dos probióticos, entre eles, pH, sais biliares, oxigênio e temperatura de armazenamento. O objetivo do trabalho foi avaliar diferentes matrizes: soro de leite em pó (S), soro em pó com inulina (SI) e soro em pó com maltodextrina (SM) na microencapsulação de duas cepas com potencial probiótico *Lactobacillus brevis* CCMA 1284 e *Lactobacillus plantarum* CCMA0359 por *spray drying*. A viabilidade das cepas microencapsuladas em sucos ácidos e biliares e durante o período de armazenamento à 7 e 25°C por 90 dias foi avaliada. As duas cepas de *Lactobacillus* sp. microencapsuladas com diferentes combinações de matrizes resultaram em alta eficiência de encapsulação (>86 %). As diferentes matrizes mantiveram a viabilidade do *L. plantarum* CCMA0359 acima de 6 log UFC/g a 7 ° C por 90 dias, enquanto que somente as micropartículas do *L. brevis* CCMA 1284 formuladas com S apresentou resultados similares. *L. brevis* CCMA 1284 apresentou maior sensibilidade às condições ácida e biliar do que *L. plantarum* CCMA 0359. A técnica de microencapsulação por *spray drying* foi utilizada com sucesso para microencapsulação das cepas *Lactobacillus* sp.. Com base nos resultados da matriz encapsulante e da cepa, o soro de leite em pó e o *L. plantarum* CCMA 0359 foram selecionados para aplicação das micropartículas em requeijão comercial. A sobrevivência de células livres e microencapsuladas de *Lactobacillus plantarum* CCMA 0359 adicionadas ao requeijão foi determinada durante o período de armazenamento a 7 °C por 90 dias, bem como em condições gastrointestinais simuladas. O efeito da adição da bactéria nas propriedades sensoriais do requeijão também foi avaliado. A população de *L. plantarum* CCMA 0359 (células microencapsuladas e livres) permaneceu em torno de 8 log UFC/g durante todo o tempo de armazenamento, no entanto, as contagens de células microencapsuladas foram ligeiramente superiores às células livres. Sob condições gastrointestinais simuladas, a técnica de microencapsulação permitiu uma proteção significativamente maior das células. A adição de *L. plantarum* CCMA 0359 (células livres e microencapsuladas) ao requeijão não afetou a aceitação do produto pelos consumidores. O requeijão mostrou-se um veículo alternativo para o potencial probiótico *L. plantarum* CCMA 0359, sem afetar a aceitação do produto.

Palavra-chaves: Microencapsulação. Viabilidade. *Lactobacillus* sp.. Alimento funcional

ABSTRACT GENERAL

The microencapsulation technique has been an alternative for the stabilization of probiotic bacteria because the encapsulating matrix provides a physical barrier against several factors that affect the viability of probiotics, including pH, bile salts, oxygen and storage temperature. The objective of this work was to evaluate different matrices: whey powder (W), whey powder with inulin (WI) and whey powder with maltodextrin (WM) in the microencapsulation of two probiotic potential strains *Lactobacillus brevis* CCMA 1284 and *Lactobacillus plantarum* CCMA0359 by spray drying. Viability of the microencapsulated strains in acid and bile juices and during 90 days of storage to 7 and 25 °C were evaluated. The two strains of *Lactobacillus* sp. microencapsulated with different matrix combinations resulted in high encapsulation efficiency (> 86%). The different matrices maintained the viability of *L. plantarum* CCMA0359 above 6 log CFU/g at 7 °C for 90 days, while only the *L. brevis* CCMA 1284 microparticles formulated with S presented similar results. *L. brevis* CCMA 1284 showed greater sensitivity to acid and biliary conditions than *L. plantarum* CCMA 0359. The spray drying microencapsulation technique was successfully used for microencapsulation of *Lactobacillus* sp. strains. Based on the results of the encapsulant matrix and the strain, whey powder and *L. plantarum* CCMA 0359 were selected for application of the microparticles in the requeijão. The survival of free and microencapsulated cells of *Lactobacillus plantarum* CCMA 0359 added in requeijão were determined during storage period at 7 °C for 90 days, as well as in simulated gastrointestinal condition. The effect of the bacteria addition in the sensorial properties of the requeijão was also evaluated. The *L. plantarum* CCMA 0359 population (microencapsulated and free cells) remained around of 8 log CFU/g during all storage time, however, the counts for microencapsulated cells were slightly higher than free cells. The addition of bacteria (microencapsulated and free cells) increased the acidity and decreased the pH of requeijão during storage, and microencapsulated cells showed more pronounced alterations of acidity and pH. Under simulated gastrointestinal condition, the microencapsulation technique allowed a significant higher protection of cells. The addition of *L. plantarum* CCMA 0359 (free and microencapsulated cells) to the requeijão did not affect the acceptance of the product by the consumers. The requeijão showed to be an alternative vehicle for the potential probiotic *L. plantarum* CCMA 0359, without affect the acceptance of the product.

Keywords: Microencapsulation. Viability. *Lactobacillus* sp.. Functional food

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO

Os lactobacilos estão entre as mais importantes bactérias láticas usadas na produção de alimentos e estão ganhando cada vez mais atenção devido as suas propriedades probióticas. De acordo com a Hill et al. (2014) probióticos são microrganismos vivos que quando ingeridos em quantidades adequadas, conferem efeito benéfico para a saúde do hospedeiro. Os potenciais benefícios nutricionais e de saúde levaram a uma maior incorporação de probióticos nos alimentos e ao desenvolvimento de ingredientes e suplementos probióticos (EL-SALAM; EL-SHIBINY, 2015).

A maioria das bactérias probióticas pertence aos gêneros *Lactobacillus* e *Bifidobacterium*, porém espécies pertencentes aos gêneros *Leuconostoc, Lactococcus, Enterococcus, Streptococcus, Pediococcus* e *Propionibacterium*, bem como levedura *Saccharomyces boulardii* também são microrganismos probióticos. Os probióticos podem desempenhar diferentes efeitos benéficos na saúde humana, por exemplo, manutenção da microbiota intestinal, proteção contra patógenos gastrointestinais, redução da intolerância à lactose, prevenção da diarreia, produção de vitaminas, níveis mais baixos de colesterol, estimulação do sistema imune, alívio da constipação, aumento da absorção de minerais, bem como efeitos anti-mutagênicos, anti-carcinogênicos e anti-hipertensivos (AMARA; SHIBL, 2015; HILL et al., 2014; RIBEIRO et al., 2014).

O mecanismo de ação dos probióticos é dependente da cepa e para que os probióticos exerçam os efeitos benéficos, eles devem ser capazes de sobreviver ao ambiente ácido do estômago e consequentemente colonizar o intestino, bem como manter a sobrevivência das células durante o processamento e armazenamento do produto (CAPELA; HAY; SHAH, 2006; PICOT; LACROIX, 2004). Além da sobrevivência no alimento, os probióticos não podem influenciar nas características sensoriais, pois são identificadas como um fator importante para aceitação de alimentos funcionais (URALA; LAHTEENMAKI, 2007).

O grande desafio para utilização de probióticos em produtos alimentícios é manter a viabilidade e a funcionalidade das células durante as condições adversas às quais são expostas. Portanto, a técnica de microencapsulação com um material de parede adequado é uma alternativa promissora para proteger os probióticos durante todo o processamento e comercialização dos alimentos até que eles atinjam o local alvo no trato gastrointestinal. Vários estudos têm utilizado diferentes técnicas de microencapsulação, entre elas, extrusão,

emulsificação e *spray drying*, sendo que a tecnologia para a formulação do pó de probióticos microencapsulados fornece um formato de distribuição mais conveniente em comparação com a formulação em gel, pois podem ser preservados por longo prazo e são utilizados em aplicações de alimentos probióticos (CHÁVARRI et al., 2010; DOLLY et al., 2011; YING et al., 2013; ZHAO et al., 2015).

Com base na importância de aumentar o número de produtos probióticos disponíveis para o mercado consumidor cada vez mais exigente, o objetivo deste estudo foi avaliar diferentes matrizes encapsulantes na microencapsulação das bactérias láticas com potencial probiótico e investigar o efeito da incorporação das micropartículas no requeijão comercial.

2 REFERENCIAL TEÓRICO

2.1 Alimentos funcionais

As indústrias de alimentos têm aumentado a expectativa em produtos que proporcionam uma alimentação mais saudável para o consumidor, entre eles, alimentos funcionais. Esses alimentos não se destinam somente em satisfazer a fome, mas também em fornecer nutrientes necessários, prevenir doenças relacionadas com a nutrição e melhorar o bem-estar físico e mental dos seres humanos (MENRAD, 2003). Na década de 1980, no Japão, foi a primeira vez em que o termo alimento funcional foi utilizado para os produtos alimentares enriquecidos com elementos especiais os quais possuem efeitos fisiológicos benéficos.

Os alimentos funcionais podem ser encontrados praticamente em todas as classes de alimentos, porém, os produtos não são homogeneamente espalhados por todos os segmentos do mercado em crescimento (SIRÓ et al., 2008). A demanda por alimentos funcionais pelos consumidores teve um grande avanço, uma vez que eles estão mais conscientes da influência da dieta na saúde (STANTON et al., 2005).

A propriedade funcional do alimento pode ser apresentada de diferentes formas, tais como produto fortificado (com nutrientes adicionais), enriquecido (adição de novos nutrientes que normalmente não são encontrados em um determinado alimento), alterado (um componente prejudicial é removido, reduzido ou substituído por outro com efeitos benéficos) e melhorado (um dos componentes tem sido naturalmente reforçado através de condições especiais de crescimento, nova composição de alimentação ou manipulação genética) (SPENCE, 2006).

O aumento da concentração, a adição ou a melhoria da biodisponibilidade de um determinado componente no alimento torna-o funcional. Os componentes de alimentos funcionais podem ser de origem animal ou vegetal, entre eles, probióticos, prebióticos, fibra solúvel, ácidos graxos poli-insaturados, ômega 3, ácido linoleico, antioxidantes vegetais, vitaminas e minerais, algumas proteínas, peptídeos e aminoácidos e fosfolipídios. Atualmente, os mais utilizados são probióticos e prebióticos, ambos são agentes seguros e eficazes que melhoram a saúde do consumidor, promovendo a microbiota do intestino. O uso de probióticos é uma maneira de repor os níveis de bactérias benéficas no intestino com microrganismos externos. Por outro lado, prebióticos não são digeríveis, permanecem intactos

através do sistema digestivo e servem de alimento para a microbiota já estabelecida no intestino (SANGWAN et al., 2011).

A maioria dos produtos probióticos é classificado como alimentos funcionais. O uso de probióticos com objetivo de aumentar o valor nutricional e terapêutico dos produtos tem crescido nos últimos anos, assim, várias estirpes probióticas têm sido estudadas e comercialmente exploradas (FRANZ et al., 2014). Os probióticos devem ser metabolicamente estáveis e ativos tanto no produto que os veiculam, quanto no hospedeiro, para alcançar os benefícios à saúde (KRASAEKOOPT; WATCHARAPOKA, 2014). A adição de probióticos nos produtos pode influenciar o aroma e o sabor, devido ao metabolismo dessas bactérias durante produção e período de armazenamento, como por exemplo, a produção de ácido acético por *Bifidobacterium* spp. Portanto, a qualidade e as propriedades sensoriais do produto final não podem ser afetadas pela cultura probiótica (MOHAMMADI; MORTAZAVIAN, 2011).

Outro desafio enfrentado pela indústria para produção de um alimento probiótico, é a manutenção da viabilidade das bactérias no alimento durante sua vida útil. A sobrevivência dos probióticos é influenciada por vários fatores, tais como o ambiente do produto (alto teor de água, baixo pH, estresse osmótico) e as condições de armazenamento (temperatura, estresse oxidativo) da maioria dos produtos alimentares, que são condições adversas para as bactérias, ocasionando a redução do número de células viáveis (BURGAIN et al., 2011; HEIDEBACH; FORST; KULOZIK, 2012; PICOT; LACROIX, 2004). Além disso, o benefício dos probióticos para o consumidor é dificultado pelo fato das bactérias encontrarem condições altamente estressantes, como acidez do estômago e pepsina e sais biliares no intestino delgado. Assim, resulta-se em número reduzido de bactérias viáveis que chegam no local alvo de atuação (intestino) (WURTH et al., 2015).

2.2 Bactérias do Ácido Lático

As bactérias do ácido lático (BAL) ou bactérias láticas constituem diverso grupo de microrganismos que são encontrados em dois filos: Firmicutes e Actinobacteria. Dentro do filo Firmicutes, as BAL pertencem à ordem *Lactobacillales* e incluem, entre outros, os seguintes gêneros: *Aerococcus*, *Alloiococcus*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Symbiobacterium*, *Tetragenococcus*, *Vagococcus* e *Weissella*. Dentro do filo Actinobacteria, as BAL pertencem aos gêneros *Atopobium* e *Bifidobacterium* (KLAENHAMMER et al., 2005; PFEILER;

KLAENHAMMER, 2007). Contudo, os gêneros *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus* e *Streptococcus* são os que formam a essência do grupo (SALMINEN; WRIGHT, 1998).

A classificação das BAL, em diferentes grupos, baseia-se no modo de fermentação em combinação com características morfológicas e fisiológicas, como faixas de temperatura para padrões de crescimento, capacidade de crescer a concentrações elevadas de sal, tolerância ácida ou alcalina e utilização de açúcares (MOTTA; GOMES, 2015).

BAL são gram-positivas, catalase negativas, anaeróbias aerotolerantes, não esporulantes, geralmente motilidade negativa, ácido-tolerantes, não reduzem nitrito, mesófilas e algumas termófilas, bacilos ou cocos (em cadeias ou individuais) e com um complexo de requerimento nutricional por aminoácidos e vitaminas. O ácido lático é o principal produto final durante a fermentação de carboidratos. Produzem um grande número de enzimas glicolíticas, lipolíticas e proteolíticas, que transformam os nutrientes fundamentais dos alimentos em compostos com propriedades sensoriais desejáveis (LIMA et al., 2009; PFEILER; KLAENHAMMER, 2007). BAL podem ser enquadradas em dois grupos distintos quanto ao modo de degradação dos carboidratos, sendo os grupos discriminados quanto aos produtos finais da fermentação. As homofermentativas produzem principalmente ácido lático a partir da degradação da glicose. As heterofermentativas, por sua vez, além do ácido lático, produzem outros compostos como o etanol, ácido acético e dióxido de carbono a partir da degradação da glicose (REDDY et al., 2008).

As BAL são encontradas em diversos habitats, desde que os substratos de carboidratos estejam disponíveis. Plantas, animais, produtos alimentícios fermentados (lácteos, vinho, cerveja, produtos de carnes e peixes), água, solo e silagem, são, geralmente, fontes nas quais se encontram as BAL. Algumas espécies também ocorrem nos tratos respiratório, intestinal e genital de humanos e animais. A capacidade para colonizar uma variedade de habitats é consequência direta da versatilidade metabólica deste grupo de bactérias (GIRAFFA, 2012; KLAENHAMMER et al., 2005).

Tradicionalmente, as BAL eram chamadas de "organismos que prejudicam o leite", e muitas vezes negativamente associadas à perda de alimentos e alimentos para animais em função da fermentação. No entanto, BAL são cada vez mais consideradas como microrganismos benéficos. BAL são amplamente utilizadas em diversas aplicações industriais, como culturas iniciadoras em indústrias de alimentos fermentados e como probióticos em suplementos alimentares. Quando aplicadas em alimentos, proporciona efeitos

benéficos aos consumidores por meio de suas propriedades funcionais e tecnológicas (KLAENHAMMER et al., 2005; MOTTA; GOMES, 2015).

A utilização das BAL como probióticos é devido ao comprovado benefício provocado no organismo hospedeiro. Capacidade de regular a microbiota intestinal, inibição da adesão de microrganismos patogênicos no trato gastrintestinal (TGI), redução do colesterol sérico, aumento da resposta imune do hospedeiro e, ainda, redução do risco de doenças alérgicas, da intolerância à lactose e da atividade carcinogênica são exemplos comprovados da atividade funcional dos probióticos (ROBERFROID, 2000).

Representantes dos gêneros *Lactobacillus* e *Bifidobacterium* são os mais amplamente utilizados e estudados como bactérias probióticas. As espécies mais conhecidas do gênero *Lactobacillus* consideradas probióticas são *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus delbrueckii, Lactobacillus paracasei, Lactobacillus plantarum, Lactobacillus reuteri, Lactobacillus rhamnosus.* As espécies de bifidobactérias consideradas probióticas são *Bifidobacterium adolescentis, Bifidobacterium animalis, Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium lactis* e *Bifidobacterium longum* (HOLZAPFEL et al., 2001). Entretanto, bactérias do gênero *Leuconostoc, Lactococcus, Enterococcus, Streptococcus, Pediococcus* também são utilizadas e estudadas como bactérias probióticas (SALMINEN; OUWEHAND; ISOLAURI, 1998; STILES; HOLZAPFEL, 1997).

Além das aplicações como probióticas, muitas BAL tem sido utilizadas com outras funções nos alimentos como preservação e segurança, qualidade no aroma, sabor, textura e aumento do valor nutricional. Os gêneros/espécies mais importantes para aplicações industriais são: *Lactobacillus* (leite, carne, vegetal, cereal), *Lactococcus* (leite), *Leuconostoc* (leite, vegetal), *Pediococcus* (carne, vegetal), *Oenococcus oeni* (vinho) e *Streptococcus thermophilus* (leite) (HOLZAPFEL; GEISEN; SCHILLINGER, 1995; KLAENHAMMER et al., 2005; LUCKE, 2000).

Com relação ao aumento do valor nutricional dos alimentos, o uso de culturas funcionais de BAL leva a produção de vitaminas do complexo B como, por exemplo, folato e riboflavina, que aumentam a qualidade do produto final (HOLZAPFEL, 1997; 2002; HUGENHOLTZ; KLEEREBEZEM, 1999; WOUTERS et al. 2002). Contudo, as BAL são empregadas, ao longo de décadas, na conservação de alimentos e utilização como probióticas, sendo amplamente consumidas por humanos e possuindo o status de GRAS (Geralmente considerado como seguro).

