Carlise Beddin Fritzen Freire

MICROENCAPSULAÇÃO DE *Bifidobacterium* BB-12 COM AGENTES PREBIÓTICOS PELO MÉTODO DE *SPRAY DRYING:* CARACTERIZAÇÃO E APLICAÇÃO DAS MICROCÁPSULAS

Tese submetida ao Programa de Pós-Graduação em Ciência dos Alimentos, do Centro de Ciências Agrárias, da Universidade Federal de Santa Catarina para a obtenção do título de Doutor em Ciência dos Alimentos.

Orientador: Profª. Drª. Elane Schwinden Prudêncio

Florianópolis

2013

Ficha de identificação da obra elaborada pelo autor,
através do Programa de Geração Automática da Biblioteca Universitária da UFSC.

Freire, Carlise Beddin Fritzen
Microencapsulação de Bifidobacterium BB-12 com agentes prebióticos pelo método de spray drying: caracterização e aplicação das microcápsulas / Carlise Beddin Fritzen Freire ; orientadora, Elane Schwinden Prudêncio - Florianópolis, SC, 2013.
170 p.

Tese (doutorado) - Universidade Federal de Santa Catarina, Centro de Ciências Agrárias. Programa de Pós-Graduação em Ciência dos Alimentos.

Inclui referências

1. Ciência dos Alimentos. 2. Microencapsulação. 3. Probióticos. 4. Prebióticos. 5. Creme de ricota. I. Prudêncio, Elane Schwinden. II. Universidade Federal de Santa Catarina. Programa de Pós-Graduação em Ciência dos Alimentos. III. Título.

FOLHA DE APROVAÇÃO

“Para conhecer as coisas, há que dar-lhes a volta.”

José Saramago

AGRADECIMENTOS

A Deus, por guiar e iluminar o meu caminho.

À minha família amada - pai (Artemio), mãe (Lourdes), irmãs (Maurícia e Lucélia), cunhados (Alexandre e Fred) e ao meu querido Alexandre - por todo apoio e por estarem sempre ao meu lado.

À Universidade Federal de Santa Catarina e ao Programa de Pós-Graduação em Ciência dos Alimentos, pela oportunidade de realizar o doutorado.

À Profa. Elane Prudêncio, pela confiança, orientação, dedicação e grande entusiasmo neste trabalho.

A todos os coautores desta tese, pela valiosa parceria.

À Profa. Carmen Müller, pela solicitude e ensinamentos na área de textura dos alimentos.

Ao Prof. Fábio Murakami, pelo auxílio com as análises térmicas.

À Profa. Cleide Vieira, pela colaboração com as análises microbiológicas.

À Profa. Renata Amboni, pela tutoria durante o pós-doutorado da Profa. Elane, pelos conhecimentos compartilhados na área de análise sensorial e pelo constante apoio.

Às queridas colegas do Laboratório de Leite e Derivados pela ajuda e boa companhia.

Ao Prof. Ernani Sant'Anna, pelo apoio e orientação no início do doutorado e por disponibilizar a estrutura do Laboratório de Biotecnologia Alimentar para a realização das análises microbiológicas.

À Fabiane Picinin de Castro Cislaghi e à Juliana Lorenz, pelos valiosos ensinamentos na área da microencapsulação.

Ao Laboratório Central de Microscopia Eletrônica (LCME) da UFSC, pela utilização do Microscópio Eletrônico de Varredura.

À banca, por aceitar o convite de participação e pelas contribuições fornecidas.

Aos julgadores da análise sensorial; a todos os professores, funcionários e colegas do Departamento de Ciência e Tecnologia de Alimentos.

À FAPESC, pela bolsa de estudo e pelo financiamento deste trabalho; ao CNPq pelo apoio financeiro.

À empresa Clariant, pela doação dos prebióticos.

A todos que contribuíram para a realização deste trabalho. Muito obrigada!

RESUMO

Este trabalho visou primeiramente o desenvolvimento e caracterização de microcápsulas contendo o micro-organismo probiótico *Bifidobacterium* BB-12, microencapsulado com substituição parcial (1:1) de leite desnatado reconstituído (LDR) por agentes prebióticos (inulina, inulina enriquecida com oligofrutose e oligofrutose) através da técnica de *spray drying*. A caracterização das microcápsulas compreendeu as seguintes análises: viabilidade, morfologia, tamanho de partícula, umidade, atividade de água, solubilidade, higroscopicidade, cor, propriedades térmicas, rendimento, sobrevivência em condições gastrintestinais simuladas e após tratamento térmico. Posteriormente, as microcápsulas que apresentaram o melhor desempenho nas análises anteriores foram aplicadas em creme de ricota, e o produto foi avaliado quanto às propriedades microbiológicas, físicas, químicas e sensoriais. A partir dos resultados obtidos, pôde-se observar uma elevada sobrevivência da bifidobactéria microencapsulada, tanto durante o processo de *spray drying*, como ao longo do armazenamento, sendo que as maiores contagens foram observadas nas microcápsulas produzidas com LDR e inulina enriquecida com oligofrutose. Além disso, de maneira geral, os resultados da caracterização física sugerem uma maior estabilidade para as microcápsulas produzidas com prebióticos. A utilização dos prebióticos inulina e inulina enriquecida com oligofrutose melhorou o rendimento da encapsulação, além de conferir maior proteção contra as condições gastrintestinais simuladas e os tratamentos térmicos e, por isso, as formulações contendo estes prebióticos foram escolhidas para a aplicação em creme de ricota. As contagens ao longo do armazenamento para as amostras de creme de ricota com bifidobactéria microencapsulada foram maiores do que para as amostras com células livres, sendo que as microcápsulas produzidas com LDR e inulina mostraram a maior viabilidade. Os valores de sólidos totais e firmeza foram maiores para as amostras com microcápsulas. Todas as amostras apresentaram um perfil similar para os valores de pH, acidez, sólidos totais e cor. De acordo com os julgadores, todas as amostras de creme de ricota apresentaram boa aceitabilidade sensorial. Desta forma, os resultados obtidos neste trabalho sugerem que a microencapsulação por *spray drying* pode ser usada como um efetivo método para manter a sobrevivência de *Bifidobacterium* BB-12, e que a combinação dos agentes encapsulantes LDR e inulina foi a mais indicada para a aplicação em creme de ricota.

Palavras-chave: microencapsulação, *spray drying*, *Bifidobacterium* BB-12, probióticos, prebióticos, creme de ricota.

ABSTRACT

This study aimed primarily the development and the characterization of microcapsules containing *Bifidobacterium* BB-12, microencapsulated by spray drying with partial replacement (1:1) of reconstituted skim milk (RSM), with prebiotics (inulin, oligofructose-enriched inulin and oligofructose). The characterization of microcapsules comprised the following analyses: viability, morphology, particle size, moisture content, water activity, solubility, hygroscopicity, color, thermal properties, yield, survival in simulated gastrointestinal conditions and after heat treatments. Subsequently, the microcapsules that showed better performance in the previous analyses were applied in ricotta cream, and the product was evaluated to microbiological, physical, chemical and sensory properties. From the results obtained, we observed a high survival of microencapsulated bifidobacteria, during the spray drying process and storage. The highest counts were observed in the microcapsules produced with LDR and oligofructose-enriched inulin. Furthermore, in general, the physical characterization results suggest a greater stability to the microcapsules produced with prebiotics. The use of prebiotics inulin and oligofructose-enriched inulin improved the encapsulation yield, besides conferring greater protection against simulated gastrointestinal conditions and heat treatments and thus, the microcapsules formulations containing these prebiotics were chosen for application in ricotta cream. The count during storage for samples of ricotta cream with microencapsulated bifidobacteria was higher than for the samples with free cells, and the microcapsules produced with LDR and inulin showed the greater viability. The values of total solids and firmness were higher for samples with microcapsules. All the samples showed a behavior similar to the values of pH, acidity, total solids and color. According to the panelists, all samples of ricotta cream showed good sensory acceptability. Thus, the results obtained in this study suggest that microencapsulation by spray drying can be used as an effective method for maintaining the survival of *Bifidobcterium* BB-12, and that the combination of encapsulating agents LDR and inulin was the most suitable for the application in ricotta cream.

Keywords: microencapsulation, spray drying, *Bifidobacterium* BB-12, probiotics, prebiotics, ricotta cream.

LISTA DE FIGURAS

Capítulo 1

Capítulo 2

Capítulo 3

Capítulo 4

LISTA DE TABELAS

LISTA DE ABREVIATURAS E SIGLAS

CFU - Colony-Forming Unit

DP - *Degree of Polymerization*

DSC - *Differential Scanning Calorimetry*

EY - *Encapsulation Yield*

LDR - Leite Desnatado Reconstituído

MEV - Microscopia Eletrônica de Varredura

MRS agar - DeMan-Rogosa-Sharpe agar

RH - *Relative Humidity*

RSM - *Reconstituted Skim Milk*

SEM - *Scanning Electron Microscope*

TGA - *Thermogravimetric Analysis*

UFC - Unidades Formadoras de Colônias

UHT - *Ultra High Temperature*

SUMÁRIO

INTRODUÇÃO

O desenvolvimento de novos produtos alimentícios torna-se cada vez mais desafiador, à medida que procura atender à demanda dos consumidores por produtos que sejam além de atrativos, também saudáveis (KOMATSU; BURITI; SAAD, 2008). Estes fatores têm colaborado para o desenvolvimento de uma série de alimentos funcionais enriquecidos com componentes fisiologicamente ativos, como os probióticos (ANNUNZIATA; VECCHIO, 2013).

Probióticos são micro-organismos vivos que, quando administrados em quantidades adequadas, conferem benefícios à saúde do hospedeiro (FAO/WHO, 2006). A influência benéfica dos probióticos sobre a microbiota intestinal humana inclui fatores como efeitos antagônicos, competição e efeitos imunológicos, resultando em um aumento da resistência contra patógenos (BOYLSTON et al., 2004). Dentre os diversos micro-organismos probióticos, os pertencentes aos gêneros *Lactobacillus* e *Bifidobacterium* figuram como os mais empregados em alimentos (KARIMI; SOHRABVANDI; MORTAZAVIAN, 2012), enquanto que os derivados lácteos são os alimentos mais utilizados para a incorporação destes micro-organismos (SAAD, 2006). Neste contexto, diferentes tipos de queijos vêm sendo utilizados como carreadores de probióticos (CRUZ et al., 2009), onde o creme de ricota, um tipo de queijo cremoso inovador, obtido a partir da homogeneização da ricota com outros ingredientes, poderia ser empregado para tal função. Além disso, Gusso et al. (2012) ressaltam as excelentes propriedades nutricionais do creme de ricota, como os

elevados teores de proteínas do soro do leite e de aminoácidos essenciais.

Para o desenvolvimento de um alimento probiótico, é fundamental que este apresente propriedades sensoriais aceitáveis e uma validade média considerada satisfatória, com uma contagem de células viáveis probióticas maior do que 6 log UFC mL^{-1} ou g^{-1} do produto (FARIA; BENEDET; GUERROUE, 2006). Entretanto, De Vos et al. (2010) relatam que os benefícios gerados à saúde dos consumidores pelos micro-organismos probióticos podem ser limitados devido à sensibilidade destes micro-organismos a elevados teores de oxigênio, sal e ácidos, bem como ao tempo e à temperatura de armazenamento dos alimentos aos quais são incorporados. Desta forma, pode-se verificar que a manutenção da funcionalidade dos probióticos na matriz láctea está relacionada a fatores do processamento e armazenamento deste tipo de alimento (GRANATO et al., 2010). Com a finalidade de superar estas limitações, métodos de microencapsulação têm sido aplicados aos micro-organismos probióticos, servindo como uma "barreira" física e, portanto, sendo uma proteção capaz de manter vivos estes micro-organismos no alimento (KOMATSU; BURITI; SAAD, 2008).