2.3 Probióticos

O termo probiótico é de origem grega e significa “para a vida”. Em 1989 Fuller definiu probióticos como suplementos alimentares com microrganismos vivos que beneficiam a saúde do hospedeiro/consumidor, melhorando e mantendo o equilíbrio microbiano intestinal. A definição atualmente mais aceita é que probióticos são microrganismos vivos (bactérias ou leveduras) que, conferem um efeito fisiológico benéfico para o consumidor, quando ingeridos em quantidades adequadas (Hill et al., 2014). A maioria dos microrganismos probióticos é de origem humana ou animal, habitantes normais do trato gastrointestinal, porém, alguns estudos demonstram que em alimentos fermentados espontaneamente também podem encontrar estirpes reconhecidas como probióticos (ALMEIDA et al., 2007; PEREIRA et al., 2012; RAMOS et al., 2010, 2013; SHREZENMEIT; DE VRESE, 2001).

Para que os microrganismos sejam considerados probióticos, devem-se avaliar não apenas suas características benéficas, mas aspectos como segurança e funcionalidade devem também ser levados em consideração. A seleção e a avaliação de potenciais candidatos a probióticos exigem uma abordagem abrangente com múltiplos passos. De modo geral, uma caracterização básica inicial de identificação da estirpe e taxonomia é seguida pela seleção daquelas com propriedades funcionais por meio de ensaios validados *in vitro* e *in vivo* (CHASSARD; GRATTEPANCHE; LACROIX, 2011).

Os testes *in vitro* são úteis para caracterizar as diferentes estirpes e conhecer o mecanismo do efeito probiótico (FAO, 2002). Os testes *in vivo*, é que se confirmam os reais benefícios à saúde do hospedeiro. Os principais testes *in vitro* atualmente utilizados em estudos de estirpes probióticas incluem resistência abaixo pH e bile, resistência a ácidos, adesão às células epiteliais humanas e linhagens celulares, atividade antimicrobiana contra bactérias potencialmente patogênicas, capacidade de reduzir a adesão de patógenos em superfícies e capacidade de aumentar a resistência da barreira epitelial humana e de linhagens celulares (MARAGKOUDAKIS et al., 2006).

Alguns critérios são avaliados para a seleção de estirpes probióticas entre eles, origem, não ser patogênico, não possuírem plasmídeos de resistência à antibióticos de fácil disseminação, exercer um efeito benéfico para a saúde do hospedeiro, sobrevivência às condições impostas pelo ambiente do TGI, funcionalidade e características pró-tecnológicas (CHASSARD; GRATTEPANCHE; LACROIX, 2011). Já os probióticos incorporados em produtos alimentares devem sobreviver ao processamento e aos estresses biológicos, entre

eles, tolerância à estresse de temperatura, pH, bem como estresse oxidativo e osmótico, além de ataque de bacteriófagos de modo que não percam a viabilidade e a funcionalidade. Culturas probióticas não devem ter efeitos adversos sobre o sabor ou o aroma do produto e não devem aumentar a acidez durante o período de vida útil do produto (GEORGIEVA et al., 2009; MILLS et al., 2011).

Os efeitos benéficos dos probióticos sobre a microbiota intestinal humana incluem efeitos antagônicos, competição e efeitos imunológicos, resultando em um aumento da resistência contra patógenos. As bactérias probióticas podem inibir o desenvolvimento de microrganismos patogênicos por excluir ou reduzir a aderência destes nas células do intestino, pela produção de ácidos, bacteriocinas e pela competição por nutrientes (PARVEZ et al., 2006). Desta forma, a utilização de culturas bacterianas probióticas estimula a multiplicação de bactérias benéficas, em detrimento às bactérias prejudiciais, reforçando os mecanismos naturais de defesa do hospedeiro. A atividade antimicrobiana dos lactobacilos difere das bifidobactérias em alguns aspectos: os lactobacilos atuam por meio da produção de ácido lático, peróxido de hidrogênio e bacteriocinas; já as bifidobactérias produzem ácido lático e acético. A produção desses ácidos provoca a redução do pH intestinal, o que restringe ou inibe o crescimento de muitos patógenos (MOLDLER et al., 1990)

Outros benefícios associados aos probióticos são redução da intolerância à lactose, prevenção da diarreia, produção de vitaminas, níveis mais baixos de colesterol, estimulação do sistema imune, alívio da constipação, aumento da absorção de minerais, bem como efeitos anti-mutagênicos, anti-carcinogênicos e anti-hipertensivos (AMARA; SHIBL, 2015; HILL et al., 2014; RIBEIRO et al., 2014). O mecanismo de ação dos probióticos é dependente da cepa e para garantir um efeito benéfico contínuo, os microrganismos probióticos devem ser ingeridos diariamente, em uma concentração mínima de 10^8-10^9 UFC/ml ou g, levando em conta o efeito do armazenamento sobre a viabilidade probiótica. O padrão para qualquer produto com probióticos com alegações de saúde deve obter pelo menos de 10^6 -10^7 UFC/mL ou g de bactérias probióticas viáveis e ser seguro para o consumidor durante o consumo (FAO/WHO, 2002; LUYER et al., 2005).

Os probióticos são administrados oralmente e são disponíveis em várias formas, tais como produtos alimentares, cápsulas, saches ou comprimidos. Nos alimentos estão presentes principalmente nos produtos lácteos fermentados, entre eles, iogurtes, kefir, queijos, leite fermentado e sorvetes o qual podem fornecer nutrientes essenciais, como cálcio, proteína e aumentar sua funcionalidade (FEUCHT; KWAK, 2013). Uma vantagem das fontes alimentares de probióticos, tais como produtos lácteos, é devido alguns fatores que favorece

sua manutenção e apresentam uma visão positiva para os consumidores. Esses fatores incluem, os produtos são mantidos a frio (4°C/8°C), vida útil relativamente curta de 28-35 dias, podem adicionalmente fornecer nutrientes como cálcio e proteína e os regulamentos para aplicações lácteas são prontamente disponíveis (MATTILA-SANDHHOLM et al., 2002; WEICHSELBAUM, 2009).

Além dos produtos lácteos, os probióticos podem ser encontrados em sucos de frutas, barras de cereais, pães, salames, chocolates e patês (MANTZOURIDOU; SPANOU; KIOSSEOGLOU, 2012; RIVERA-ESPINOZA; GALLARDO-NAVARRO, 2010; YING et al., 2013). Contudo, o desenvolvimento bem sucedido de alimentos que contêm probióticos não é um processo simples uma vez que vários aspectos tecnológicos devem ser examinados (CHAMPAGNE; GARDNER; ROY, 2005). As estirpes selecionadas devem apresentar algumas características que incluem, boa multiplicação no alimento, propriedades sensoriais adequadas e estabilidade/viabilidade durante o armazenamento. Além disso, devem ser adequadas para a produção em escala industrial e manter sua funcionalidade durante a produção e armazenamento das culturas liofilizadas ou secas (TRIPATHI; GIRI, 2014).

A sobrevivência dos probióticos é influenciada pelo stress ambiental (oxigênio, pH ácido e ambiente no sistema digestivo) que depende de vários fatores. Esses fatores incluem a cepa utilizada, a interação entre espécies presentes, condições de cultivo, produção de peróxido de hidrogênio (metabolismo bacteriano), acidez final do produto, concentração de ácidos láctico e acético, disponibilidade de nutrientes, promotores e inibidores de crescimento, concentração de açúcar, oxigênio dissolvido e a permeação de oxigênio através da embalagem, inoculação, temperatura de incubação, tempo de fermentação e armazenamento (GREGUREK, 1999; KRASAEKOOPT; BHANDARI; DEETH, 2003).

2.3 Prebióticos

Inicialmente prebiótico foi definido como um ingrediente alimentar não digerível que estimula seletivamente o crescimento e/ou atividade das bactérias no cólon, beneficiando a saúde do hospedeiro. A definição atual é que prebiótico é um ingrediente fermentado seletivamente que confere benefícios na saúde e no bem-estar do hospedeiro, através das alterações específicas, tanto na composição quanto na atividade da microbiota do TGI (GIBSON; ROBERFROID, 1995; GIBSON et al., 2004; GIBSON et al., 2017). As alterações induzidas na composição da microbiota intestinal do hospedeiro (especialmente no cólon) que são responsáveis pelos efeitos benéficos na saúde e não o prebiótico por si só. O efeito de um

prebiótico é indireto porque se alimenta seletivamente um número limitado de microrganismos, principalmente as bactérias dos gêneros bifidobactérias e lactobacilos promovendo, assim, uma modificação seletiva na microbiota intestinal (TEITELBAUM; WALKER, 2002).

O uso de prebióticos tem atraído grande interesse do ponto de vista científico e industrial sendo na maioria das vezes aplicados para oferecer benefício duplo como, melhor qualidade sensorial e composição nutricional mais equilibrada, podendo ser utilizados tanto pelas suas vantagens nutricionais ou propriedades tecnológicas (FRANCK, 2002; ROBERFROID, 2007).

A classificação de um ingrediente alimentar para ser considerado como prebiótico deve apresentar alguns critérios como, resistência à atividade gástrica e hidrólise por enzimas de mamíferos, fermentação pela microbiota intestinal e estimulação seletiva do crescimento e /ou atividade das bactérias intestinais. Diferentes componentes alimentares, entre eles, os oligossacarídeos não digeríveis frutanos e galactanos foram atribuídos por terem atividades prebióticas. Substratos que afetam a composição da microbiota através de mecanismos que não envolvem a utilização seletiva por microrganismos hospedeiros não são prebióticos. Esses substratos incluem antibióticos, minerais, vitaminas e bacteriófagos, que não são substratos de crescimento, embora sua ingestão possa alterar a microbiota e a composição metabólica (GIBSON et al., 2017; ROBERFROID, 2007).

Os prebióticos são de origem vegetal e podem ser encontrados em alimentos naturais, sendo as principais fontes soja, cereais, raiz de chicória, grãos integrais e aveia. Os prebióticos mais utilizados incluem inulina, frutooligossacarídeos (FOS), galactooligossacarídeos (GOS), oligossacarídeos de soja, xilo-oligossacarídeos, pirodextrina, isomalto-oligossacárideos e lactulose. Além desses, pecticoligossacarídeos, lactosacarose, álcoois de açúcar, glico-oligossacarídeos, amido resistente, levanas e polissacarídeos têm sido classificados como prebióticos (GIBSON; ROBERFROID, 1995; ROBERFROID, 2007; SANGWAN et al., 2011).

Entre os prebióticos, inulina e oligofrutose, foram investigados por sua importância biológica em promover benefícios na saúde humana. A inulina é um tipo de polímero de frutose cujas unidades são ligadas por ligações glicosídicas (2-1) e que o grau de polimerização desses frutanos pode chegar até 60 unidades. Esse polímero pode ser hidrolisado em oligofrutose pela inulinase (YANG et al., 2016). Essas fibras prebióticas foram classificadas pela Food and Drug Administration (FDA) como seguras para o consumo

humano e têm sido aplicadas em associação com outros agentes encapsulantes na microencapsulação de cepas probióticas (PINTO et al., 2015; VERRUCK et al., 2018).

Os prebióticos para serem utilizados como ingredientes de alimento funcional, devem ser quimicamente estáveis a tratamentos durante o processamento dos alimentos, como o calor, baixo pH e as condições da reação de Maillard. Isto é, um prebiótico deixaria de proporcionar a seleção de microrganismos benéficos se for degradado em componentes como monossacarídeos e dissacarídeos ou quimicamente alterados de modo que eles não estarão disponíveis para o metabolismo bacteriano (HUEBNER et al., 2008; WANG, 2009). No entanto, o que permite a esses carboidratos chegarem intactos no cólon, resultando em enriquecimento seletivo das bactérias por fornecerem a elas uma vantagem competitiva, é a capacidade de resistirem durante as condições de processamento do alimento (WANG; GIBSON, 1993).

O uso de prebióticos é uma estratégia para aumentar a sobrevivência e o crescimento das bactérias probióticas no ecossistema do TGI humano. Vários trabalhos têm utilizado a estratégia de co-encapsulação de probióticos e prebióticos para aumentar a viabilidade das bactérias probióticas durante a passagem pelo TGI e período de armazenamento do produto (KRASAEKOOPT; WATCHARAPOKA, 2014; VALERO-CASES; FRUTOS, 2015). Os probióticos utilizam os prebióticos como fonte de carbono e energia, resultando no aumento do número de células e a colonização no trato intestinal promovendo modificação da microbiota intestinal e consequentemente efeitos benéficos para o hospedeiro (ANN et al., 2007; OZER; AKIN; OZER, 2005).

Okuro, et al (2013) utilizaram a co-encapsulação do probiótico *Lactobacillus acidophilus* (LAC 04) com a adição de componentes prebióticos, inulina ou polidextrose os quais verificaram maior proteção e melhor estabilidade do probiótico com polidextrose, que aumentou o potencial simbiótico.

Sathyabama et al, (2014), avaliaram o processo de co-encapsulação das cepas probióticas *Staphylococcus succinus* (MAbB4) e *Enterococcus fecium* (FIdM3) com a adição dos prebióticos, beterraba e chicória separadamente. Concluíram que a co-encapsulação ofereceu o potencial aumento na eficácia da sobrevivência dos probióticos. Outros pesquisadores utilizaram o processo de co-encapsulação e perceberam que os prebióticos protegiam as células contra as condições adversas (ETCHEPARE et al., 2016a, 2016b; FRITZEN-FREIRE et al., 2012; VERRUCK et al., 2018).

2.4 Microencapsulação de probióticos

Microencapsulação refere-se a uma tecnologia em que os componentes bioativos, tais como probióticos são completamente envoltos e revestidos por um material de parede encapsulante (matriz) sem qualquer saliência dos componentes bioativos (PICOT; LACROIX, 2004). Essa técnica permite reduzir a lesão celular ou a perda de células retendo as mesmas dentro da membrana encapsulante. Os principais elementos que devem ser considerados com relação ao encapsulamento de probióticos são: mantê-los vivos até atingirem o local alvo e liberação efetiva dos microrganismos aprisionados (EL-SALAM; EL-SHIBINY, 2015; FEUCHT; KWAK, 2013).

A microcápsula compreende uma membrana semipermeável ou não permeável, esférica, fina e forte, envolvendo um núcleo sólido/líquido com tamanhos que variam de alguns micrômetros até alguns milímetros (EL-SALAM; EL-SHIBINY, 2012). Os sinônimos para microcápsulas são microesferas, esferas de gel (se a cápsula tiver uma estrutura semelhante a gel) e micropartículas, uma vez que as microesferas têm normalmente uma forma esférica para elíptica. Rachaduras na superfície das microcápsulas não são incomuns, porém uma extensão dessas rachaduras leva à formação de poros e provavelmente pode reduzir a eficiência da encapsulação (FEUCHT; KWAK, 2013).

A principal finalidade da microencapsulação de probiótico é proteger as células contra um ambiente desfavorável (temperatura, pH, oxigênio e umidade) e permitir sua liberação num estado viável e metabolicamente ativo no intestino para exercer seus efeitos probióticos. As propriedades físico-químicas das cápsulas que incluem tipo e concentração do material encapsulante, tamanho da partícula, número de células iniciais e cepas bacterianas são alguns dos parâmetros que podem afetar a sobrevivência das células microencapsuladas. As cápsulas devem ser formadas no tamanho que não tenha qualquer influência negativa em relação aos aspectos sensoriais do produto alimentício (HEIDEBACH et al., 2012; PICOT; LACROIX, 2004).

Microencapsulação de células fornece alguns benefícios, como proteção contra bacteriófagos e fatores prejudiciais durante os processos de liofilização, congelamento e armazenamento permitindo o aumento da viabilidade celular. As células podem ser convertidas em forma de pó aumentando sua distribuição homogênea em todo o produto por apresentar uma forma fácil de ser utilizada (MORTAZAVIAN et al., 2007; SULTANA et al., 2000). Outras vantagens que a tecnologia apresenta incluem estimulação da produção e a

excreção dos metabólitos secundários e a reutilização contínua. Além disso, pode aumentar a sobrevivência microbiana e a eficiência operacional durante o processo de fermentação (CHAMPAGNE; LACROIX; SODINI-GALLOT, 1994).

Diferentes matrizes são utilizadas para a microencapsulação de probióticos incluindo polímeros naturais ou sintéticos que estão diretamente em contato com a célula viva de modo que deve ser biocompatível e biodegradável. As matrizes encapsulantes mais utilizadas são proteínas (gelatina, proteínas do leite, caseinato de sódio e proteína de soja), carboidratos (emulsionantes (alginato), amidos, lactose e maltodextrina), gomas (goma arábica, xantana). Esses polímeros são todos naturais, biocompatíveis e apresentam o status de GRAS que podem ser usados em aplicações de alimentos (GENTILE et al., 1995; MORTAZAVIAN et al., 2007; NAZZARO et al., 2012).

A escolla dos materias da cápsula é um elemento importante para microencapsulação bem sucedida de probióticos e o uso de probióticos em alimentos funcionais (HUQ et al., 2013). Em geral, as cápsulas devem ser resistentes às condições ácidas e cumprir os requisitos para a liberação de probióticos retidos na parte alvo do trato gastrointestinal. No caso dos probióticos direcionados ao intestino delgado, as matrizes devem ser decompostas após submetê-los ao pH deste intestino ou enzimas pancreáticas, porém para aqueles que visam o intestino grosso, as cápsulas devem ser tolerantes a essas condições (EL-SALAM; EL-SHIBINY, 2015).

Proteínas do leite são bons candidatos para matrizes de encapsulamento, devido as suas propriedades estruturais, físico-químicas e alto valor nutricional. Essas propriedades incluem propriedades de gelificação, interações com outros polímeros para formar complexos, biocompatibilidade e biodegradabilidade (LIVNEY, 2010). Muitas proteínas do leite tal como proteína do soro de leite e proteínas da membrana dos glóbulos de gordura do leite, foram relatadas como materiais de encapsulamento (DOHERTY et al., 2012; SINGH, 2006).

Soro de leite é um subproduto líquido produzido durante a fabricação de queijos e caseína na indústria de laticínios. Como matéria-prima, tem muitas aplicações nas indústrias de alimentos e farmacêutica em função das propriedades funcionais e nutricionais (BALDASSO; BARROS; TESSARO, 2011). Apresenta importância relativa na indústria de lacticínios, por ser produzido em grandes quantidades, baixo custo, facilmente disponível e devido à sua composição nutricional, que é principalmente lactose e proteínas solúveis. É considerado como efluente de laticínios que causa sérios problemas ambientais, pelo fato de sua demanda de oxigênio biológico e químico. Assim, a utilização do soro de leite líquido como matriz de encapsulamento pode reduzir a quantidade de soro de leite descartado como

subproduto, reduzindo os danos ambientais (CARVALHO; PRAZERES; RIVAS, 2013; PINTO et al., 2015).