Dentre os métodos de microencapsulação existentes, o mais utilizado pela indústria de alimentos é o de *spray drying* porque apresenta baixo custo e alta capacidade de produção de cápsulas (GHARSALLAOUI et al., 2007). Neste método, o agente encapsulante, que exercerá a função de "barreira" física ao micro-organismo, deve possuir alta solubilidade em água, baixa viscosidade a altas concentrações e ser um emulsionante eficiente. Corcoran et al. (2004) relatam que compostos prebióticos, como a inulina e a oligofrutose,

poderiam funcionar como bons agentes encapsulantes de probióticos no processo de microencapsulação pelo método de *spray drying* e com isso, auxiliar na sobrevivência destes micro-organismos durante o processamento e armazenamento dos alimentos. No entanto, pesquisas envolvendo a caracterização e a manutenção de concentrações adequadas do micro-organismo probiótico *Bifidobacterium* BB-12 microencapsulado com diferentes agentes prebióticos pelo método de *spray drying*, e a aplicação destas microcápsulas em creme de ricota, são inexistentes. Desta forma, este trabalho, apresentado na forma de artigos, está dividido nos seguintes capítulos:

(a) Capítulo 1: **Revisão Bibliográfica**, abordando os principais temas envolvidos no trabalho, ou seja, probióticos, microencapsulação, agentes encapsulantes, caracterização das microcápsulas obtidas por *spray drying*, aplicação de probióticos microencapsulados em derivados lácteos.

(b) Capítulo 2: **Microencapsulação de bifidobactéria por *spray drying* na presença de prebióticos**, cujo objetivo foi avaliar o efeito da substituição parcial (1:1) do leite desnatado reconstituído (LDR) por agentes prebióticos (inulina, inulina enriquecida com oligofrutose e oligofrutose) na viabilidade de *Bifidobacterium* BB-12, microencapsulada por *spray drying*, durante o armazenamento por 180 dias a 4 °C e a - 18 °C, bem como caracterizar as microcápsulas em relação às suas propriedades físicas.

(c) Capítulo 3: **Efeito da microencapsulação na sobrevivência de *Bifidobacterium* BB-12 exposta a condições gastrintestinais simuladas e ao tratamento térmico**, cujo objetivo foi avaliar a sobrevivência de *Bifidobacterium BB-12* microencapsulada por *spray drying* com substituição parcial (1:1) do LDR por agentes prebióticos (inulina, inulina enriquecida com oligofrutose e oligofrutose), durante o processo de encapsulação (rendimento), em condições gastrintestinais simuladas e após tratamentos térmicos.

(d) Capítulo 4: **Efeito da aplicação de *Bifidobacterium* BB-12 microencapsulada com prebióticos por *spray drying* nas propriedades de creme de ricota**, cujo objetivo foi avaliar o efeito da aplicação de *Bifidobacterium* BB-12 microencapsulada com LDR e prebióticos (inulina e inulina enriquecida com oligofrutose), nas propriedades microbiológicas, físico-químicas, de textura, de cor e sensoriais de creme de ricota durante 60 dias de armazenamento.

Vale ressaltar que a escolha das microcápsulas (com LDR e os prebióticos inulina e inulina enriquecida com oligofrutose) para aplicação no creme de ricota (Capítulo 4), foi realizada de acordo com os resultados obtidos na caracterização de todas as microcápsulas estudadas (Capítulos 2 e 3).

Os artigos publicados em revistas indexadas (Anexos A, B e C) e os comprovantes dos trabalhos parciais publicados em eventos científicos da área de Ciência dos Alimentos (Anexo D) estão apresentados em anexo.

CAPÍTULO 1

Revisão Bibliográfica

1 Revisão Bibliográfica

1.1 Probióticos

Probióticos são definidos como micro-organismos vivos, administrados em quantidades adequadas, que conferem benefícios à saúde do hospedeiro (FAO/WHO, 2006). O termo probiótico significa "para vida" e é utilizado para designar a presença de bactérias benéficas para o organismo humano. No começo do século XX, o médico francês Henry Tissier observou que crianças com diarreia apresentavam, em suas fezes, um baixo número de bifidobactérias, sendo que essas bactérias eram abundantes em crianças saudáveis. Ele sugeriu que as bifidobactérias poderiam ser isoladas e administradas em pacientes com diarreia, com a finalidade de ajudar a restaurar a microbiota intestinal saudável, prevenindo assim maiores infecções (STANTON et al., 2005).

Os micro-organismos probióticos mais empregados em alimentos são pertencentes aos gêneros *Lactobacillus* e *Bifidobacterium* (KARIMI; SOHRABVANDI; MORTAZAVIAN, 2012). Estes gêneros têm sido isolados de todas as porções do trato gastrintestinal saudável, sendo que o íleo terminal e o cólon parecem ser, respectivamente, os locais de preferência para a colonização intestinal dos lactobacilos e das bifidobactérias (BIELECKA; BIEDRZYCKA; MAJKOWSKA, 2002).