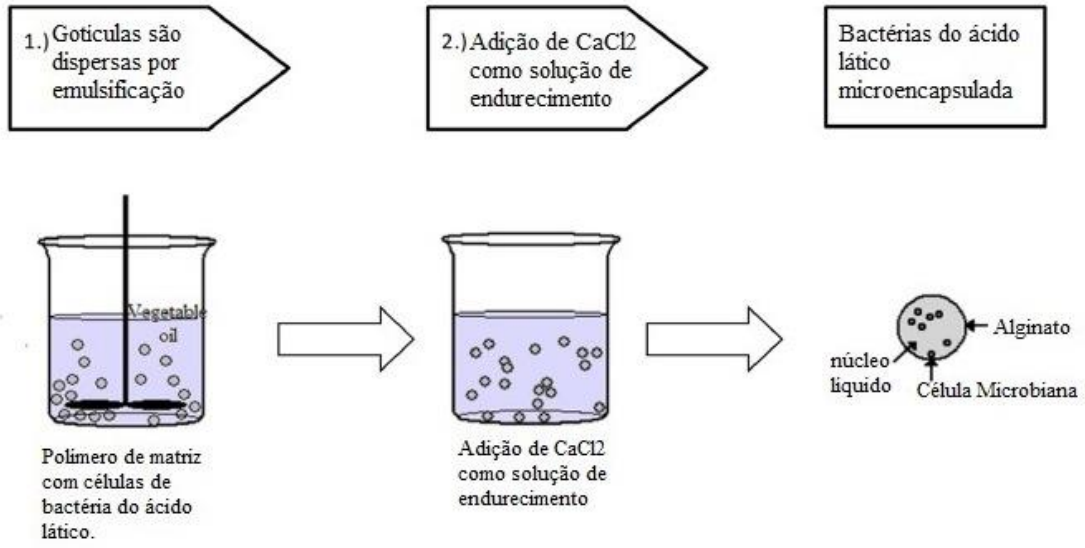
A maltodextrina é um produto obtido a partir da hidrólise do amido constituído por unidades de D-glicose. A sua utilização como material de parede de ingredientes alimentícios tem sido pesquisada, pois oferece vantagens como custo relativamente baixo, aroma e sabor neutros, excelentes propriedades de bloqueio de oxigênio, altamente solúvel e de baixa higroscopicidade. Além disso, na microencapsulação por *spray drying*, a adição de maltodextrina pode aumentar a concentração de sólidos na alimentação e reduzir a umidade do pó produzido (FERNANDES; BORGES; BOTREL, 2014; PHISUT, 2012).

2.4.1 Técnicas de microencapsulação

Diferentes técnicas são utilizadas na microencapsulação de probióticos, entre elas, emulsão, extrusão, *spray drying*, liofilização e *spray chilling*. A transformação de culturas bacterianas em pós secos concentrados foi uma das primeiras tecnologias de encapsulamento para melhorar a vida útil dos alimentos contendo probióticos (GBASSI; VANDAMME, 2012). A seleção do método de microencapsulação é realizada em função do tamanho médio da micropartícula; das propriedades físicas do agente encapsulante; da espécie bacteriana; da aplicação das micropartículas; do mecanismo de liberação; do custo e da eficiência da microencapsulação (ANAL; SINGH, 2007).

A técnica de microencapsulação por emulsão foi desenvolvida no início dos anos 80 para imobilizar células vivas sensíveis e é, portanto, certamente um dos métodos mais aplicados para gerar microcápsulas contendo LAB até hoje. A técnica de emulsão é baseada na relação entre a fase descontínua (solução encapsulante com células probióticas) e a fase contínua (óleo, tais como óleo de soja, óleo de girassol, óleo de canola ou óleo de milho). Fase descontínua é adicionada na fase contínua e a mistura é homogeneizada para formar uma emulsão água em óleo. Após a formação da emulsão, o polímero solúvel em água deve ser insolubilizado para formar pequenas partículas de gel dentro da fase de óleo (FIGURA 1). Esferas geradas durante o processo são recolhidas por filtração ou centrifugação. O tamanho da esfera é controlado pela velocidade de agitação, podendo variar de 25 µm a 2 mm. É uma técnica de fácil realização, apresenta alta taxa de sobrevivência das células bacterianas e pode obter microcápsulas de diâmetro reduzido. No entanto, a principal desvantagem dessa técnica é que não há padronização no tamanho e no formato das microcápsulas (KRASAEKOOPT; BHANDARI; DEETH, 2003; MORTAZAVIAN et al., 2007).

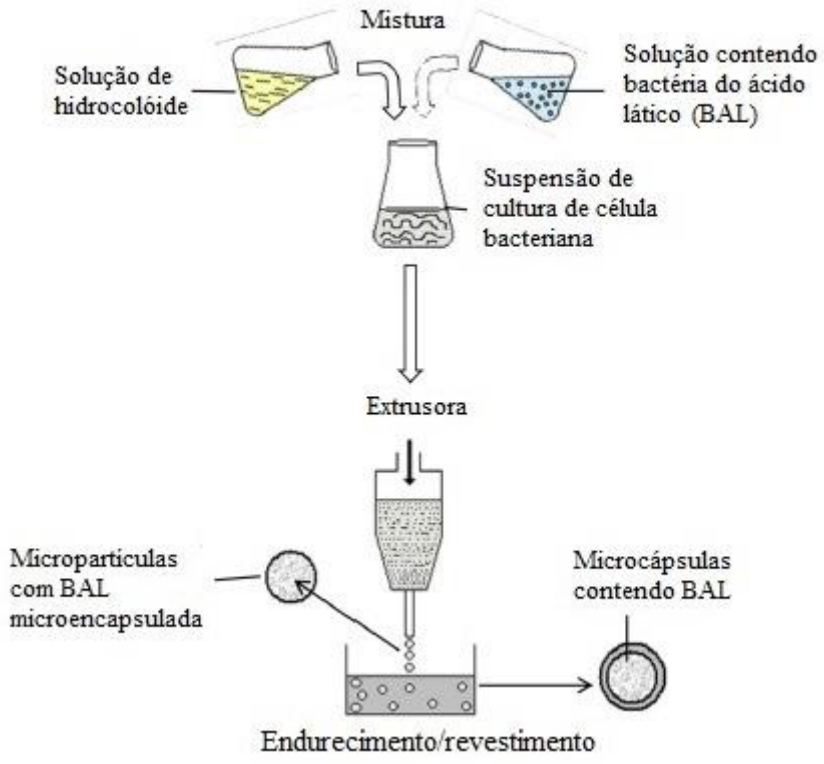
Figura 1 - Microencapsulação pela técnica de emulsão.



Fonte: Feucht and Kwak (2013)

A técnica de extrusão é a abordagem mais antiga e mais comum para produzir cápsulas com hidrocolóides (alginato, carragena e pectina). Essa técnica envolve a preparação de uma solução aquosa de hidrocolóide, com a adição de microrganismos a ela e a extrusão da mistura de células-hidrocolóide ocorre através de uma agulha de seringa sob a forma de gotículas em queda livre em uma solução de endurecimento (FIGURA 2). O tamanho e a forma das esferas dependerão do diâmetro da agulha e a distância de queda livre, respectivamente. Este método é o mais popular devido à sua facilidade, simplicidade, baixo custo e as condições de formulação suave garantindo alta retenção da viabilidade celular. Além disso, a técnica de extrusão é capaz de produzir microcápsulas de forma mais uniforme por ser gerada em comparação com a técnica de emulsão. Porém possui a desvantagem de ser de difícil aplicação em escala industrial, devido à formação das microcápsulas ser muito lenta (BURGAIN et al., 2011; KRASAEKOOPT; BHANDARI; DEETH, 2003; NAZARRO et al., 2012).

Figura 2 - Microencapsulação pela técnica de extrusão



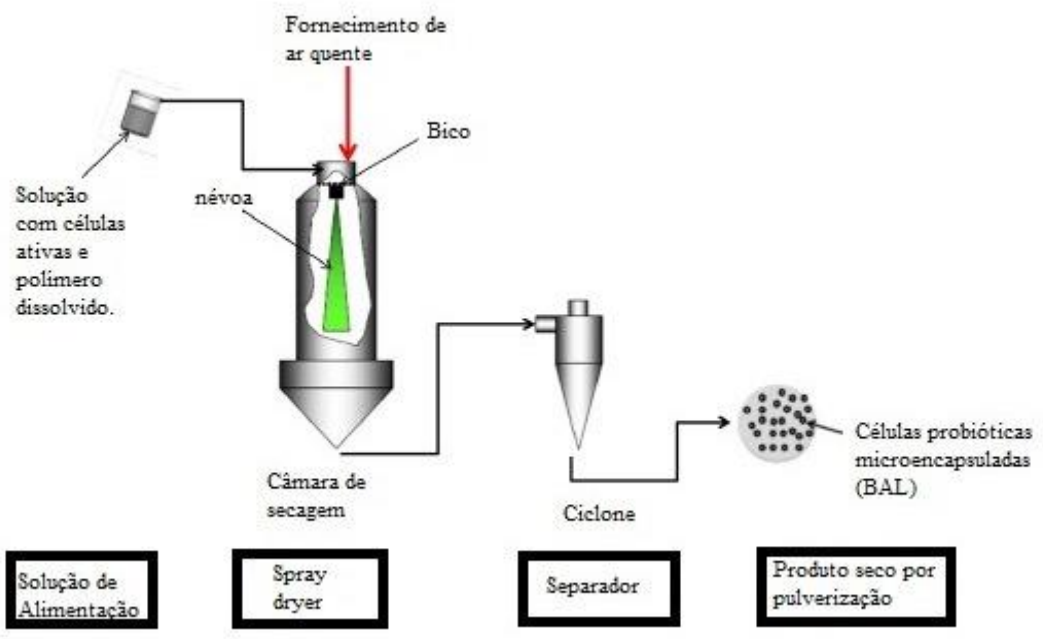
Fonte: Feucht and Kwak (2013).

Na indústria alimentícia, o *spray drying* é um método de microencapsulação comumente aplicado, produzindo grandes quantidades de microcápsulas em uma etapa contínua do processo (KAILASAPATHY, 2002; PICOT; LACROIX, 2004). Este método é muito adequado quando probióticos microencapsulados precisam ser secos para permitir o armazenamento por um longo período. A técnica por *spray drying* (secagem por atomização) consiste em converter uma dispersão líquida (matriz encapsulante e suspensão de células probióticas) num produto seco através da aspersão do líquido em contato com o ar quente numa câmara de secagem com rápida evaporação do solvente (FIGURA 3) (MARTÍN et al., 2015).

No *spray drying* são produzidas micropartículas de diâmetro inferior a 100 μm, a custos reduzidos. Além da rapidez do procedimento, esta técnica é altamente reprodutível e adequada para aplicações industriais. Uma desvantagem é o uso de alta temperatura, que não é compatível com a sobrevivência das bactérias. No entanto, o ajuste e o controle das condições de processamento, tais como temperaturas de entrada e saída podem atingir culturas encapsuladas viáveis com uma distribuição de micropartículas de tamanho desejado. Além disso, podem ser adicionados protetores como amido, fibra solúvel ou trealose no meio antes

da secagem para melhorar a sobrevivência dos probióticos. As micropartículas podem ser revestidas por uma camada adicional, a fim de proteger contra as condições adversas (HEIDEBACH; FORST; KULOZIK, 2012; MARTÍN et al., 2015; SEMYONOV et al., 2010).

Figura 3 - Microencapsulação pela técnica *spray drying*.



Fonte: Feucht and Kwak (2013).

O grande desafio do processo de microencapsulação é selecionar a técnica e o material de encapsulamento adequados. Tamanho das microcápsulas também é uma característica importante; quando as microcápsulas são maiores, podem afetar a textura e propriedades sensoriais dos produtos o qual são adicionadas. Além disso, a liberação das bactérias probióticas microencapsuladas viáveis no local de ação (intestino) é um importante ponto de discussão. Pois diferentes fatores podem interferir na liberação do probiótico microencapsulado, tais como mudanças de pH, estresse mecânico, temperatura, atividade enzimática, tempo e força osmótica). Contudo, a microencapsulação é uma valiosa tecnologia de processo para facilitar a adição de ingredientes, especialmente de probióticos na forma de LAB aos alimentos (SOLANKI et al., 2013; FEUCHT; KWAK, 2013).

CONSIDERAÇÕES FINAIS

As BAL constituem diverso grupo de microrganismos que são usados na produção de alimentos, apresentam o status de GRAS e estão ganhando cada vez mais atenção devido as suas propriedades probióticas. Representantes dos gêneros *Lactobacillus* e *Bifidobacterium* são os mais amplamente utilizados na aplicação industrial. Probióticos são microrganismos amplamente utilizados como suplementos alimentares ou adicionados a produtos alimentícios, no intuito de gerar benefícios à saúde. A busca por esses alimentos teve um grande avanço, uma vez que os consumidores estão mais conscientes da influência da dieta na saúde.

O desafio para a produção de alimentos probióticos é manter a viabilidade das células durante a passagem no trato gastrointestinal e vida útil do produto. As bactérias probióticas são sensíveis ao ambiente ácido e aos sais biliares, temperaturas e estresse oxidativo encontrados durante a passagem no trato gastrintestinal, processamento e armazenamento dos produtos. Esses são os principais obstáculos enfrentados pelas bactérias ingeridas e adicionadas aos produtos para manterem sua sobrevivência e causarem benefício ao hospedeiro. Dentro deste contexto, a técnica de microencapsulação protege as bactérias probióticas quando expostas as condições adversas, pois a matriz de encapsulamento fornece uma barreira física contra as condições ambientais adversas, podendo contribuir para o desenvolvimento de novos alimentos probióticos. Diferentes técnicas e matrizes são utilizadas nesse processo, no entanto a escolha da técnica e da matriz é importante para uma eficiente microencapsulação probiótica.

As cepas probióticas são principalmente adicionadas a produtos lácteos, como iogurtes, kefir, queijos, leite fermentado e sorvetes, que podem fornecer nutrientes essenciais, como cálcio, proteína e aumentar sua funcionalidade. Estes produtos são considerados os principais veículos para o fornecimento de bactérias probióticas ao trato gastrointestinal e possuem algumas vantagens que favorecem a sobrevivência probiótica, incluindo baixa temperatura de armazenamento (4 °C/8 °C) e uma vida útil relativamente curta de 28-35 dias. Além disso, os regulamentos para aplicações em laticínios para produtos probióticos estão facilmente disponíveis.

REFERÊNCIAS

ALMEIDA, E. G.; RACHID, C. C. T. C.; SCHWAN, R. F. Microbial population present in fermented beverage cauim produced by Brazilian Amerindians. **International Journal of Food Microbiology**, v. 120, p. 146–151, 2007.

AMARA, A. A.; SHIBL, A. Role of Probiotics in health improvement, infection control and disease treatment and management. **Saudi Pharmaceutical Journal**, v. 23, n. 2, p. 107-114, 2015.

ANAL, A. K.; SINGH, H. Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. **Trends in Food Science and Technology**, v. 18, n. 5, p. 240–251, 2007.

ANN, E. Y. et al. Microencapsulation of *Lactobacillus acidophilus* ATCC 43121 with prebiotic substrates using a hybridisation system. **International Journal of Food Science and Technology**, v. 42, n. 4, p. 411–419, 2007.

BALDASSO, C.; BARROS, T. C.; TESSARO, I. C. Concentration and purification of whey proteins by ultrafiltration. **Desalination**, v. 278, p. 381–386, 2011.

BURGAIN, J. et al. Encapsulation of probiotic living cells: From laboratory scale to industrial applications. **Journal of Food Engineering**, v. 104, n. 4, p. 467–483, 2011.

CAPELA, P.; HAY, T. K. C.; SHAH, N. P. Effect of cryoprotectants, prebiotics and microencapsulation on survival of probiotic organisms in yoghurt and freeze-dried yoghurt. **Food Research International**, v. 39, n. 2, p. 203–211, 2006.

CARVALHO, F.; PRAZERES, A. R.; RIVAS, J. Cheese whey waste water: Characterization and treatment. **Science of the Total Environment**, v. 445–446, p. 385–396, 2013.

CHAMPAGNE, C. P.; GARDNER, N. J.; ROY, D. Challenges in the addition of probiotic cultures to foods. **Critical reviews in food science and nutrition**, v. 45, n. 1, p. 61–84, 2005.

CHAMPAGNE, C. P.; LACROIX, C.; SODINI-GALLOT, I. Immobilized cell technologies for the dairy industry. **Critical Reviews in Biotechnology**, v. 14, n. 2, p. 1994, 1994.

CHÁVARRI, M. et al. Microencapsulation of a probiotic and prebiotic in alginate-chitosan capsules improves survival in simulated gastro-intestinal conditions. **International Journal of Food Microbiology**, v. 142, n. 1–2, p. 185–189, 2010.

CHASSARD, C.; GRATTEPANCHE, F.; LACROIX, C. Probiotics and health claims: challenges for tailoring their efficacy. In: KNEIFEL, W.; SALMINEN, S. (Ed.). **Probiotics and health claims**. Oxford: Wiley-Blackwell, 2011. p. 49-74.

DOHERTY, S. B. et al. Survival of entrapped *Lactobacillus rhamnosus* GG in whey protein micro-beads during simulated ex vivo gastro-intestinal transit. **International Dairy Journal**, v. 22, n. 1, p. 31–43, 2012.

DOLLY, P. et al. Microencapsulation of *Lactobacillus plantarum* (mtcc 5422) by spray-freeze-drying method and evaluation of survival in simulated gastrointestinal conditions. **Journal of microencapsulation**, v. 28, n. 6, p. 568–574, 2011.

EL-SALAM, M. H. A.; EL-SHIBINY, S. Preparation and properties of milk proteins-based encapsulated probiotics: a review. **Dairy Science & Tecnhology**, v. 95, p. 393-412, 2015.

EL-SALAM, M. H. A.; EL-SHIBINY, S. Formation and potential uses of milk proteins as nano delivery vehicles for nutraceuticals: A review. **Internatinal Journal of Dairy Tecnhology**, v. 65, n. 1, p. 13–21, 2012.

ETCHEPARE, M. DE A. et al. Effect of resistant starch and chitosan on survival of *Lactobacillus acidophilus* microencapsulated with sodium alginate. **LWT - Food Science and Technology**, v. 65, p. 511–517, 2016a.

ETCHEPARE, M. DE A. et al. Effect of resistant starch (Hi-maize) on the survival of *Lactobacillus acidophilus* microencapsulated with sodium alginate. **Journal of Functional Foods**, v. 21, p. 321–329, 2016b.

FAO/WHO. Guidelines for the Evaluation of Probiotics in Food. **London, Ontario, Canada, April 30 and May 1**, p. 1–11, 2002.

FERNANDES, R. V. D. B.; BORGES, S. V.; BOTREL, D. A. Gum arabic/starch /maltodextrin /inulin as wall materials on the microencapsulation of rosemary essential oil. **Carbohydrate Polymers**, v. 101, p. 524–532, 2014.

FEUCHT, A.; KWAK, H. Microencapsulation of Lactic Acid Bacteria (LAB). **Korean Journal Food Sciense**, v. 33, n. 2, p. 229–238, 2013.

FRANCK, A. Technological functionality of inulin and oligofructose. **The British Journal of Nutrition**, v. 87, n. S2, p. S287–S291, 2002.

FRANZ, C. M. A. P. et al. African fermented foods and probiotics. **International Journal of Food Microbiology**, v. 190, p. 84–96, 2014.

FRITZEN-FREIRE, C. B. et al. Microencapsulation of bifidobacteria by spray drying in the presence of prebiotics. **Food Research International**, v. 45, n. 1, p. 306–312, 2012.

GENTILE, F. T. et al. Polymer science for microencapsulation of cells for central nervous system transplantation. **Reactive Polymers**, v. 25, n. 2–3, p. 207–227, 1995.

GEORGIEVA, R. et al. Technological properties of candidate probiotic *Lactobacillus plantarum* strains. **International Dairy Journal**, v. 19, n. 11, p. 696–702, 2009.

GIBSON, G. R. et al. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. **Nutrition Research Reviews**, v. 17, p. 259–275, 2004.

GIBSON, G. R. et al. Expert Consensus Document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. **Nature Reviews Gastroenterology & Hepatology**, v. 14, n. 8, p. 491–502, 2017.

GIBSON, G. R.; ROBERFROID, M. B. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. **The Journal of nutrition**, v. 125, n. 6, p. 1401–1412, 1995.

GIRAFFA, G. Selection and design of lactic acid bacteria probiotic cultures. **Engineering in Life Sciences**, n. 4, p. 391–398, 2012.

GREGUREK, L. Effect of level of starter culture on viability of probiotic bacteria in yoghurts. **Mljekarstvo**, v. 49, n. 1, p. 3–8, 1999.

HEIDEBACH, T.; FORST, P.; KULOZIK, U. Microencapsulation of probiotic cells for food applications. **Critical reviews in Food Science and Nutrition**, v. 52, n. 4, p. 291–311, 2012.

HILL, C. et al. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. **Nature Reviews Gastroenterology & Hepatology**, v. 11, p. 506–514, 2014.

HOLZAPFEL, W. Use of starter cultures in fermentation on a household scale. **Food Control**, v. 8, n. 5/6, p. 241–258, 1997.

HOLZAPFEL, W. H. et al. Taxonomy and importants features of probiotic microorganisms in food and nutrition. **American Society for Clinical Nutrition**, v. 73, p. 365S–373S, 2001.

HOLZAPFEL, W. H. Appropriate starter culture technologies for small-scale fermentation in developing countries. **International Journal of Food Microbiology**, v. 75, p. 197–212, 2002.

HOLZAPFEL, W. H.; GEISEN, R.; SCHILLINGER, U. Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. **International Journal of Food Microbiology**, v. 24, n. 3, p. 343–362, 1995.

HUEBNER, J. et al. Effect of processing conditions on the prebiotic activity of commercial prebiotics. **International Dairy Journal**, v. 18, n. 3, p. 287–293, 2008.

HUGENHOLTZ, J.; KLEEREBEZEM, M. Metabolic engineering of lactic acid bacteria: overview of the approaches and results of pathway rerouting involved in food fermentations. **Current Opinion in Biotechnology**, v. 10, n. 5, p. 492–497, 1999.

HUQ, T.; KHAN, A., KHAN, R. A., RIEDL, B., LACROIX, M. Encapsulation of probiotic bacteria in biopolymeric system. **Critical Reviews in Food Science and Nutrition**, v. 53, p. 909-916, 2013.

KAILASAPATHY, K.; CHIN, J. Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp.. **Immunology and Cell Biology**, v. 78, n. 1, p. 80–88, 2000.

KLAENHAMMER, T. R. et al. Genomic features of lactic acid bacteria effecting bioprocessing and health. **FEMS Microbiology Letters**, v. 29, p. 393–409, 2005.

KRASAEKOOPT, W.; BHANDARI, B.; DEETH, H. Evaluation of encapsulation techniques of probiotics for yoghurt. **International Dairy Journal**, v. 13, n. 1, p. 3–13, 2003.

KRASAEKOOPT, W.; WATCHARAPOKA, S. Effect of addition of inulin and galactooligosaccharide on the survival of microencapsulated probiotics in alginate beads coated with chitosan in simulated digestive system, yogurt and fruit juice. **LWT - Food Science and Technology**, v. 57, n. 2, p. 761–766, 2014.

LIVNEY, Y. D. Milk proteins as vehicles for bioactives. **Current Opinion in Colloid & Interface Science**, v. 15, n. 1, p. 73–83, 2010.

LUCKE, F. Utilization of microbes to process and preserve meat. **Meat Science**, v. 56, n. 2, p. 105–115, 2000.

LUYER, M. D. et al. Strain-Specific effects of probiotics on gut barrier integrity following hemorrhagic shock. **Infection and Immunity**, v. 73, n. 6, p. 3686–3692, 2005.

MALMO, C.; STORIA, A. LA; MAURIELLO, G. Microencapsulation of *Lactobacillus reuteri* DSM 17938 cells coated in alginate beads with chitosan by spray drying to use as a probiotic cell in a chocolate soufflé. **Food and Bioprocess Technology**, 2011.

MANTZOURIDOU, F.; SPANOU, A.; KIOSSEOGLOU, V. An inulin-based dressing emulsion as a potential probiotic food carrier. **Food Research International**, v. 46, n. 1, p. 260–269, 2012.

MARAGKOUDAKIS, P. A. et al. Probiotic potential of *Lactobacillus* strains isolated from dairy products. **International Dairy Journal**, v. 16, p. 189–199, 2006.

MARTÍN, M. J. et al. Microencapsulation of bacteria: A review of different technologies and their impact on the probiotic effects. **Innovative Food Science and Emerging Technologies**, v. 27, p. 15–25, 2015.

MATTILA-SANDHHOLM, T. et al. Technological challenges for future probiotic foods. **International Dairy Journal**, v. 12, p. 173–182, 2002.

MENRAD, K. Market and marketing of functional food in Europe. **Journal of Food Engineering**, v. 56, p. 181–188, 2003.

MILLS, S. et al. Enhancing the stress responses of probiotics for a lifestyle from gut to product and back again. **Microbial Cell Factories**, v. 10, p. 1–15, 2011.

MODLER, H. W.; MCKELLAR, R. C.; YAGUCHI, M. *Bifidobacteria* and Bifidogenic Factors. **Canadian Institute of Food Science and Technology**, v. 23, n. 1, p. 29–41, 1990.

MOHAMMADI, R.; MORTAZAVIAN, A. M. Review Article: Technological aspects of prebiotics in probiotic fermented milks. **Food Reviews International**, v. 27, n. 2, p. 192–212, 2011.

MORTAZAVIAN, A. et al. Principles and methods of microencapsulation of probiotic microorganisms. **Iranian Journal of Biotechnology**, v. 5, n. 1, p. 1–18, 2007.

NAZZARO, F. et al. Microencapsulation in food science and biotechnology. **Current Opinion in Biotechnology**, v. 23, n. 2, p. 182–186, 2012.

MOTTA, A. De S.; GOMES, M. Da S. M. Technological and functional properties of lactic acid bacteria: the importance of these microorganisms for food. **Revista do Instituto de Laticínios Cândido Tostes**, v. 70, n. 3, p. 172-184, 2015.

OKURO, P. K. et al. Co-encapsulation of *Lactobacillus acidophilus* with inulin or polydextrose in solid lipid microparticles provides protection and improves stability. **Food Research International**, v. 53, n. 1, p. 96–103, 2013.

OZER, D.; AKIN, S.; OZER, B. Effect of inulin and lactulose on survival of *Lactobacillus acidophilus* LA-5 and *Bifidobacterium bifidum* BB-02 in acidophilus-bifidus yoghurt. **Food Science and Technology International**, v. 11, n. 1, p. 19–24, 2005.

PARVEZ, S. et al. Probiotics and their fermented food products are beneficial for health. **Journal of Applied Microbiology**, v. 100, n. 6, p. 1171–1185, 2006.

PEREIRA, G. V. DE M. et al. Cocoa fermentations and screening of yeast and bacterial strains to develop a defined starter culture. **Applied and Environmental Microbiology**, v. 78, n. 15, p. 5395–5405, 2012.

PFEILER, E. A.; KLAENHAMMER, T. R. The genomics of lactic acid bacteria. **TRENDS in Microbiology**, v. 15, n. 12, p. 546–553, 2007.

PHISUT, N. Spray drying technique of fruit juice powder: some factors influencing the properties of product. **International Food Research Journal**, v. 19, n. 4, p. 1297– 1306, 2012.

PICOT, A.; LACROIX, C. Encapsulation of bifidobacteria in whey protein-based microcapsules and survival in simulated gastrointestinal conditions and in yoghurt. **International Dairy Journal**, v. 14, n. 6, p. 505–515, 2004.

PINTO, S. S. et al. Influence of microencapsulation with sweet whey and prebiotics on the survival of *Bifidobacterium*-BB-12 under simulated gastrointestinal conditions and heat treatments. **LWT - Food Science and Technology**, v. 64, n. 2, p. 1004–1009, 2015.

RAMOS, C. L. et al. Determination of dynamic characteristics of microbiota in a fermented beverage produced by Brazilian Amerindians using culture-dependent and culture-independent methods. **International Journal of Food Microbiology**, v. 140, n. 2–3, p. 225–231, 2010.

RAMOS, C. L. et al. Strain-specific probiotics properties of *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Lactobacillus brevis* isolates from Brazilian food products. **Food Microbiology**, v. 36, n. 1, p. 22–29, 2013.

REDDY, G. et al. Amylolytic bacterial lactic acid fermentation — A review. **Biotechnology Advances**, v. 26, n. 1, p. 22–34, 2008.

RIBEIRO, M. C. E. et al. Effect of microencapsulation of *Lactobacillus acidophilus* LA-5 on physicochemical, sensory and microbiological characteristics of stirred probiotic yoghurt. **Food Research International**, v. 66, p. 424–431, 2014.

RIVERA-ESPINOZA, Y.; GALLARDO-NAVARRO, Y. Non-dairy probiotic products. **Food Microbiology**, v. 27, n. 1, p. 1–11, 2010.

ROBERFROID, M. Prebiotics: The Concept Revisited.**The Journal of nutrition**, v. 137, n. 1, p. 830S–837S, 2007.

ROBERFROID, M. B. Prebiotics and probiotics: are they functional foods? **American Journal of Clinical Nutrition**, v. 71, n. 6, p. 1682S–1687S, 2000.

SALMINEN, S.; OUWEHAND, A. C.; ISOLAURI, E. Clinical applications of probiotic bacteria. **International Dairy Journal**, v. 8, n. 5–6, p. 563–572, 1998.

SALMINEN, S.; WRIGHT, A. VON. Current Probiotics – Safety Assured? **Microbial Ecology in Health and Disease**, v. 10, n. 2, p. 68-77, 1998.

SANGWAN, V. et al. Galactooligosaccharides: novel components of designer foods. **Journal of Food Science**, v. 76, n. 4, 2011.

SATHYABAMA, S. et al. Co-encapsulation of probiotics with prebiotics on alginate matrix and its effect on viability in simulated gastric environment. **LWT - Food Science and Technology**, v. 57, n. 1, p. 419–425, 2014.

SEMYONOV, D. et al. Microencapsulation of *Lactobacillus paracasei* by spray freeze drying. **Food Research International**, v. 43, n. 1, p. 193–202, 2010.

SINGH, H. The milk fat globule membrane — A biophysical system for food applications. **Current Opinion in Colloid & Interface Science**, v. 11, p. 154–163, 2006.

SIRÓ, I. et al. Functional food. Product development, marketing and consumer acceptance-A review. **Appetite**, v. 51, n. 3, p. 456–467, 2008.

SOLANKI, H. K. et al. Development of microencapsulation delivery system for long-term preservation of probiotics as biotherapeutics agent. **BioMed Research International**, v. 2013, 2013.

SPENCE, J. T. Challenges related to the composition of functional foods. **Journal of Food Composition and Analysis**, v. 19, p. S4–S6, 2006.

STANTON, C. et al. Fermented functional foods based on probiotics and their biogenic metabolites. **Current Opinion in Biotechnology**, v. 16, p. 198–203, 2005.

STILES, M. E.; HOLZAPFEL, W. H. Lactic acid bacteria of foods and their current taxonomy. **International Journal of Food Microbiology**, v. 36, n. 1, p. 1–29, 1997.

SULTANA, K. et al. Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. **International Journal of Food Microbiology**, v. 62, n. 1–2, p. 47–55, 2000.

TEITELBAUM, J. E.; WALKER, W. A. Nutritional impact of pre- and probiotics as protective gastrointestinal organisms. **Annual Review Nutrition**, v. 22, p. 107–138, 2002.

TRIPATHI, M. K.; GIRI, S. K. Probiotic functional foods: Survival of probiotics during processing and storage. **Journal of Functional Foods**, v. 9, n. 1, p. 225–241, 2014.
URALA, N.; LAHTEENMAKI, L. Consumers' changing attitudes towards functional foods. **Food Quality and Preference**, v. 18, p. 1–12, 2007.

VALERO-CASES, E.; FRUTOS, M. J. Effect of different types of encapsulation on the survival of *Lactobacillus plantarum* during storage with inulin and in vitro digestion. **LWT - Food Science and Technology**, v. 64, n. 2, p. 824–828, 2015.

VERRUCK, S. et al. Effect of full-fat goat's milk and prebiotics use on *Bifidobacterium* BB-12 survival and on the physical properties of spray-dried powders under storage conditions. **Food Research International**, v. 119, p.643-652, 2018.

WANG, X.; GIBSON, G. R. Effects of the in vitro fermentation of oligofructose and inulin by bacteria growing in the human large intestine. **The Journal of applied bacteriology**, v. 75, n. 4, p. 373–380, 1993.

WANG, Y. Prebiotics: Present and future in food science and technology. **Food Research International**, v. 42, n. 1, p. 8–12, 2009.

WEICHSELBAUM, E. Probiotics and health: A review of the evidence. **Nutrition Bulletin**, v. 34, n. 4, p. 340–373, 2009.

WOUTERS, J. T. M. et al. Microbes from raw milk for fermented dairy products. **International Dairy Journal**, v. 12, n. 2/3, p. 91–109, 2002.

YANG, J.-K. et al. Genetic modification and optimization of endo-inulinase for the enzymatic production of oligofructose from inulin. **Journal of Molecular Catalysis B: Enzymatic**, v.134, p. 225–232, 2016.

YING, D. et al. Microencapsulated *Lactobacillus rhamnosus* GG in whey protein and resistant starch matrices: Probiotic survival in fruit juice. **Journal of Functional Foods**, v. 5, n. 1, p. 98–105, 2013.

ZHAO, M. et al. Microencapsulation of *Lactobacillus acidophilus* CGMCC1.2686: Correlation between bacteria survivability and physical properties of microcapsules. **Food Biophysics**, v. 10, n. 3, p. 292–299, 2015.

SEGUNDA PARTE – ARTIGOS

ARTIGO 1

STABILITY OF MICROENCAPSULATED LACTIC ACID BACTERIA UNDER ACIDIC AND BILE JUICE CONDITIONS

Artigo aceito para publicação na Revista International Journal of Food Science and Technology

Abstract

The probiotic strains *Lactobacillus brevis* CCMA1284 and *Lactobacillus plantarum* CCMA0359 were microencapsulated by spray drying using different matrices: whey powder (W), whey powder with inulin (WI) and whey powder with maltodextrin (WM). Viability of the microencapsulated strains in acid and bile juices and during 90 days of storage (7 and 25 °C) was evaluated. The two strains showed high encapsulation efficiency (> 86%) by spray drying. The different matrices kept *L. plantarum* viability above of 6 log CFU/g at 7 °C for 90 days, whereas only W showed similar results for *L. brevis*. The use of inulin as matrix of encapsulation did not enhance bacterial viability in the evaluated conditions. In general, the use of W and WM as matrices were effective for *L. plantarum* viability, while only W was effective for *L. brevis* for the evaluated conditions. Spray drying technique was successfully used for the encapsulation of *L. plantarum* CCMA0359 and *L. brevis* CCMA1284 strains.

Keywords: spray drying**,** viability, *Lactobacillus brevis*, *Lactobacillus plantarum*, milk proteins

1. Introduction

Probiotics are "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Hill *et al.*, 2014). The most of the probiotic strains are from human or animal source, normal inhabitants of the gastrointestinal tract (GIT), however several probiotic microorganisms have been isolated from spontaneously fermented foods. Spontaneous fermentations such as cocoa fermentation and indigenous fermented foods are rich sources of microorganisms that may present great a great potential for industrial and health application. The strains *Lactobacillus plantarum* CCMA0359 and *L. brevis* CCMA1284 were previously isolated and characterized in regarding their probiotic potential and will be employed in the present work (Ramos *et al*., 2013).

To be used as probiotic, the bacterial strains must survive stressful conditions such as the acid environment, bile salts, high temperatures, moisture and oxidative stress imposed during passage through the gastrointestinal tract, processing and storage of the products using as probiotic vehicle (Barbosa and Teixeira, 2016). Microencapsulation technology is a promising proposal to increase the viability of probiotic strains during adverse environmental conditions and provide a more favorable anaerobic environment for sensitive probiotic bacteria. This technology employs an encapsulating matrix that protects cells during food storage and allows the release of probiotic bacteria into a viable and metabolically active state in the intestine (Picot and Lacroix, 2004; Martín *et al.*, 2015). Among the microencapsulation techniques of probiotic cells, it is highlighted that spray drying is a highly efficient and reproducible technique, with relatively low cost, producing powder with low moisture content and suitable for industrial applications (Martín *et al*., 2015).

The microencapsulation matrices include natural or synthetic polymers that are directly in contact with the living cell and must be biocompatible, biodegradable and safe (Nazzaro *et al*., 2012). Milk proteins are important candidates of encapsulation matrices due to high nutritional value in addition to their structural and physicochemical properties such as gelling, interactions with other polymers to form complexes, biocompatibility and biodegradability. Milk proteins comprise of casein, whey proteins and milk globule membrane proteins. Among them, whey protein has been widely studied for the encapsulation of lactic acid bacteria (LAB) (Livney, 2010).