Os componentes do gênero *Bifidobacterium* são classificados como bastonetes Gram-positivos, não formadores de esporos, sem motilidade e anaeróbios estritos (ANAL; SINGH, 2007). A aparência dos bastonetes é bastante variável e pode ser influenciada pelas condições nutricionais do meio, enquanto o grau de tolerância ao

oxigênio e o meio de crescimento, dependem da espécie e da morfologia (CORBO et al., 2001; TAMIME, 2002). Dentre os micro-organismos deste gênero, as espécies mais utilizadas industrialmente são *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium longum* e *Bifidobacterium animalis*, incluindo a cepa BB-12 (MASCO et al., 2005).

Segundo Saad (2006), a correção das propriedades da microbiota desbalanceada constitui a racionalidade da terapia por probióticos. Dentre os benefícios à saúde do hospedeiro atribuídos à ingestão de culturas probióticas, os que mais se destacam são a diminuição da população de patógenos (KOTZAMPASSI; GIAMARELLOS-BOURBOULIS, 2012), a estabilização da microbiota intestinal (SAAD et al., 2013), o aumento da absorção de minerais (NITSCHKE; UMBELINO, 2002), o alívio da constipação (WEICHSELBAUM, 2009), a estimulação do sistema imune (AURELI et al., 2011), a prevenção de infecções urogenitais (ANUKAM et al., 2006), os efeitos inibitórios sobre a mutagenicidade (COMMANE et al., 2005), a diminuição do risco de doença cardiovascular, a redução dos níveis séricos de colesterol e os efeitos anti-hipertensivos (NAGPAL et al., 2012). As bifidobactérias ainda são capazes de produzir as vitaminas B1, B2, B6, B12, ácido nicotínico e fólico (KOLIDA; TUOHY; GIBSON, 2002) e têm ação adjunta no metabolismo de aminoácidos (MARTÍÑEZ-VILLALUENGA et al., 2006). Entretanto, é a resistência contra patógenos a característica mais promissora no desenvolvimento de produtos probióticos eficazes (SAAD, 2006). A modulação da microbiota intestinal pelos micro-organismos probióticos ocorre através de um mecanismo denominado “exclusão competitiva”. Este mecanismo

impede a colonização da mucosa intestinal por micro-organismos potencialmente patogênicos, através da competição por nutrientes e/ou por produção de compostos antimicrobianos (GUARNER; MALAGELADA, 2003).

A seleção de bactérias probióticas tem como base os seguintes critérios preferenciais: (a) segurança para uso humano, (b) a estabilidade frente ao ácido e à bile, (c) a capacidade de aderir na mucosa intestinal e (d) a capacidade de colonizar, ao menos temporariamente, o trato gastrintestinal humano (OLIVEIRA et al., 2002). Outros critérios fundamentais são a segurança para uso humano, o histórico de não patogenicidade e não estarem associadas a outras doenças (STANTON et al. 2005).

Na produção de um alimento probiótico é fundamental que o micro-organismo possa ser cultivado em escala industrial, sendo que o produto final deve ter vida média satisfatória, propriedades sensoriais aceitáveis e um número de células viáveis presentes no produto maior do que 6 log UFC mL^{-1} ou g^{-1} durante toda a sua validade (FARIA; BENEDET; GUERROUE, 2006). Saad (2006) afirma que a necessidade de manter um número elevado de micro-organismos viáveis no produto final é decorrente da dose mínima diária recomendada, cerca de 8 – 9 log células viáveis, obtidas pelo consumo de 100 gramas de produto com 6 -7 log células viáveis mL^{-1} ou g^{-1}, sendo que estes produtos devem ser consumidos regularmente para manter o efeito dos micro-organismos na composição da microbiota intestinal.

Os micro-organismos probióticos têm sido amplamente adicionados em produtos lácteos, como iogurtes (AWAISHEH; HADDADIN; ROBINSON, 2005; KAILASAPATHY; HARMSTORF;

PHILLIPS, 2008), sorvetes (AKIN; AKIN; KIRMACI, 2007; AKALIN; ERISIR, 2008; TURGUT; CAKMAKCI, 2009), sobremesas (ARAGON-ALEGRO et al., 2007; BURITI; CASTRO; SAAD, 2010) e diferentes tipos de queijo (VINDEROLA et al., 2009; BERGAMINI et al., 2010; FRITZEN-FREIRE et al., 2010; ALVES et al., 2013). No entanto, Granato et al. (2010) relatam que a manutenção da funcionalidade dos probióticos na matriz láctea depende de várias condições intrínsecas do processamento e armazenamento, como ambientes ácidos, elevados níveis de oxigênio, tempo e temperatura, além do trânsito através do trato gastrintestinal. Objetivando solucionar estas limitações, diversas pesquisas têm focado a proteção das culturas probióticas durante a produção, armazenamento e passagem do alimento pelo trato gastrintestinal através da utilização de técnicas de microencapsulação (RODRÍGUEZ-HUEZO et al., 2007; ANNAN; BORZA; HANSEN, 2008; KIM et al., 2008; DING; SHAH, 2009; SEMYONOV et al., 2010).

1.2 Microencapsulação

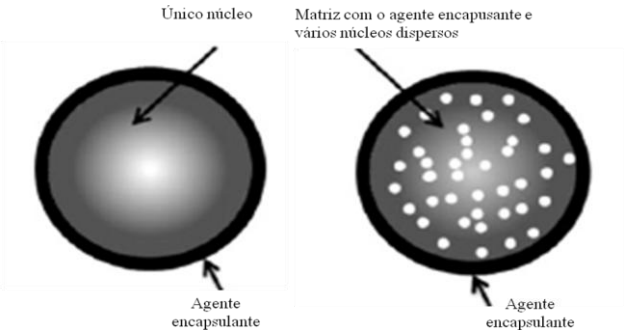
O conceito de microencapsulação tem sua origem na idealização do modelo celular, no qual o núcleo é envolvido por uma membrana semipermeável que o protege do meio externo e ao mesmo tempo controla a entrada e a saída de substâncias na célula (RÉ, 2006). De modo semelhante, a microcápsula consiste em uma camada de material de cobertura que atua como um filme protetor, isolando a substância ativa e evitando os efeitos de sua exposição inadequada (ROCHA; FÁVARO-TRINDADE; GROSSO, 2012).