The prebiotics use is a strategy to increase the survival and growth of probiotic bacteria in the human GTI ecosystem. Probiotic strains use prebiotics as a source of nutrients and energy, resulting in increased numbers of cells and colonization of intestinal tract which

promote modification of the intestinal microbiota and consequently beneficial effects to the host (Gibson, *et al.*, 2017).

The encapsulation technique and matrix selection are of great importance to improve and maintain the viability of probiotic cells during stressful conditions such as food processing and storage, in addition to adverse GIT conditions, allowing the microorganism to reach the intestine in viable populations and adequate to perform their beneficial functions. Several food products such as yogurt, cheese, chocolate, ice cream and juice may be used as carriers of microencapsulated probiotics adding functional value to the product. The aim of this work was to study the effect of different matrices: whey powder, whey powder added with prebiotic inulin, and whey powder added with maltodextrin in microencapsulation by spray drying of two strains previously recognized as probiotics (*L. plantarum* and *L. brevis*). Survival of the microencapsulated strains was evaluated in acid and bile juices conditions and during the storage period at 7 and 25 ° C.

2. Material and Methods

2.1. Microorganisms and culture conditions

The strains *L. plantarum* CCMA0359 and *L. brevis* CCMA1284 previously characterized as potential probiotics (Ramos *et al*., 2013) and belonging to the Culture Collection of Agricultural Microbiology (CCMA) of the Federal University of Lavras (Lavras, Brazil) were employed in this study. The bacteria cells were cultured in MRS broth (Himedia, Mumbai, India) at 37 °C for 12 and 21 h, respectively, and were subcultured two more times, at same conditions, for each assay. For the assays, the bacteria were recovered by centrifugation at 7100x *g*, 4 °C for 10 min, washed twice and resuspended in 0.1% (w / v) peptone water.

2.2. Microencapsulation by spray drying

The feed solutions were prepared according to Fritzen-Freire *et al*. (2012), with modifications. Three different encapsulating matrices were used in the final concentration of 20% (w/v): whey powder (Laticínios Porto Alegre, Mutum, MG, Brazil) (W); whey powder and inulin (degree of polymerization> 10, Orafti®GR, BENEO-Orafti, Tienen, Belgium) (WI, 1:1 ratio); and whey powder and maltodextrin (Maltogil DE10, Cargill, São Paulo, Brazil)

(WM, 1:1). All feed solutions were homogenized in sterile distilled water, pasteurized (75 °C for 30 min) and cooled (4°C until use). Then, 100 mL/L of the suspension of each bacterium (10^8 CFU/mL) were separately added to each feed solution (W, WI and WM). The two LAB strains were microencapsulated in a pilot scale spray dryer (model MSD 1.0; Labmaq do Brasil, Ribeirão Preto, Brazil), at constant air inlet temperature at 150 °C and outlet temperature at 80 °C. The feed solutions were maintained under magnetic stirring at room temperature (approximately 24 °C) and fed into the main chamber through a peristaltic pump, with feed flow of 0.7 mL/min and compressed air flow of 35 L/min. The resulting powder (microcapsules) was collected at the base of the cyclone and stored in sterile sealed vials which were kept at 4 °C for further analysis.

2.3. Microcapsules characterization

2.3.1. Particle morphology and size

The morphology and size of the microparticles were evaluated using a scanning electron microscope (SEM) (Jeol model JSM 6360 LV) with an acceleration voltage of 20 kV. The microparticles were mounted on aluminum bases, using double-sided adhesive tape and were coated with gold using a vacuum spraying applicator. The microparticles diameter was measured by SEM micrographs at their original magnification using the AxionVision Microcopy Software - Zeiss. The diameter of 225 particles of each different formulation was registered (Etchepare *et al*., 2016).

2.3.2. Moisture content

The percentage of microparticles moisture was determined using a halogen moisture analyzer (Model IV 3000; Ind. Com. Eletro-Eletronica Gehaka, Brazil).

2.4. Encapsulation efficiency of LAB strains

The encapsulated bacteria were released according to Kim *et al*. (2008). One gram of the powder was resuspended in 9 mL of phosphate buffer (0.1 M, pH 7.0) and homogenized for 10 min on magnetic stirrer. The viable cell count was evaluated by plating the cultures on MRS agar and incubated at 37 °C for 48 h. Encapsulation efficiency (EE) was calculated as

follows: EE = (N/N_0) x 100. Where N is the number of viable (log CFU/g) of dry matter in powder and N_0 is the number of viable cells (log CFU/g) of dry matter in the feed solutions (before spray drying) (Picot and Lacroix, 2004; Verruck *et al.*, 2017). The evaluation was performed in triplicate.

2.5. Evaluation of viability of encapsulated LAB during storage at 7 and 25 °C

The viability of the two LAB strains was monitored for 90 days of microparticles storage at 7 and 25 °C. The microparticles (30 g) were stored in 50 mL plastic tubes which were rapidly opened to withdraw the samples for analysis. The experiment was carried out in triplicate. Viability was determined by plating on MRS agar.

2.6. Survival of LAB strains in acid and bile juices

The survival of the two probiotic LAB strains in in acid and bile juices was evaluated according to Dolly *et al.* (2011), with modifications. The encapsulated cells (1 g) and/or free cells (1 mL) were inoculated in 9 mL of MRS broth containing 0.3% (v/v) pepsin (3000 μg/g, Sigma-Aldrich Co., St. Louis, MO) and pH was adjusted to 2.0 with 1M HCL, and incubated at 37 °C for 2 h at 100 rpm. After 0, 1 and 2 h of incubation the viable cell contents were quantified by pour plate method using MRS agar. After 2 hours of incubation in acid juice, the cells were recovered by centrifuged at 7100x *g*, 4 °C for 10 min and inoculated in 10 mL of bile juice. For preparation of bile juice, 0.3% (v/v) oxgall (Himedia, Mumbai, India) was added in MRS broth and pH was adjusted to 7.5 with 1 M NaOH. Cultures were incubated at same conditions. After 0, 1 and 2 h of incubation the number of viable cells was quantified by pour plate method using MRS agar. Free cells (without encapsulations) were used as a control.

2.7. Statistical analysis

The results were expressed as mean of three replicates. Data were submitted to analysis of variance (ANOVA) and significant differences between means values were determined by the Scott-Knott test at 5% level of significance using Sisvar software version 5.6.

3. Results and Discussion

3.1. Microparticles characterization

The microparticles of *L. brevis* and *L. plantarum* produced by spray drying using different matrices were evaluated for moisture content after drying, morphology and particle size by SEM. The microparticles presented similar moisture content ($p > 0.05$) among the different matrices, with mean values of 4.51%, 4.53% and 5.00% for W, WM and WI, respectively, values recommended to guarantee the stability of the cells during the storage process (Chávez and Ledeboer, 2007). Values of moisture ranging from 3 to 6 % are considered common for this type of process. Verruck et al. (2018), which used full-fat goat's milk powder with or without inulin found values around 3.51%, while Maciel et al., 2014 using sweet whey powder and powdered milk, and Rajam and Anandharamakrishnan (2015) using serum protein isolate and prebiotic (FOS) as wall materials observed higher moisture of microparticles (4 – 5 %), similar to those found in the present work.

Fig. 1 shows SEM micrographs of the microparticles of the two strains of *Lactobacillus* produced by different combinations of encapsulating matrices. The SEM showed that the microparticles produced by spray drying presented spherical shape, different diameters and there was no evident presence of free bacteria, confirming the maintenance of the microorganisms encapsulated inside the matrix for all the different combinations of encapsulant. The microparticles produced with S showed the smooth surface, while the microparticles formulated with WI and WM showed concavities, shrunken and wrinkled surface and some cracks. The formation of concavities on the surface of the atomized particles can be attributed to shrinkage during the drying process, which is typical characteristic of atomized products (Pinto *el al*., 2015b). According to Ré (1998), the formation of wrinkles or concavities occurs when there is slow film formation during the drying of the atomized droplets. Similar morphology was observed by Fritzen-Freire *et al*. (2012) in microparticles of *Bifidobacterium* BB-12 with commercial skimmed milk powder and prebiotics as wall material for spray drying and also by Pinto *et al*. (2015b) and De Castro-Cislaghi *et al*. (2012), on microparticles of *Bifidobacterium* BB-12 produced by spray drying using liquid whey. In addition, there is no occurrence of crack in the structure of the microparticles in any of treatments. This fact contributes to a greater protection of microencapsulated microorganisms.

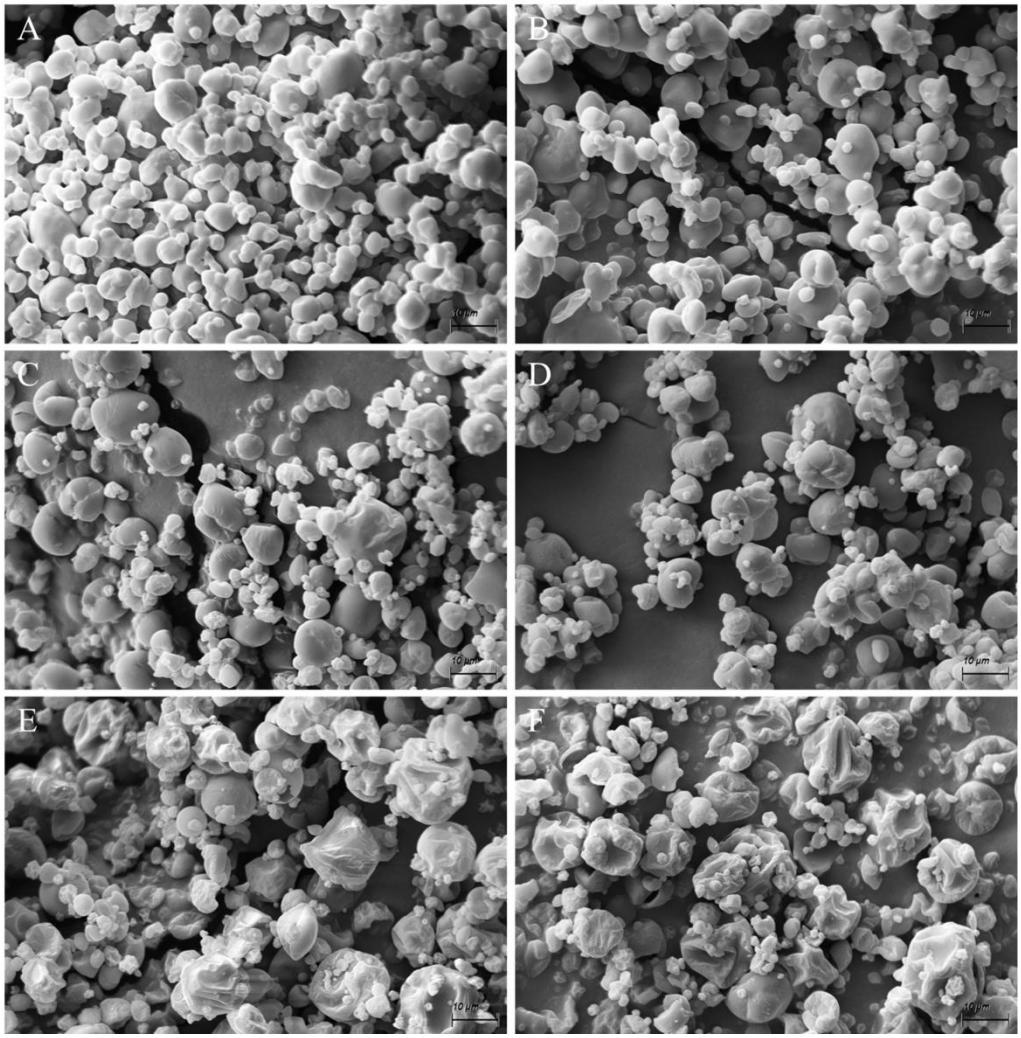


Fig. 1: SEM micrographs of the microparticles of *L. brevis* CCMA1284 (A, C, E) and *L. plantarum* CCMA0359 (B, D, F). Cells encapsulated with W (A, B), WI (C, D) and WM (E, F). Whey powder (W), whey powder with inulin (WI) and whey powder with maltodextrin (WM).

Microparticle size is an important quality parameter because size can be correlated with the degree of protection of cells during adverse conditions (Bustamante *et al*., 2017). The different combinations of encapsulating matrices and the strains of *Lactobacillus* affected significantly ($p< 0.05$) the mean size of the microparticles. The size of the microparticles produced with WM was higher ($p< 0.05$) when compared to the microparticles of W and WI. The microparticles of *L. plantarum* CCMA0359 produced with W and WI were larger than the microparticles of *L. brevis* CCMA1284 in the same matrices. The average of *L. plantarum* CCMA0359 microparticle size was 7.20, 7.28 and 7.38 μm and of *L. brevis* CCMA1284 was 6.74, 6.86 and 7.41 μm for the microparticles produced with W, WI and WM, respectively. According to Annan *et al.* (2008), diameters smaller than 100 μm are required for most of industrial applications.

3.2. Encapsulation efficiency of LAB strains

The bacterial count for the two strains of *Lactobacillus* microencapsulated with different matrices was evaluated before and after drying (Table 1). The concentration of bacterial cells in the powder produced by spray drying ranged from 6.91 - 7.14 log CFU/g for *L. brevis* CCMA1284, and 7.29 - 7.85 log CFU/g for *L. plantarum* CCMA0359, showing probiotic cells population above of the minimum recommended level (6.0 log CFU/g) by FAO/WHO (2006). These data show that the technique was satisfactory for microencapsulation of bacteria, even using a very high temperature of 150 °C during the encapsulation procedure. There was interaction between the strains of *Lactobacillus* sp. and the encapsulating matrices ($p < 0.05$) regarding the counts after drying. The microparticles of *L. plantarum* CCMA0359 produced with W and WM showed higher counts after drying when compared to the microparticles produced with WI and also of *L. brevis* CCMA1284 microparticles produced with the three different matrices. This fact suggests that *L. brevis* CCMA1284 was more sensitive to the spray drying conditions used in the present study.

Table 1. Viable cells count before (N_0) and after (N) drying by spray drying, encapsulation efficiency (EE), moisture (%) and size (μm) of microparticles encapsulated with different matrices combination.

Strains	Matrices	Viable cells (log CFU/g)		EE (%)	Moisture (%)	Size (μm)
		N_0	N			
	W	8.09± 0.18[a]	7.14± 0.14[b]	88.3± 3.35[a]	4,83± 0.05[a]	6,74± 0.30[b]
L. brevis	WI	8.09± 0.12[a]	6.98± 0.13[b]	86.3± 0.68[a]	4,73± 0.05[a]	6,86± 0.12[b]
CCMA1284	WM	8.01± 0.10[a]	6.91± 0.14[b]	86.3± 2.77[a]	4,90± 0.65[a]	7,41± 0.30[a]
L.	W	8.33± 0.28[a]	7.46± 0.21[a]	89.7± 4.62[a]	4,23± 0.25[a]	7,20± 0.05[b]
plantarum	WI	8.26± 0.30[a]	7.29± 0.20[b]	88.3± 4.37[a]	4,30± 0.05[a]	7,28± 0.07[b]
CCMA0359	WM	8.32± 0.22[a]	7.85± 0.20[a]	94.3± 4.85[a]	5,10± 0.30[a]	7,38± 0.19[a]

Means ± standard deviation followed by same letter in the column did not differ ($p< 0,05$) by Scott-Nott test. Whey powder (W), whey powder with inulin (WI) and whey powder with maltodextrin (WM).

The influence of the encapsulation procedure on the number of viable cells after the drying process was expressed in terms of the encapsulation efficiency (Table 1). The results showed that there was non-significant difference (p>0.05) between the encapsulating matrices and also between the *Lactobacillus* strains in the survival of the cells during spray drying. All combinations of wall materials resulted in high encapsulation efficiency, around 89, 87 and 90% for W, WI and WM, respectively. Although the number of viable cells of *L. plantarum* was higher (p< 0.05) when using the W and WM matrices, there was no difference (p> 0.05) in the encapsulation efficiency when using the prebiotics for both bacteria, showing that the different matrices did not affect the survival of the bacteria during the drying process. Similar results were observed by Pinto *et al*. (2015b) who verified that the presence of prebiotic inulin or polyhydrextrose in the encapsulating matrix (liquid whey) did not increase the *Bifidobacterium* BB-12 count after drying.

Although no statistical differences were found between matrices containing or not containing prebiotic, it is known that the encapsulating matrix is largely related to obtaining high encapsulation efficiencies. Some authors suggest that dairy products are very efficient in maintaining the viability of the cells during the drying process due to the presence of milk components, especially lactose (Ananta *et al.*, 2005). Lactose can interact with components of the cell membrane (phospholipids and proteins), avoiding membrane leakage during the removal of water in the spray drying procedure which increase the storage period (Ananta *et al*., 2005). A similar behavior was observed by Pinto *et al*. (2015a) who achieved high encapsulation efficiency (95.43%), by using whey concentrate as an encapsulating agent in the microencapsulation of *Bifidobacterium* BB-12 by spray drying method.

The encapsulation efficiency of the present study was higher when compared to similar studies. Maciel *et al.* (2014) obtained encapsulation efficiency of approximately 76.58% in the microencapsulation of *Lactobacillus acidophilus* La-5 by spray drying using whey powder and skimmed milk as an encapsulating materials. Dimitrellou *et al.* (2016) found encapsulation efficiency of 72.5% using milk powder and *Lactobacillus casei* ATCC 393 in microencapsulation by spray drying. Efficiency variations can occur because of the physicochemical properties of the capsules including encapsulating material type and concentration, particle size, number of initial cells and bacterial strains are the parameters that may affect the survival of encapsulated cells (Picot and Lacroix, 2004).

3.3. Viability of encapsulated LAB during storage at 7 and 25 °C

The viable cell counts of *L. plantarum* CCMA0359 and *L. brevis* CCMA1284 microparticles stored at 7 and 25 °C were evaluated during 90 days of storage (Fig. 2). The microparticles containing both *L. plantarum* CCMA0359 and *L. brevis* CCMA1284 showed higher counts ($p< 0.05$) when stored at 7°C. The survival of microorganisms during storage is affected by storage temperature, since the stability of microencapsulated probiotics during storage is higher at low temperatures, such as refrigeration, than at room temperature (Martín *et al.*, 2015). Under low temperatures, the microorganisms are kept in a latent state, avoiding the rearrangement of the wall material, thus preventing inadequate exposure of the microorganisms which results in an increase in microparticulate shelf-life (Albertini *et al.*, 2010).

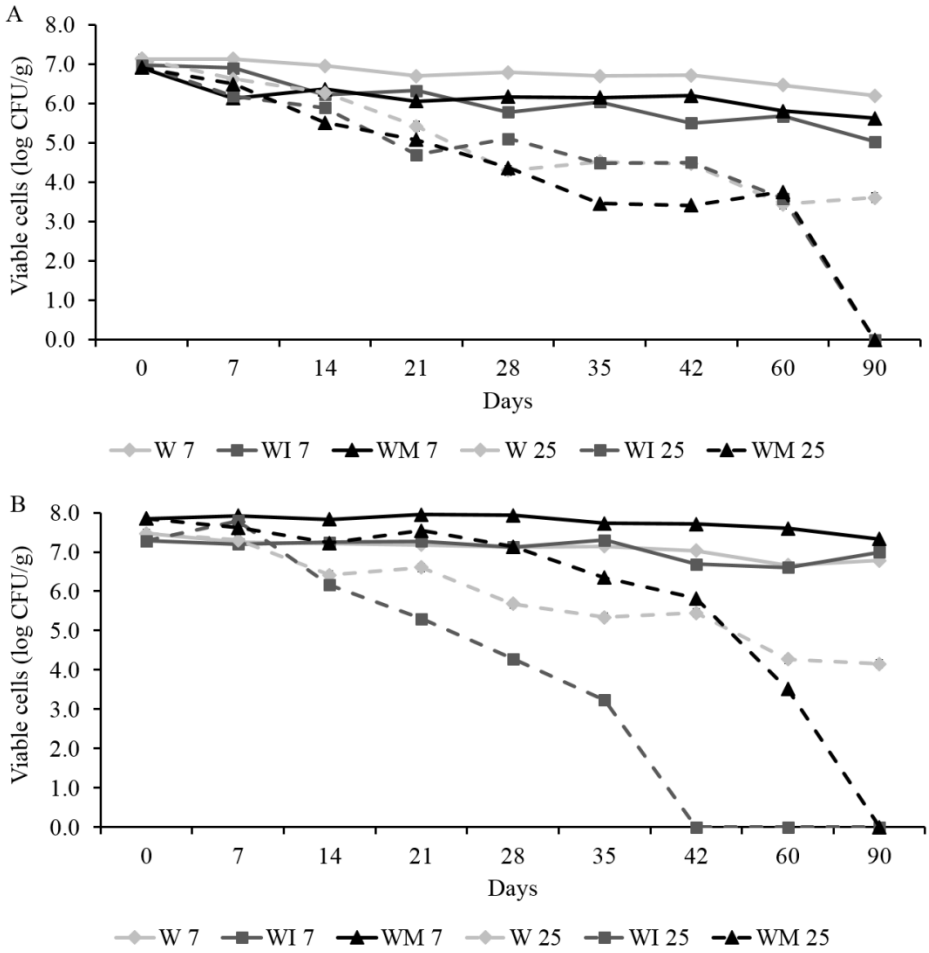


Fig. 2: Viability of microencapsulated cells of *L. brevis* CCMA1284 (A) and *L. plantarum* CCMA0359 (B) during storage at 7 and 25 °C. Whey powder (W), whey powder with inulin (WI) and whey powder with maltodextrin (WM). Mean values of viability at different temperatures were compared $p< 0.05$ by the Scott-Knott test.

In general, there was a decrease ($p< 0.05$) in the count of *Lactobacillus* strains during the 90 days of storage in the different assays. However, at the end of storage, *L. plantarum* CCMA0359 had a viable cell count above 6 log CFU/g for the microparticles kept at 7 °C, which is recommended to exert their beneficial effect as probiotic. On the other hand, only *L. brevis* CCMA1284 microparticles formulated with S showed count above 6 log CFU/g during storage at 7 °C. At 25 °C, none of the *Lactobacillus* strains in all different assays were able to keep their viability above the recommended value (6 log CFU/g). The decrease in the viability at 25 °C may be due the fact that the bacteria metabolically active within the microparticles may produce and excrete metabolites such as acids, bacteriocins or lack of nutrients which will affect their owner cells (Pinto *et al.*, 2015b). *L. plantarum* is a homofermentative bacteria, which produces only lactic acid from glucose fermentation, while *L. brevis* is a heterofermentative bacteria, producing acetic acid and/or ethanol and carbon dioxide in addition to lactic acid (Stiles and Holzapfel, 1997). This fact may explain the lower viability of *L. brevis* when compared to *L. plantarum*.

Although the addition of prebiotic inulin showed no positive effect on encapsulation efficiency, significant differences ($p< 0.05$) were observed between the encapsulating matrices for both *Lactobacillus* strains during storage period (Fig. 2). The highest count of *L. plantarum* CCMA0359 and *L. brevis* CCMA1284 was obtained with the microparticles produced with WM and W, respectively. The encapsulating matrices have different chemical characteristics as well as physical properties. Thus, matrices may exert different effects on the protection of encapsulated cells (Etchepare *et al.,* 2016). The presence of prebiotic inulin did not increase the LAB cells viability during storage of the microparticles. The same occurred in the study performed by Pinto *et al.* (2015a), who observed that the presence of inulin or polydextrose in feed solutions did not affect the viability of *Bifidobacterium* BB-12 during the storage period. Despite this, some studies have shown that co-encapsulation of probiotic and prebiotic is a strategy to increase probiotic viability during storage (Fritzen-Freire *et al.,* 2012, Valero-Cases and Frutos, 2015). The present work demonstrated a positive effect of using maltodextrin as a matrix, mainly for *L. plantarum* CCMA0359 (Fig. 2), demonstrating that the type of matrix can affect differently for the different strains.

3.4. Survival of LAB strains in acid and bile juices

The viability of the LAB strains in acid and bile juices is shown in (Fig. 3). The microencapsulation with different combinations of matrices were effective to protect the two

LAB strains in the acid and bile conditions when compared to free cells, indicating that microencapsulation is an interesting alternative to increase the probiotic resistance in acid and bile conditions. These data corroborate with Maciel *et al.* (2014) who observed higher protection of *L. acidophilus* La-5 cells microencapsulated with whey powder or skimmed milk as spray-dried wall than free cells.

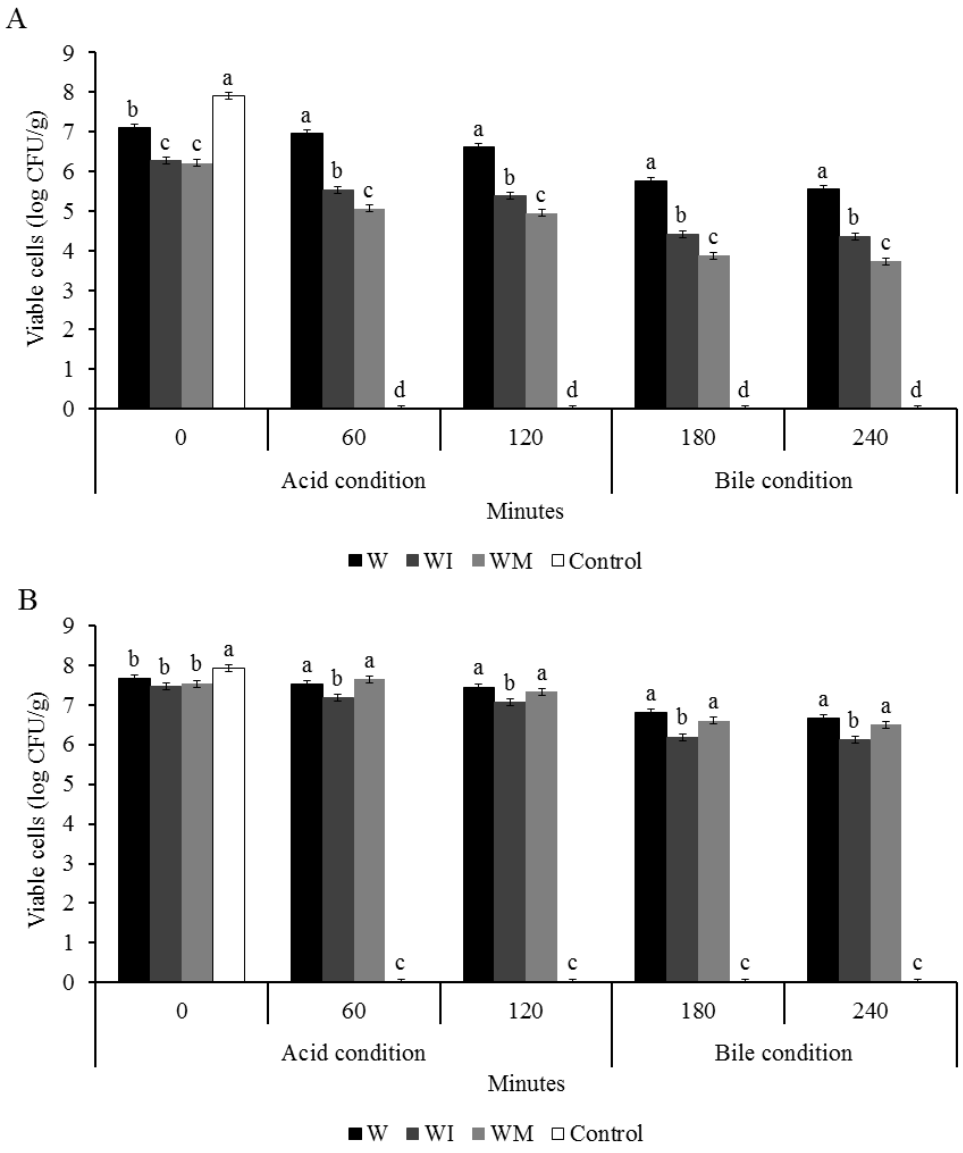


Fig. 3: Viability of microencapsulated cells of *L. brevis* CCMA1284 (A) and *L. plantarum* CCMA0359 (B) exposed to simulated GIT conditions. Whey powder (W), whey powder with inulin (WI) and whey powder with maltodextrin (WM). The mean values of the simulated TGI conditions were compared for each strain, values followed by different lowercase letters are significant different at $p< 0.05$ by the Scott-Knott test.

In the present study, the different combinations of matrices provided a significant protection ($p< 0.05$) for the two *Lactobacillus* strains. The *L. plantarum* CCMA0359 microparticles produced with W and WM showed higher protection than microparticles

produced with WI. On the other hand, the highest protection of *L. brevis* CCMA1284 was observed for the microparticles produced with W. As demonstrated for storage assay, the presence of the prebiotic inulin in the encapsulant matrix did not increase the resistance of the two *Lactobacillus* strains in acid and bile juices. On the other hand, the inulin used as an encapsulating matrix may have a positive effect after the releasing of cells in the intestine, serving as nutrient for the probiotic, a fact that needs to be studied.

The number of cells decreased significantly ($p< 0.05$) during the sequential incubation time in acid and bile juices. After 60 minutes of incubation in the acid juice, there was no significant reduction in the viability of both *Lactobacillus* strains, except for free cells that did not survive. Probably, few cells were released from the microparticles in the acidic condition, According to Heidebach *et al.* (2012), the release of probiotic cells occurs in the gut rather than in the stomach, thus ensuring passage through the gastrointestinal tract. In the intestinal condition, the number of cells decreased significantly ($p< 0.05$) and the reduction was higher for *L. brevis* CCMA1284 with a decrease of 1.54, 1.91 and 2.49 log CFU/g, while *L. plantarum* CCMA0359 showed decrease levels of 1.02, 1.34 and 1.04 log CFU/g for the W, WI and WM matrices, respectively. Some studies have found that the resistance of probiotic bacteria to low pH and bile salts is specific for each strain (Ramos *et al*., 2013; Bono *et al*., 2015), which corroborates the results of the present study. The *L. plantarum* CCMA0359 microparticles showed higher ($p< 0.05$) protection than *L. brevis* CCMA1284 microparticles.

At the end of incubation in bile juice, the number of viable cells of the two LAB strains differed significantly ($p< 0.05$) (Fig. 3). The *L. plantarum* CCMA0359 population was 6.66, 6.51 and 6.13 log CFU/g for W, WM and WI, respectively, resulting in a higher percentage of survival (85.5, 86.3 and 82.0% for W, WM and WI, respectively) than *L. brevis* CCMA1284 (78.3, 69.7 and 60.0% for W, WI and WM, respectively). Although the viable cell count (5.55, 4.36 and 3.72 log CFU/g for W, WI and WM, respectively) of *L. brevis* CCMA 1284 after 240 minutes of sequential incubation was not above that recommended for the probiotic to exert its beneficial effects on host health, the microencapsulation technique increased survival in acid and bile juices. These results suggest that a larger population of *L. brevis* CCMA1284 should be used initially for microencapsulation to obtain more viable cells at the end of the passage throughout GIT.

4. Conclusion

The spray drying technique was successfully used for the encapsulation of potential probiotics strains *L. plantarum* CCMA0359 and *L. brevis* CCMA1284 strains. *L. brevis* CCMA1284 showed higher sensitivity to the acid and bile juices conditions and storage period than *L. plantarum* CCMA0359. The viability of the two LAB strains during acid and bile juices conditions and storage period was influenced by different encapsulating matrices and storage temperature. Furthermore, the incorporation of the prebiotic inulin into the matrix did not provide additional protection for both strains in acid and bile juices and storage period. The use of whey powder and whey powder with maltodextrin as matrices were effective to maintain viability of *L. plantarum* strain CCMA0359 in acid and bile juices and during storage under refrigeration (7 °C). Only whey powder was effective in maintaining the viability of *L. brevis* CCMA1284 under the same conditions. Therefore, studies and standardization of the microencapsulation technique should be performed for each strain.

Acknowledgements

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5. References

Albertini, B., Vitali, B., Passerini, N., Cruciani, F., Di Sabatino, M., Rodriguez, L., & Brigidi, P. (2010). Development of microparticulate systems for intestinal delivery of *Lactobacillus acidophilus* and *Bifidobacterium lactis*. *European Journal of Pharmaceutical Sciences*, **40**, 359–366.

Ananta, E., Volkert, M., & Knorr, D. (2005). Cellular injuries and storage stability of spray-dried *Lactobacillus rhamnosus* GG. *International Dairy Journal*, **15**, 399–409.

Annan, N. T., Borza, A. D., & Hansen, L. T. (2008). Encapsulation in alginate-coated gelatin microspheres improves survival of the probiotic *Bifidobacterium adolescentis* 15703T during exposure to simulated gastro-intestinal conditions. *Food Research International*, **41**, 184–193.

Barbosa, J., & Teixeira, P. (2016). Development of probiotic fruit juice powders by spraydrying: A review. *Food Reviews International*, **33**, 335–358.

Bono, Y. Del, Pendino, B., Mario, C., & Vinderola, G. (2015). Variability in the content of *Lactobacillus casei* in different commercial lots of fermented milks. *International Journal of Food Science & Technology*, **50**, 263–267.

Bustamante, M., Oomah, B. D., Rubilar, M., & Shene, C. (2017). Effective *Lactobacillus plantarum* and *Bifidobacterium infantis* encapsulation with chia seed (*Salvia hispanica* L.) and flaxseed (*Linum usitatissimum* L.) mucilage and soluble protein by spray drying. *Food Chemistry*, **216**, 97–105.

Chávez, B. E., & Ledeboer, A. M. (2007). Drying of probiotics: Optimization of formulation and process to enhance storage survival. *Drying Technology*, **25**, 1193–1201.

De Castro-Cislaghi, F. P., Silva, C. D. R., Fritzen-Freire, C. B., Lorenz, J. G., & Sant'Anna, E. S. (2012). *Bifidobacterium* Bb-12 microencapsulated by spray drying with whey: Survival under simulated gastrointestinal conditions, tolerance to NaCl, and viability during storage. *Journal of Food Engineering*, **113**, 186–193.

Dimitrellou, D., Kandylis, P., Petrovic, T., Dimitrijevic-Brankovic, S., Levi, S., Nedovic, V., & Kourkoutas, Y. (2016). Survival of spray dried microencapsulated *Lactobacillus casei* ATCC 393 in simulated gastrointestinal conditions and fermented milk. *LWT - Food Science and Technology*, **71**, 169–174.

Dolly, P., Anishaparvin, a, Joseph, G. S., & Anandharamakrishnan, C. (2011). Microencapsulation of *Lactobacillus plantarum* (mtcc 5422) by spray-freeze-drying method and evaluation of survival in simulated gastrointestinal conditions. *Journal of Microencapsulation*, **28**, 568–574.

Etchepare, M. de A., Raddatz, G. C., de Moraes Flores, É. M., Zepka, L. Q., Jacob-Lopes, E., Barin, J. S., & de Menezes, C. R. (2016). Effect of resistant starch and chitosan on survival of *Lactobacillus acidophilus* microencapsulated with sodium alginate. *LWT - Food Science and Technology*, **65**, 511–517.

FAO/WHO (2006). Probiotics in food. Health and nutritional properties and guidelines for evaluation. FAO Food and Nutrition Paper, Vol. 85.

Fritzen-Freire, C. B., Prudêncio, E. S., Amboni, R. D. M. C., Pinto, S. S., Negrão-Murakami, A. N., & Murakami, F. S. (2012). Microencapsulation of bifidobacteria by spray drying in the presence of prebiotics. *Food Research International*, **45**, 306–312.

Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., Scott, K., Stanton, C., Swanson, K. S., Cani, P. D., Verbeke, k., & Reid, G. (2017).

Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology & Hepatology*, **14**, 491–502.

Heidebach, T., Forst, P., & Kulozik, U. (2012). Microencapsulation of probiotic cells for food applications microencapsulation of probiotic cells for food applications. *Critical Reviews in Food Science and Nutrition*, **52**, 291–311.

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., Morelli, L., Canani, R. B., Flint, H. J., Salminen, S., Calder, P. C., & Sanders, M. E. (2014). The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology*, **11**, 506–514.

Kim, S., Cho, S. Y., Kim, S. H., Song, O., Shin, I., Cha, D. S., & Park, H. J. (2008). Effect of microencapsulation on viability and other characteristics in *Lactobacillus acidophilus* ATCC 43121. *LWT - Food Science and Technology*, **41**, 493–500.

Livney, Y. D. (2010). Milk proteins as vehicles for bioactives. *Current Opinion in Colloid & Interface Science*, **15**, 73–83.

Maciel, G. M., Chaves, K. S., Grosso, C. F., & Gigante, L. (2014). Microencapsulation of *Lactobacillus acidophilus* La-5 by spray-drying using sweet whey and skim milk as encapsulating materials. *Journal of Dairy Science*, **97**, 1991–1998.