Microencapsulação é o processo de empacotamento de materiais sólidos, líquidos ou gasosos em cápsulas extremamente pequenas, as quais podem liberar o conteúdo de forma controlada e sob condições específicas (ANAL; STEVENS, 2005). O material a ser encapsulado é denominado núcleo ou material ativo, enquanto o material que forma o revestimento é chamado de material de parede, carreador ou agente encapsulante (MADENE et al., 2006). O tamanho das microcápsulas pode variar de alguns poucos micrômetros até milímetros (GHARSALLAOUI et al., 2007), a forma também é bastante variável em função do método, do material ativo e do agente encapsulante utilizados para prepará-las (FÁVARO-TRINDADE; PINHO; ROCHA, 2008). Dentre os diferentes grupos morfológicos obtidos pelos métodos de microencapsulação, dois deles são mais comumente visualizados: (1) mononucleares: onde o material ativo fica concentrado próximo ao centro, rodeado pelo agente encapsulante ou, (2) multinucleares: onde o agente encapsulante forma uma rede tridimensional com vários materiais ativos, que podem estar adsorvidos, incorporados ou ligados covalentemente à matriz (Figura 1) (FANG; BHANDARI, 2010).

A microencapsulação tem sido utilizada com sucesso na área de alimentos visando à proteção de substâncias sensíveis à luz, oxigênio, tempo e temperatura de armazenamento, além de impedir interações com outros compostos, estabilizando o produto, e consequentemente, aumentando a sua validade (DESAI; PARK, 2005). Entre os materiais que podem ser encapsulados para aplicação na indústria alimentícia destacam-se as vitaminas, aminoácidos, hidrolisados proteicos, ácidos, aromatizantes, minerais, corantes, enzimas e micro-organismos (FÁVARO-TRINDADE; PINHO; ROCHA, 2008). Outro objetivo da

microencapsulação é permitir a liberação controlada do material ativo (ANAL; SINGH, 2007). No entanto, as características de liberação variam de acordo com a natureza do agente encapsulante e normalmente ocorrem devido a mecanismos como: variação de temperatura e de pH, ruptura mecânica, dissolução em solventes, ação de força osmótica, ação de enzimas, difusão e permeabilidade seletiva (GOUIN, 2004).

Figura 1 - Representação da estrutura física das microcápsulas (a) mononucleares e (b) multinucleares.



Fonte: Burgain et al. (2011).

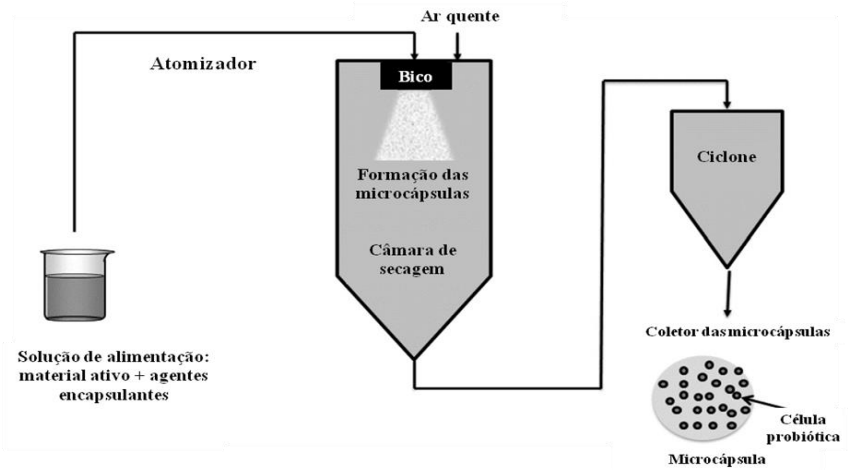
Vários métodos de microencapsulação têm sido empregados na indústria de alimentos, sendo que a seleção de cada método é dependente da aplicação que será dada à microcápsula, do tamanho desejado, do mecanismo de liberação e das propriedades físico-químicas, tanto do material ativo, quanto do agente encapsulante (FÁVARO-TRINDADE; PINHO; ROCHA, 2008) (Tabela 1).

Tabela 1 – Principais métodos utilizados para microencapsulação, com as respectivas faixas de tamanho das partículas.

Métodos de encapsulação	**Materiais encapsuláveis**	**Faixa de tamanho (μm)**
Métodos físicos		
Extrusão estacionária	Líquido/sólido/gás	1.000 - 6.000
Extrusão centrífuga	Líquido/sólido/gás	125 - 3.000
Spray drying	Líquido/sólido	5 - 150
Spray chilling e *spray cooling*	Líquido/sólido	20 - 200
Leito fluidizado	Sólido	>100
Co-cristalização	Sólido/líquido	-
Liofilização	Líquido	-
Métodos químicos		
Polimerização interfacial	Líquido/sólido	1 - 500
Inclusão molecular	Líquido	5 - 50
Polimerização *in situ*	Líquido/sólido	1 - 500
Métodos físico-químicos		
Coacervação	Líquido/sólido	1 - 500
Lipossomas	Líquido/sólido	0,02 - 3
Lipoesferas	Líquido/sólido	0,02 - 10
Evaporação do solvente	Líquido/sólido	1 - 5.000

Fonte: Desai e Park (2005) e Madene et al. (2006).

Figura 2 - Representação esquemática da microencapsulação no equipamento *spray dryer*.



Fonte: Burgain et al. (2011).