Martín, M. J., Lara-Villoslada, F., Ruiz, M. A., & Morales, M. E. (2015). Microencapsulation of bacteria: A review of different technologies and their impact on the probiotic effects. *Innovative Food Science and Emerging Technologies*, **27**, 15–25.

Nazzaro, F., Orlando, P., Fratianni, F., & Coppola, R. (2012). Microencapsulation in food science and biotechnology. *Current Opinion in Biotechnology*, **23**, 182–186.

Picot, A., & Lacroix, C. (2004). Encapsulation of bifidobacteria in whey protein-based microcapsules and survival in simulated gastrointestinal conditions and in yoghurt. *International Dairy Journal*, **14**, 505–515.

Pinto, S. S., Fritzen-freire, C. B., Benedetti, S., Murakami, F. S., Petrus, J. C., Prudêncio, E. S., & Amboni, R. D. M. C. (2015a). Potential use of whey concentrate and prebiotics as carrier agents to protect *Bifidobacterium* -BB-12 microencapsulated by spray drying. *Food Research International*, **67**, 400–408.

Pinto, S. S., Verruck, S., Vieira, C. R. W., Prudêncio, E. S., Amante, E. R., & Amboni, R. D. M. C. (2015b). Influence of microencapsulation with sweet whey and prebiotics on the

survival of *Bifidobacterium*-BB-12 under simulated gastrointestinal conditions and heat treatments. *LWT - Food Science and Technology*, **64**, 1004–1009.

Rajam, R., & Anandharamakrishnan, C. (2015). Microencapsulation of *Lactobacillus plantarum* (MTCC 5422) with fructooligosaccharide as wall material by spray drying. *LWT - Food Science and Technology*, **60**, 773–780.

Ramos, C. L., Thorsen, L., Schwan, R. F., & Jespersen, L. (2013). Strain-specific probiotics properties of *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Lactobacillus brevis* isolates from Brazilian food products. *Food Microbiology*, **36**, 22–29.

Ré, M. I. (1998). Microencapsulation by Spray drying. *Drying Technology: An International Journal*, **16**(6), 1195–1236.

Stiles, M. E., & Holzapfel, W. H. (1997). Lactic acid bacteria of foods and their current taxonomy. *International Journal of Food Microbiology*, **36**, 1–29.

Valero-Cases, E., & Frutos, M. J. (2015). Effect of different types of encapsulation on the survival of *Lactobacillus plantarum* during storage with inulin and in vitro digestion. *LWT - Food Science and Technology*, **64**, 824–828.

Verruck, S., Carvalho, M. W. De., Liz, G. R. De., Amante, E. R., Vieira, C. R. W., Amboni, R. D. De. M. C., & Prudencio, E. S. (2017). Survival of *Bifidobacterium* BB-12 microencapsulated with full-fat goat's milk and prebiotics when exposed to simulated gastrointestinal conditions and thermal treatments. *Small Ruminant Research*, **153**, 48–56.

Verruck, S., Liz, G. R. De, Dias, C. O., Amboni, R. D. D. M. C., & Prudencio, E. S. (2018). Effect of full-fat goat's milk and prebiotics use on *Bifidobacterium* BB-12 survival and on the physical properties of spray-dried powders under storage conditions. *Food Research International*. https://doi.org/10.1016/j.foodres.2018.10.042

ARTIGO 2

SURVIVAL AND SENSORIAL EVALUATION OF *Lactobacillus plantarum* CCMA 0359 IN REQUEIJÃO

ABSTRACT

The survival of free and microencapsulated cells of *Lactobacillus plantarum* CCMA 0359 added in requeijão were determined during storage period at 7 °C for 90 days, as well as in simulated gastrointestinal condition. The effect of the bacteria addition in the sensorial properties of the cream cheese was also evaluated. The *L. plantarum* CCMA 0359 population (microencapsulated and free cells) remained around of 8 log CFU/g during all storage time, however, the counts for microencapsulated cells were slightly higher than free cells. The addition of bacteria (microencapsulated and free cells) increased the acidity and decreased the pH of requeijões during storage, and microencapsulated cells showed more pronounced alterations of acidity and pH. Under simulated gastrointestinal condition, the microencapsulation technique allowed a significant higher protection of cells. The addition of *L. plantarum* CCMA 0359 (free and microencapsulated cells) to the requeijão did not affect the acceptance of the product by the consumers. The requeijão showed to be an alternative vehicle for the potential probiotic *L. plantarum* CCMA 0359, without affect the acceptance of the product.

Keywords: *Spray drying*, Viability, Probiotic requeijão

1 INTRODUCTION

Food products with addition of probiotics, which benefits the health of the consumer in addition nurturing, has attracted great interest from the scientific and commercial point of view. Demand for these products has increased due consumers are more aware of the relationship of good nutrition and health. To obtain success in the consumer's market, the product should supply the consumer needs. Probiotic products have been accepted by the general population in regards of health and well-being promotion (BAMPI et al., 2016; MANTZOURIDOU et al., 2012).

Lactic acid bacteria (LAB) are considered safe (GRAS-Generally Recognized as Safe) to be applied in food industries and are highly valued for probiotic properties. They promote positive physiological effects, improving and activating the intestinal functions. The most common species used as probiotics in the food market belong to the genera *Lactobacillus* and *Bifidobacterium* (JIANG et al., 2016; SHORI, 2016). Among them, strains of *L. plantarum* specie have shown satisfactory results in the treatment or prevention of gastrointestinal disorders, including irritable bowel syndrome, ulcerative colitis and diarrheal diseases such as antibiotic-associated diarrhea and *Clostridium difficile* (DUCROTT et al., 2012).

One of the main challenges to produce probiotic foods is to maintain cell viability during digestion and colonization in the intestine. In addition, to ensure that their properties will be maintained during the conditions of processing and storage of the product. In this context, the microencapsulation technique which protects probiotic bacteria when exposed to adverse conditions, may contribute to the development of new probiotic foods (NAZZARO et al., 2012). Spray drying is the most widely used microencapsulation technique in the food industry. This technique is convenient for the long-term preservation of probiotic cells and their use in functional food applications. The drying process is fast, relatively low cost, simple and has continuous operation capability (DOLLY et al., 2011: MARTÍN et al., 2015). The use of dairy matrices as wall material for the microencapsulation of probiotic microorganisms have been extensively studied and showed promising results (EL- SALAM; EL-SHIBINY, 2015).

Probiotics strains are mainly added to dairy products, such as yogurts, kefir, cheeses, fermented milk and ice creams which can provide essential nutrients such as calcium, protein and increase its functionality (VINDEROLA et al., 2011). These products are considered the main vehicles for the supply of probiotic bacteria to the gastrointestinal tract and have some advantage that favor the probiotic survival including low temperature of storage (4°C/8°C)

and a relatively short shelf life of 28-35 days. Further, regulations for dairy applications for probiotic products are easily available (MATTILA-SANDHHOLM et al., 2002; WEICHSELBAUM, 2009).

Requeijão is a typical Brazilain product, which technology and characteristics differ from region to region. According to the Brazilian legislation "Requeijão is the product obtained from the fusion of the curd paste, cooked or uncooked, desorbed and washed, obtained by acid and / or enzymatic coagulation of the milk optionally added with cream and / or butter and/or anhydrous fat milk or *butteroil* (BRASIL, 1997). Requeijão can be a good vehicle alternative for probiotics because of its higher pH, oxygen level, fat content, storage conditions and for having a solid matrix, presenting better protection for microorganisms.

Based on the importance of increasing the number of probiotic products available to the increasingly demanding consumer market, the objective of this study was to investigate the effect of the incorporation of potential probiotic *Lactobacillus plantarum* CCMA 0359 (De ANDRADE et al., 2019; RAMOS et al., 2013) microencapsulated by spray drying in the requeijão and to evaluate the survival of the culture during storage and simulated gastrointestinal condition. The influence of culture addition on the sensory properties of requeijão was also evaluated.

2 MATERIAL AND METHODS

2.1 Microorganism and culture conditions

The LAB strain *L. plantarum* CCMA 0359 previously characterized as probiotic (De ANDRADE et al., 2019; RAMOS et al., 2013) and belonging to Culture Collection of Agricultural Microbiology (CCMA) from Biology Department of Federal University of Lavras, Lavras, MG, was employed in the present study. *L. plantarum* was reactivated from frozen stock cultured in 1L MRS broth (Himedia, Mumbai, India) at 37 °C for 12 h and subcultured twice at same conditions before each assay. For each assay, the bacteria were recovered by centrifugation at 7100 x *g*, 4 °C for 10 min and washed twice with sterile peptone water 0.1 % (w/v) at same condition.

2.2 Production of *L. plantarum* CCMA 0359 microparticles by *spray drying*

The encapsulating matrix to prepare the feed solution was whey powder 20 % (w/v) (Laticínios Porto Alegre, Mutum, MG, Brazil). The feed solution was homogenized in sterile distilled water, pasteurized (75 °C for 30 min) and cooled (4 °C until use). The bacterial suspension in the concentration of 10^{10} CFU/g was added to the feed solution and microencapsulated using a pilot scale spray dryer (model MSD 1.0; Labmaq do Brasil, Ribeirão Preto, Brazil), operating at constant air inlet temperature at 150 °C and outlet temperature at 80 °C. The feed solution containing *L. plantarum* was maintained under magnetic stirring at room temperature (approximately 25 °C) and fed into the main chamber through a peristaltic pump, with feed flow of 0.7 ml/min and compressed air flow of 35 L/min. The resulting powder (microparticles) was collected at the base of the cyclone and stored in a sealed sterile vial which was maintained at 4 °C for further analysis.

2.3 Encapsulation efficiency of *L. plantarum* CCMA 0359 cells

Sample of 1g of the microparticles (powder) containing *L. plantarum* cells was resuspended in 9 mL of phosphate buffer (0.1 M, pH 7.0) followed by homogenization on magnetic stirrer. The viable cell count was determined by serial dilutions, plated on MRS agar (Himedia, Mumbai, India) and incubated at 37 °C for 48 h. The encapsulation efficiency (EE) was obtained according to described by De Andrade et al., 2019.

2.4 Addition and survival of *L. plantarum* CCMA 0359 in requeijão during storage period

Free cells and microencapsulated cells of *L. plantarum* were separately added in Vimilk (Perdões-MG, Brazil) commercial requeijão. Samples of 50g of cream cheese were inoculated with microcapsules or free cells in the concentration of 10^{8} CFU/g, homogenized and stored at 7 °C for 90 days. Samples were withdrawn on days 0, 15, 30, 60 and 90 days of storage for microbiological and physicochemical analysis. The viability of microencapsulated and free cells of *L. plantarum* during storage period of requeijão was determined by plating in MRS agar, as described in the section 2.3.

2.5 Physicochemical determinations

The physicochemical parameters pH, titratable acidity and moisture content of requijão were evaluated during storage period. The pH values were determined using a pHmeter (Model AC-100; MS Tecnopon, Brazil). Titratable acidity was determined by titration with 0.1N NaOH solution and phenolphthalein as indicator and expressed as g of lactic acid per 100 g of cream cheese. The moisture content was determined using a halogen moisture analyzer (Model IV 3000; Ind. Com. Electro-Eletronica Gehaka, Brazil) and expressed in percentage.

2.6 Viability of *L. plantarum* CCMA 0359 in Brazilian cream cheese evaluated in simulated gastrointestinal condition

The viability of *L. plantarum* CCMA 0359 in requeijão in simulated gastrointestinal condition was evaluated during the refrigerated storage period (7 °C at 0, 15, 30, 60 and 90 days) according to Ribeiro et al. (2014) and Wang et al. (2009), with modifications. Saline solution 0.9 % (w/v of NaCl) with pH 2.0 adjusted with 1M HCL and pepsin (3000 μg/g) (Sigma-Aldrich Co., St. Louis, MO) filter-sterilized at the final concentration of 0.3 % (v/v) were used to simulate the acid condition of gastrointestinal tract. Ten grams of the cream cheese was dissolved in 100 mL of the simulated acid solution and incubated at 37 °C for 2 h under 100 rpm. The viable cells were quantified at 0 and 2 h of incubation by plating on MRS agar. After 2 h of incubation in simulated acid condition, 0.95 g/L of pancreatin and bile (Himedia, Mumbai, India) at a final concentration of 0.3 % (v/v) were added and the pH was adjusted to 7.0 using 1 M NaOH, obtaining the simulated intestinal solution. The samples were incubated under the same conditions above for 2 h and new aliquots were removed for evaluation of the viability of the bacterium. The requeijão inoculated with free cells was also evaluated as a control.

2.7 Sensorial analysis

Acceptability and purchase intention evaluations were performed by 100 consumers randomly recruited, 72 females and 28 males, over 18 years of age. Each consumer received 40g of each requeijão (at 7 °C) in plastic cups randomly coded with three-digit numbers. Water at room temperature was used to rinse the mouth in the intervals between the

evaluations. For acceptance test, the consumers were asked to evaluate the sensory attributes (appearance, aroma, taste, texture and overall appearance) using a structured nine-point hedonic scale (9 = extremely liked; 1 = extremely disliked). The purchase intention form presented a hedonic scale of 5 points (5 = certainly buy; 1 = certainly would not buy) (VILLANUEVA; Da SILVA, 2009). The sensorial evaluation of the requeijões samples was approved by the Research Ethics Committee (Federal University of Lavras, Lavras, MG, Brazil (n 02537318.1.0000.5148).

2.8 Statistical analysis

The results were expressed as mean of three replicates. Data were submitted to analysis of variance (ANOVA) and significant differences between the mean values were determined by the Scott-Knott test with a significance level of 5 % using Sisvar software version 5.6. (FERREIRA, 2011).

3 RESULTS AND DISCUSSION

3.1 *L. plantarum* CCMA 0359 viability after spray drying and encapsulation efficiency

L. plantarum CCMA 0359 viable cells were obtained before and after spray drying technique. The cell concentration into de microparticles was 9.97 log CFU/g, showing a reduction of 0.07 log UFC/g from initial number (10.04 log CFU/g) of cells. Eckert et al., 2017 observed a reduction of 0.08 to 0.90 log CFU/g of *L. plantarum* ATCC8014 microencapsulated with whey as wall material. This reduction is probable due the inactivation of cells by heating treatment employed during spray drying as observed by other authors (GOLOWCZYC et al., 2011; RANADHERA et al., 2015).

The bacterial cell concentration found in the present study was satisfactory, it is above recommended for a product to be considered probiotic. This result corroborates with Tripathi and Giri (2014) which describe that probiotics products should contain a minimum concentration of 10^8 - 10^9 CFU/g or mL of viable probiotic cells, at the time of consumption, to ensure that an enough therapeutic dosage (around 10^6 CFU) can reach the colon and provide benefits to the consumer's health.

Encapsulation efficiency is one of the most important parameters indicating the effect of the microencapsulation process and the selected encapsulant matrix on the probiotic cells

(SHAMAEI et al., 2017). It is known that the choice of a suitable encapsulating matrix is directly related to obtaining high encapsulation efficiencies. The present work demonstrated a positive effect when using whey powder as wall material, resulting in high encapsulation efficiency of 99.27%. The protection of cell viability during the spray drying process is probably due to the presence of lactose and milk proteins in the matrix as also observed by other authors (IHA et al., 2015; RAJAM; ANANDHARAMAKRISHNAN, 2015; VERRUCK et al., 2017). These components coat the bacterial cell wall, preventing cell membrane rupture during the drying process and consequently increasing encapsulation efficiency (CORCORAN et al, 2004). Furthermore, the use of the cells in the stationary phase may also contribute for the survival of the cells during the drying process since they are less susceptible than bacteria in logarithmical phase.

3.2 *L. plantarum* CCMA 0359 survival during requeijão storage period

The viability of microencapsulated and free *L. plantarum* CCMA 0359 cells added in the requeijão was evaluated at 7 °C for 90 days of storage (Table 1). Initial cell numbers were 8.69 log CFU/g and 8.64 log CFU/g for microencapsulated and free cells, respectively. As observed in Table 1, there was a slight reduction of 0.38 log CFU/g for microencapsulated bacteria and 0.58 log CFU/g for free cells after 90 days of requeijão storage. However, the bacterial population in the product remained around 8 log CFU/g, which is recommended (≥ 6 log CFU/g or mL) to exert their beneficial effect on the health of the consumer.

Although the methodology of microencapsulation had direct effect on the cell survival, the bacterial species and the food carrier product should also be considered. Cells of *L. plantarum* ATCC 8014 (ECKERT et al., 2017) and *Bifidobacterium* BB-12 (DE CASTRO-CISLAGHI et al., 2012) were microencapsulated by spray drying using whey as matrix, however, *L plantarum* ATCC 8014 cells were inoculated in milk while *Bifidobacterium* BB-12 was inoculated into a dairy dessert. Both products were stored at 4 °C for 42 days, however *L. plantarum* ATCC 8014 cells remained stable showing counts above 9 log CFU/g, while *Bifidobacterium* BB-12 showed a reduction of 1.16 log CFU/g, but the count still above than 7 log CFU/g. In general, dairy products are considered the main vehicles for the supply of probiotic strains since they contain nutrients such as carbohydrates (lactose) and proteins that can be used as a source of energy by probiotic microorganism. This fact may explain the suitable maintenance of cell viability during storage period.

Table 1. Viable cells count, values of pH, titratable acidity and humidity of the requeijão inoculated with microencapsulated and free cells and stored at 7 ° C for 90 days.

Samples	Days	Viable cells count (log CFU/g)	pH	Acidity (% lactic acid)	Humidity (%)
Microencapsulated	0	8.69 ± 0,05[a]	5,80 ± 0,19[a]	0,81 ± 0,00[a]	33,0 ± 1,65[a]
	15	8.03 ± 0,01[a]	6,17 ± 0,12[a]	0,87 ± 0,05[a]	39,9 ± 1,08[a]
	30	8.11 ± 0,03[a]	6,20 ± 0,13[a]	0,90 ± 0,00[a]	36,4 ± 0,76[a]
	60	8.13 ± 0,04[a]	4,85 ± 0,10[b]	1,23 ± 0,14[b]	40,5 ± 0,96[b]
	90	8.31 ± 0,04[a]	4,76 ± 0,12[b]	1,32 ± 0,19[b]	45,1 ± 1,46[b]
Free	0	8.64 ± 0,04[a]	5,85 ± 0,14[a]	0,81 ± 0,09[a]	31,7 ± 1,21[a]
	15	8.00 ± 0,03[a]	6,08 ± 0,15[a]	0,84 ± 0,05[a]	38,4 ± 0,56[a]
	30	7.91 ± 0,07[b]	6,07 ± 0,24[a]	0,84 ± 0,05[a]	37,0 ± 0,71[a]
	60	8.11 ± 0,03[a]	5,59 ± 0,21[a]	0,99 ± 0,16[a]	35,7 ± 0,81[a]
	90	8.07 ± 0,04[b]	5,44 ± 0,17[a]	1,14 ± 0,05[a]	38,6 ± 0,36[a]

Means followed by the same lowercase letter in the column do not differ from each other ($p < 0.05$) by the Scott-Nott test.