A formação das microcápsulas por *spray drying* é resultado da rápida perda de umidade da gotícula aspergida pelo atomizador e formação de uma matriz rígida composta pelo agente encapsulante. O processo resulta em partículas esféricas devido à suspensão livre das gotículas líquidas no meio gasoso (GHARSALLAOUI et al., 2007). Por outro lado, a eficiência na retenção do material ativo no núcleo está relacionada à parâmetros do processo (temperatura de secagem e tamanho de gotícula formada), características do agente encapsulante (tamanho das moléculas, solubilidade) e características do material ativo (polaridade, pressão de vapor, tamanho de molécula) (RÉ, 1998).

De acordo com Pérez-Alonso et al. (2003), os agentes encapsulantes utilizados no método de *spray drying* devem possuir alta

solubilidade em água, baixa viscosidade a altas concentrações e serem emulsionantes eficientes. Estes autores relatam ainda que a seleção dos agentes encapsulantes para microencapsulação por *spray drying* tem tradicionalmente
envolvido procedimentos de tentativa e erro. Assim, a medida que as microcápsulas são produzidas, avaliações do rendimento da microencapsulação, das propriedades físicas, da estabilidade ao longo do armazenamento e do comportamento de liberação *in vitro* tornam-se necessárias (FÁVARO-TRINDADE; PINHO; ROCHA, 2008).

Devido à sua versatilidade e ao pequeno tempo de residência dos produtos na câmara de secagem, o *spray drying* tornou-se um importante método para a microencapsulação de materiais que apresentam sensibilidade ao calor, como os micro-organismos probióticos (ROKKA; RANTAMÄKI, 2010). Estudos têm mostrado que os probióticos podem ser significativamente protegidos através do método de *spray drying* em uma série de agentes encapsulantes, incluindo o leite desnatado reconstituído (LDR) (SIMPSON et al., 2005; SILVA et al., 2011) e alguns carboidratos, como por exemplo, os prebióticos (CORCORAN et al., 2004; ANANTA; VOLKERT; KNORR, 2005; CHEN et al., 2005). No entanto, Meng et al. (2008), ressaltam a importância do controle da temperatura de saída das microcápsulas da câmara de secagem do equipamento, já que este é o parâmetro que mais influencia na taxa de sobrevivência dos micro-organismos durante o processo.

1.3 Agentes encapsulantes

Dentre os agentes encapsulantes mais testados (sozinhos ou em associações) pelo método de *spray drying*, destacam-se os biopolímeros provenientes de várias fontes, como as gomas naturais (goma arábica, alginatos, carragenas), maltodextrinas, amidos, gelatina e derivados do leite, como o leite desnatado reconstituído (GHARSALLAOUI et al., 2007).

O leite desnatado reconstituído (LDR) é o agente encapsulante que tem mostrado melhor efeito na sobrevivência de micro-organismos probióticos durante o processo de *spray drying* (FU; CHEN, 2011). Este agente, resultante da solubilização em água do leite desnatado em pó, apresentou melhores efeitos protetivos sobre probióticos em concentrações entre 10 % e 20 %, quando comparado à goma arábica (LIAN; HSIAO; CHOU, 2002), gelatina (LIAN; HSIAO; CHOU, 2002), amido (LIAN; HSIAO; CHOU, 2002), maltodextrina (REDDY; MADHU; PRAPULLA, 2009) e polidextrose (ANANTA; VOLKERT; KNORR, 2005). Corcoran et al. (2004) atribui às características químicas do meio o efeito protetor do LDR, uma vez que é uma solução aquosa rica em proteínas, lactose e minerais.

De acordo com Silva et al. (2011), o leite desnatado é capaz de prevenir as injúrias celulares dos micro-organismos, através da estabilização da membrana celular, devido à presença das proteínas do leite, que fornecem uma camada protetora às células. No entanto, estes autores também relatam que a proteção conferida pelo LDR é dependente do micro-organismo utilizado e dos parâmetros empregados no *spray dryer*. Por outro lado, Ananta, Volkert e Knorr (2005) sugerem

que a substituição parcial deste agente encapsulante por outros, como os prebióticos, poderia aumentar a proteção dos micro-organismos tanto durante o *spray drying*, como durante o armazenamento das microcápsulas.

1.3.1 Prebióticos

O termo prebiótico foi introduzido por Gibson e Roberfroid (1995) e passou a englobar ingredientes alimentares não digeríveis com atividade bifidogênica, ou seja, capazes de estimular o crescimento e/ou a atividade de algumas bactérias presentes no cólon, afetando beneficamente o hospedeiro. Segundo estes autores, carboidratos não digeríveis (oligossacarídeos e polissacarídeos), alguns peptídeos e proteínas e certos lipídeos (ésteres) são considerados ingredientes prebióticos.

Os seguintes efeitos são atribuídos aos prebióticos: (a) estimulação seletiva da multiplicação das bactérias benéficas no cólon; (b) potencial para reprimir patógenos e limitar virulência por imunoestimulação; (c) modulação de funções fisiológicas, como a absorção de cálcio e o metabolismo lipídico e; (e) redução do risco de câncer de cólon. (SAAD et al., 2013). Os prebióticos atuam mais frequentemente no intestino grosso, embora possam ter algum efeito no intestino delgado (KOLIDA; TUOHY; GIBSON, 2002). Após a fermentação dos prebióticos, alguns metabólitos são liberados pelas bactérias do trato intestinal, em especial os ácidos graxos de cadeia curta como, por exemplo, o acético, o butírico e o propiônico. Estes ácidos podem atuar diretamente ou indiretamente sobre as células intestinais

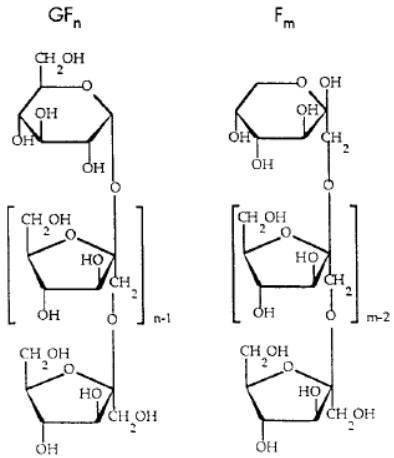
participando do controle de processos de inflamação, carcinogênese e eliminação de compostos nitrogenados (SAAD, 2006).

Os micro-organismos alvos de prebióticos são espécies dos gêneros *Lactobacillus* e *Bifidobacterium* (VULEVIC; RASTALL; GIBSON, 2004), enquanto que os frutanos, inulina e oligofrutose, são os prebióticos mais utilizados como ingredientes funcionais em alimentos (KOLIDA; TUOHY; GIBSON, 2002). O notável interesse pelos frutanos advém do fato desses compostos serem resistentes às enzimas digestivas e, portanto, não digeridos pelo organismo humano, atuando como fibras alimentares solúveis. Consequentemente, chegam ao intestino grosso intactos e são fermentados por bifidobactérias presentes no cólon (ROBERFROID, 2007). Devido ao potencial sinérgico entre probióticos e prebióticos, a combinação destes ingredientes é definida como simbiótica, a qual beneficia o hospedeiro devido ao aumento da sobrevivência e implantação dos micro-organismos vivos no sistema gastrintestinal (CASIRAGHI et al., 2007). A interação entre o probiótico e o prebiótico *in vivo* pode ser favorecida por uma adaptação do probiótico ao substrato prebiótico anterior ao consumo. Isto pode, em alguns casos, resultar em uma vantagem competitiva para o probiótico, se ele for consumido juntamente com o prebiótico (SAAD, 2006).

Dentre os prebióticos, a inulina e a oligofrutose são os mais estudados e com propriedades bem estabelecidas (GIBSON et al., 2004). A inulina é um carboidrato de reserva naturalmente presente nas plantas da família *Asteraceae* (RONKART et al., 2009). No entanto, a espécie *Cichorium intybus* (chicória) é a mais utilizada para a extração industrial de inulina (ROBERFROID, 2007), enquanto a oligofrutose é obtida a partir da inulina (GIBSON et al., 2004).

Quimicamente, os frutanos do tipo inulina são carboidratos polidispersos compostos por cadeias lineares de unidades de frutose, ligadas entre si por ligações β 2-1, com ou sem uma unidade final de glicose (Figura 3) (ROBERFROID, 2007). Dependendo do comprimento da cadeia, definido pelo número de unidades de monossacarídeos, denominado grau de polimerização (DP), tem-se a inulina e a oligofrutose (SAAD, 2006). O grau de polimerização (DP) da inulina extraída da chicória varia entre 2 e 60. A oligofrutose (DP 2-9) é produzida pela hidrólise parcial da inulina utilizando uma endoinulinase (EC 3.2.1.7) (BIEDRZYCKA; BIELECKA, 2004).

Figura 3 - Estrutura química dos frutanos do tipo inulina. G= glicose; F= frutose; n ou m indicam o número de unidades de frutose nas moléculas.



Fonte: Roberfroid, Van Loo e Gibson (1998).

Grande parte dos prebióticos atualmente comercializados no mercado mundial é produzida pela empresa belga Beneo-Orafti (ROBERFROID, 2007). Além do processo enzimático para a obtenção da oligofrutose (Orafti® oligofrutose), a empresa também utiliza técnicas de separação física, visando eliminar todos os oligômeros com DP < 10, e com isso, produzir frutanos do tipo inulina com alta massa molar (DP entre 10 e 60). Este tipo de inulina é comercializado com a denominação Orafti® HP (*high performance*) e apresenta-se termicamente mais estável e menos solúvel do que a inulina nativa (FRANCK, 2002). A partir de uma combinação específica da inulina HP e da oligofrutose (1:1), obtêm-se a "inulina enriquecida com oligofrutose", denominada comercialmente como Orafti® Sinergy 1, que foi formulada a fim de melhorar a digestibilidade e a absorção de cálcio (ROBERFROID, 2007). No entanto, os efeitos prebióticos dos frutanos do tipo inulina dependem tanto da composição da microbiota intestinal de cada indivíduo, como do grau de polimerização das cadeias de frutose (COUDRAY et al., 2003). Biedrzycka e Bielecka (2004) mostraram que frutanos de cadeia curta, como a oligofrutose, atuam mais intensamente na região proximal do cólon, enquanto a inulina é mais efetiva na parte distal. Estes autores ainda sugerem o uso de misturas de oligofrutose e inulina com o objetivo de melhorar a atividade prebiótica no intestino.

O comprimento da cadeia também pode afetar algumas propriedades tecnológicas dos frutanos do tipo inulina. A oligofrutose é muito mais solúvel e doce do que a inulina nativa, com cerca de um terço do poder adoçante da sacarose e com um baixo valor calórico (1–2 kcal/g) (FRANCK, 2002). Por estas propriedades, tem sido usada como substituta parcial/total da sacarose em alimentos (DE CASTRO et al.,

2009; VILLEGAS et al., 2010). Já a inulina, tem a habilidade de formar microcristais, imperceptíveis na boca, quando misturada à água ou ao leite, promovendo uma textura finamente cremosa, semelhante a da gordura (FRANCK, 2002). Desta maneira, tem sido utilizada como substituta de gordura (KIP; MEYER; JELLEMA, 2006; PASEEPHOL; SMALL; SHERKAT, 2008).

Finalmente, outro benefício dos frutanos do tipo inulina seria relativo ao seu uso como agentes encapsulantes na microencapsulação de probióticos, podendo melhorar a sobrevivência destes micro-organismos durante o processamento e armazenamento de alimentos (CORCORAN et al., 2004; CHEN et al., 2005).