As observed in Table 1, the microencapsulated cells showed higher ($p<0.05$) counts than free cells, 8.31 log CFU/g and 8.07 log CFU/g, respectively, which may be related to added protection provided by microencapsulation. Other authors have also reported similar findings (BRINQUES; AYUB, 2011; DIMITRELLOU et al., 2016; MOUSA et al., 2014; ORTAKCI et al., 2012; RIBEIRO et al., 2014).

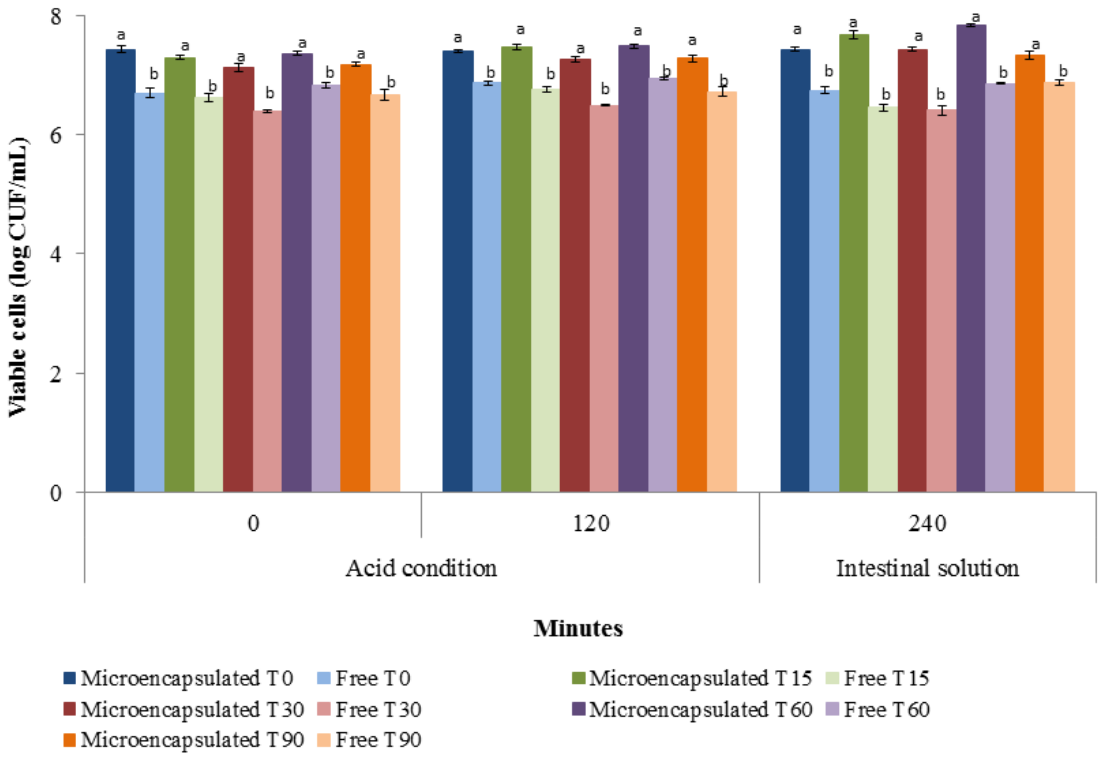
During the 90 days of storage, the physical-chemical characteristics of the requeijões inoculated with microencapsulated and free cells were evaluated and the results are shown in Table 1. Changes in the titratable acidity, pH and humidity of the requeijões were significantly different ($p < 0.05$) between the samples containing microencapsulated and free cells at 60 and 90 days. The titratable acidity increased 0.81 to 1.32 and 0.81 to 1.14 g/100 g of requeijão to the microencapsulated and free cells samples, respectively, while the pH decreased during storage. The lowest pH value and highest acidity and humidity were detected at 60 and 90 days of storage for microencapsulated cells. However, the cells viability was not affected by these modifications, indicating that the microencapsulation technique protected the cells into the product.

Higher humidity value for the requeijão added with microencapsulated cells may be due the presence of the matrix in the composition of the product. Although these slight variations in the physical-chemical characteristics of the product were observed, the humidity value is in accordance with the legislation (BRAZIL, 1997) that maximum humidity for requeijão is 65 %.

3.3 Viability of *L. plantarum* CCMA 0359 cells in gastrointestinal conditions using requeijão as probiotic carrier

The microencapsulation technologies have been employed to improve the bacterial survival during passage in the gastrointestinal tract (ECKERT et al., 2017; RIBEIRO et al., 2014). In this sense, the viability of microencapsulated and free cells of *L. plantarum* CCMA 0359 carried by requeijão stored from 0 to 90 days at 7 °C, were evaluated in a simulated gastrointestinal condition (Figure 1). As observed in the Fig. 2, the microencapsulated cells showed higher ($p<0.05$) viability than free cells during the passage in the gastric and intestinal simulated juices when carried by requeijão stored from 0 to 90 days. These data shown that microencapsulation may be an important tool to maintain probiotic viability from requeijão storage under refrigeration until to reach the intestine, where they will provide benefits to consumer's health.

Figure 1 - Cell viability of *L. plantarum* CCMA 0359 microencapsulated and free exposed to simulated gastrointestinal sequential conditions. Means followed by the same lowercase letter do not differ from each other ($p <0.05$) by the Scott-Nott test.



In general, the viability of *L. plantarum* CCMA 0359 showed a slight increase of around 0.33 Log CFU/g and 0.19 Log CFU/g, for microencapsulated and free cells

respectively, during simulated gastrointestinal passage. It is known that food matrices used to carry probiotic strains may improve their survival during gastrointestinal passage. It is due the buffer effect of the food on the stomach, increasing the pH to approximately 4.5 (ORTAKCI et al., 2012; TULUMOGLU et al., 2014).

Although minor variations in the bacterial viability were observed during storage and simulated gastrointestinal passage, they were maintained above to the minimum recommendation (≥ 6 log CFU/g) to exert their benefits to the host health. However, microencapsulated cells showed an advantage of approximately 1 Log CFU/g higher than free cells, which increase the possibility of a probiotic to dominate and display its benefits.

3.4 Sensory analysis

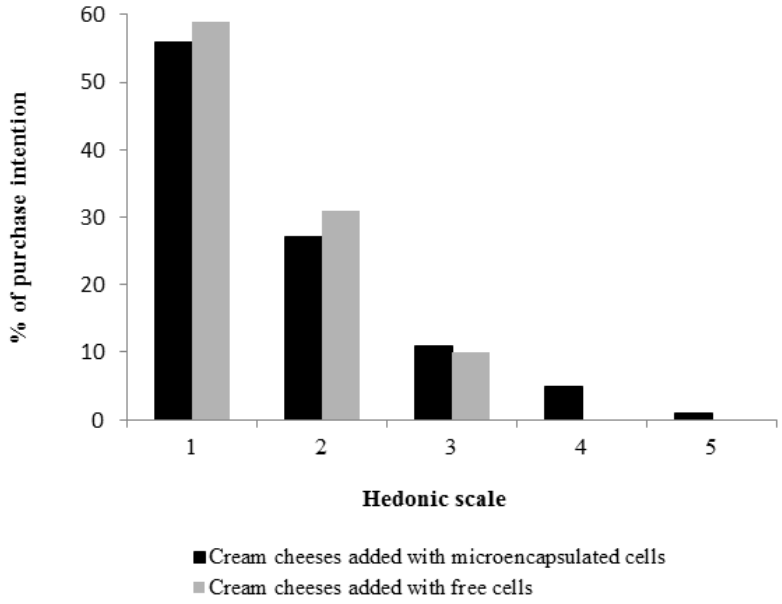
The sensory evaluation of the requeijões added with microencapsulated and free *L. plantarum* CCMA 0359 cells is showed in Table 2. The scores obtained by consumer's evaluations regarding the appearance, aroma, taste and texture attributes were between 8 and 9 in the hedonic scale of 9 points, which corresponding to "liked very much" and "extremely liked". These data suggest that the addition of microencapsulated and free *L. plantarum* CCMA 0359 cells in the requeijão had no negative effect on the sensory properties and acceptance of the product by consumers.

Table 2: Sensory analysis of requeijão added with microencapsulated and free cells of *L. plantarum* CCMA 0359.

Atributes	Requeijão with microencaspulated *L. plantarum* CCMA 0359 cells	Requeijão with free *L. plantarum* CCMA 0359 cells
Appearance	8.06 ± 1.02	8.53 ± 0.70
Aroma	7.99 ± 1.18	7.93 ± 1.16
Taste	7.90 ± 1.25	8.24 ± 0.98
Texture	8.37 ± 0.92	8.44 ± 0.81
Overall appearance	8.11 ± 1.03	8.35 ± 0.77

Figure 3 shows the purchase intention of the cream cheeses added with microencapsulated and free *L. plantarum* CCMA 0359 cells. According to the results, 90 % of the consumers "certainly would buy" or "probably would buy" the requeijão containing free cells, while the percentage for the requeijão added with microencapsulated cells was 83%.

Figure 2 - Purchase intention of requeijões added with microencapsulated and free cells of *L. plantarum* CCMA 0359.



4 CONCLUSION

The present study showed that microencapsulation of *L. plantarum* CCMA 0359 by spray drying using whey powder as wall material was successfully used to improve cell survival during refrigerated storage (7 °C) and simulated gastrointestinal conditions when added in requeijão. The populations of microencapsulated and free *L. plantarum* CCMA 0359 cells added in the requeijão remained above of 8 log CFU/g after 90 days of storage, which is above of the minimum recommended for probiotic food products. The addition of microencapsulated or free *L. plantarum* CCMA 0359 cells did not affect the acceptability of the requeijão as the consumers classified the products as “liked very much” and “extremely liked”. Furthermore, more than 50 % of the consumers “certainly would buy” the requeijões added with microencapsulated of free probiotic cells. Therefore, o requeijão is an alternative for a new vehicle for microencapsulated or free probiotic cells showing high acceptability by the consumers.

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5 REFERENCES

BAMPI, G. B. et al. Spray Chilling Microencapsulation of *Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. lactis and Its Use in the Preparation of Savory Probiotic Cereal Bars. **Food and Bioprocess Technology**, v. 9, p. 1422–1428, 2016.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Departamento de Inspeção de Produtos de Origem Animal. *Regulamento técnico de identidade e qualidade do requeijão*. Portaria nº 359 de 04 de setembro de 1997. Disponível em: <http://www.defesa.agricultura.sp.gov.br/legislacoes/portaria-ma-359-d,675.html>. Acesso em: 10 Dez. 2018.

BRINQUES, G. B.; AYUB, M. A. Z. Effect of microencapsulation on survival of *Lactobacillus plantarum* in simulated gastrointestinal conditions, refrigeration and yogurt. **Journal of Food Engineering**, v. 103, n. 2, p. 123–128, 2011.

CORCORAN, B. M. et al. Comparative survival of probiotic lactobacilli spray-dried in the presence of prebiotic substances. **Journal of Applied Microbiology**, v. 96, n. 5, p. 1024–1039, 2004.

DE ANDRADE, D. P. et al. Stability of microencapsulated lactic acid bacteria under acidic and bile juice conditions. **International Journal of Food Science and Technology**, 2019.

DE CASTRO-CISLAGHI, F. P. et al. *Bifidobacterium* Bb-12 microencapsulated by spray drying with whey: Survival under simulated gastrointestinal conditions, tolerance to NaCl and viability during storage. **Journal of Food Engineering**, v. 113, n. 2, p. 186–193, 2012.

DIMITRELLOU, D. et al. Survival of spray dried microencapsulated *Lactobacillus casei* ATCC 393 in simulated gastrointestinal conditions and fermented milk. **LWT - Food Science and Technology**, v. 71, p. 169–174, 2016.

DOLLY, P. et al. Microencapsulation of *Lactobacillus plantarum* (mtcc 5422) by spray-freeze-drying method and evaluation of survival in simulated gastrointestinal conditions. **Journal of microencapsulation**, v. 28, n. 6, p. 568–574, 2011.

DUCROTTÉ, P.; SAWANT, P.; JAYANTHI, V. Clinical trial: *Lactobacillus plantarum* 299v (DSM 9843) improves symptoms of irritable bowel syndrome. **World Journal of Gastroenterology**, v. 18, n. 30, p. 4012–4018, 2012.

ECKERT, C. et al. Microencapsulation of *Lactobacillus plantarum* ATCC 8014 through spray drying and using dairy whey as wall materials. **LWT - Food Science and Technology**, v. 82, p. 176–183, 2017.

EL-SALAM, M. H. A.; EL-SHIBINY, S. Preparation and properties of milk proteins-based encapsulated probiotics: a review. **Dairy Science & Technology**, p. 1–20, 2015.

FERREIRA, D. F. Sisvar: a computer statistical analysis system. **Ciência e Agrotecnologia**, v. 35, p. 1039–42, 2011.

GOLOWCZYC, M. A. et al. Cellular injuries of spray-dried *Lactobacillus* spp . isolated from kefir and their impact on probiotic properties. **International Journal of Food Microbiology**, v. 144, n. 3, p. 556–560, 2011.

ILHA, E. C. et al. *Lactobacillus paracasei* isolated from grape sourdough : acid, bile, salt and heat tolerance after spray drying with skim milk and cheese whey. **European Food Research and Technology**, v. 240, p. 977–984, 2015.

JIANG, M. et al. Evaluation of probiotic properties of *Lactobacillus plantarum* WLPL04 isolated from human breast milk. **Journal of Dairy Science**, v. 99, p. 1–11, 2016.

MANTZOURIDOU, F.; SPANOU, A.; KIOSSEOGLOU, V. An inulin-based dressing emulsion as a potential probiotic food carrier. **Food Research International**, v. 46, n. 1, p. 260–269, 2012.

MARTÍN, M. J. et al. Microencapsulation of bacteria: A review of different technologies and their impact on the probiotic effects. **Innovative Food Science and Emerging Technologies**, v. 27, p. 15–25, 2015.

MATTILA-SANDHHOLM, T. et al. Technological challenges for future probiotic foods. **International Dairy Journal**, v. 12, p. 173–182, 2002.

MOUSA, A. et al. Evaluation of physiochemical, textural, microbiological and sensory characteristics in set yogurt reinforced by microencapsulated *Bifidobacterium bifidum* F-35. **International Journal of Food Science and Technology**, v. 49, n. 7, p. 1673–1679, 2014.

NAZZARO, F. et al. Microencapsulation in food science and biotechnology. **Current Opinion in Biotechnology**, v. 23, n. 2, p. 182–186, 2012.

ORTAKCI, F.; SERT, S. Stability of free and encapsulated *Lactobacillus acidophilus* ATCC 4356 in yogurt and in an artificial human gastric digestion system. **Journal of Dairy Science**, v. 95, n. 12, p. 6918–6925, 2012.

PEDROSO, D. DE L. et al. Protection of *Bifidobacterium lactis* and *Lactobacillus acidophilus* by microencapsulation using spray-chilling. **International Dairy Journal**, v. 26, n. 2, p. 127–132, 2012.

PICOT, A.; LACROIX, C. Encapsulation of bifidobacteria in whey protein-based microcapsules and survival in simulated gastrointestinal conditions and in yoghurt. **International Dairy Journal**, v. 14, n. 6, p. 505–515, 2004.

RAJAM, R.; ANANDHARAMAKRISHNAN, C. Microencapsulation of *Lactobacillus plantarum* (MTCC 5422) with fructooligosaccharide as wall material by spray drying. **LWT - Food Science and Technology**, v. 60, n. 2, p. 773–780, 2015.

RAMOS, C. L. et al. Strain-specific probiotics properties of *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Lactobacillus brevis* isolates from Brazilian food products. **Food Microbiology**, v. 36, n. 1, p. 22–29, 2013.

RANADHEERA, C. S. et al. Microencapsulation of *Lactobacillus acidophilus* LA-5, *Bifidobacterium animalis* subsp . lactis BB-12 and *Propionibacterium jensenii* 702 by spray drying in goat's milk. **Small Ruminant Research**, v. 123, n. 1, p. 155–159, 2015.

RIBEIRO, M. C. E. et al. Effect of microencapsulation of *Lactobacillus acidophilus* LA-5 on physicochemical, sensory and microbiological characteristics of stirred probiotic yoghurt. **Food Research International**, v. 66, p. 424–431, 2014.

SHAMAEI, S. et al. Microencapsulation of walnut oil by spray drying: Effects of wall material and drying conditions on physicochemical properties of microcapsules. **Innovative Food Science and Emerging Technologies**, v. 39, p. 101–112, 2017.

SHORI, A. B. Influence of food matrix on the viability of probiotic bacteria: A review based on dairy and non-dairy beverages. **Food Bioscience**, v. 13, p. 1–8, 2016.

TRIPATHI, M. K.; GIRI, S. K. Probiotic functional foods: Survival of probiotics during processing and storage. **Journal of Functional Foods**, v. 9, n. 1, p. 225–241, 2014.

TULUMOGLU, S.; KAYA, H. I.; SIMSEK, O. Probiotic characteristics of *Lactobacillus fermentum* strains isolated from Tulum cheese. **Anaerobe**, v. 30, p. 120–125, 2014.

VERRUCK, S. et al. Survival of *Bifidobacterium* BB-12 microencapsulated with full-fat goat's milk and prebiotics when exposed to simulated gastrointestinal conditions and thermal treatments. **Small Ruminant Research**, v. 153, n. February, p. 48–56, 2017.

VILLANUEVA, N.D.M.; SILVA, M.A.A.P.da. Comparative performance of the nine- point hedonic, hybrid and self-adjusting scales in the generation of internal preference maps. **Food quality and preference**, v. 20, p. 1–12, 2009.

VINDEROLA, G. et al. Cell viability and functionality of probiotic bacteria in dairy products. **Frontiers in Microbiology**, v. 2, p. 1–6, 2011.

WANG, J. et al. Fermentation characteristics and transit tolerance of probiotic *Lactobacillus casei* Zhang in soymilk and bovine milk during storage. **Journal of Dairy Science**, v. 92, n. 6, p. 2468–2476, 2009.

WEICHSELBAUM, E. Probiotics and health: A review of the evidence. **Nutrition Bulletin**, v. 34, n. 4, p. 340–373, 2009.