1.4 Caracterização das microcápsulas obtidas por *spray drying*

A utilização de diferentes agentes encapsulantes na produção de microcápsulas pelo método de *spray drying* pode resultar em pós com diferentes propriedades, dependendo da estrutura de cada agente encapsulante (TONON et al., 2009). Segundo Barbosa-Cánovas e Juliano (2005), o conhecimento das propriedades das microcápsulas é essencial para otimizar processos, funcionalidades e para reduzir custos.

Propriedades físicas como umidade, atividade de água e higroscopicidade são essenciais no que diz respeito à estabilidade e armazenamento das microcápsulas, enquanto a solubilidade está diretamente relacionada à reconstituição dos pós (TONON et al., 2009). A microestrutura, por sua vez, está ligada à funcionalidade e às propriedades de escoamento. O método mais utilizado para análise da microestrutura das microcápsulas é a microscopia eletrônica de

varredura (MEV), através da qual é possível visualizar a eficiência da microencapsulação (ALLAN-WOJTAS; HANSEN; PAULSON, 2008). Segundo Jobin et al. (2005) a utilização da MEV no estudo da microencapsulação pode contribuir com informações sobre a faixa de tamanho das microcápsulas, além de possíveis alterações na matriz contendo os agentes encapsulantes. De acordo com O'hagan et al. (2005), o tamanho das partículas também está diretamente relacionado à capacidade de reidratação, solubilidade e dispersibilidade, bem como a mistura de componentes. Estes autores também relatam que o tamanho das partículas pode influenciar o sabor, a cor, a textura e o odor do produto final, características que irão determinar se um produto será viável economicamente. Propriedades térmicas, como a calorimetria e a termogravimetria, são ferramentas importantes para a caracterização de microcápsulas, já que através destas técnicas é possível obter informações relacionadas à variação de massa, estabilidade térmica, água livre, pureza, ponto de fusão, ponto de ebulição, calores de transição, entre outras (IONASHIRO, 2004). Por outro lado, o conhecimento das características das microcápsulas em condições gastrintestinais simuladas e após tratamento térmico é essencial em pesquisas envolvendo a microencapsulação de probióticos. Mandal, Puniya e Singh (2006) afirmam que para as bactérias probióticas manterem a alta viabilidade e exercerem as suas atividades no intestino, devem suportar as barreiras naturais do trato gastrintestinal, além das altas temperaturas de processamento em que determinados alimentos são submetidos. Além disso, Cook et al. (2012) relatam que após a administração dos probióticos, ocorre uma considerável perda de células viáveis durante o contato com o baixo pH estomacal e com as elevadas

concentrações de bile no intestino. Desta forma, estes autores sugerem que a microencapsulação de probióticos seria um método efetivo para reduzir a perda de viabilidade o longo do trato gastrintestinal.

1.5 Aplicação de probióticos microencapsulados em derivados lácteos

Pesquisas estão proporcionando à indústria o desenvolvimento de técnicas capazes de controlar e melhorar a estrutura física e a composição química de produtos alimentares funcionais (URALA; LÄHTEENMÄKI, 2007). Sandoval-Castilla et al. (2010) estudaram a microencapsulação do micro-organismo probiótico *Lactobacillus casei* com alginato de sódio e pectina pela técnica de extrusão, com aplicação em iogurte, e obtiveram um produto com maior resistência ao ácido e à bile e com maior estabilidade durante o armazenamento. Homayouni et al. (2008) também observaram uma sobrevivência maior de *Lactobacillus casei* e *Bifidobacterium lactis* em sorvetes, quando microencapsulados com uma combinação de amido resistente e alginato, pela técnica de emulsificação. Özer et al. (2009), em um estudo realizado com o queijo branco turco, observaram uma elevada viabilidade dos micro-organismos *Lactobacillus acidophilus* e *Bifidobacterium bifidum*, microencapsulados pelas técnicas de extrusão e emulsificação, utilizando alginato de sódio e carragena, respectivamente. Assim como estes autores que obtiveram resultados positivos no emprego de micro-organismos probióticos microencapsulados, Zomorodi et al. (2011) verificaram a viabilidade de três micro-organismos probióticos na forma livre e microencapsulada

(por extrusão com alginato de sódio), em queijo branco iraniano durante 60 dias, e constataram uma maior sobrevivência dos micro-organismos microencapsulados ao longo do armazenamento. Além disso, Buriti, Cardarelli e Saad (2008) relatam que a manutenção da viabilidade de micro-organismos probióticos em queijos cremosos durante o armazenamento torna-se um desafio, o que estimula a incorporação destes micro-organismos na forma microencapsulada.

O creme de ricota é um tipo de queijo cremoso que surge no mercado como uma opção saudável e ao mesmo tempo saborosa para os consumidores de derivados lácteos (GUSSO et al., 2012). Este tipo de queijo é obtido a partir da homogeneização de uma massa de queijo ricota juntamente com outros ingredientes (leite, soro, manteiga, creme, hidrocolóides, sal, entre outros). A ricota, empregada como massa base de queijos cremosos, contém excelentes propriedades nutricionais, devido ao seu baixo teor de gordura e elevado teor de proteínas do soro do leite (CONCEIÇÃO et al., 2009). Este queijo é obtido pela precipitação das proteínas do soro por meio de acidificação, adicionado ou não de 20% de leite, seguido de aquecimento a 92 ºC (RIBEIRO et al., 2005).

O processo de fabricação de queijos cremosos requer agitação e aquecimento da mistura de ingredientes (DIMITRELI; THOMAREIS, 2004). Segundo Kalab (1985) a homogeneização com aquecimento é uma etapa essencial para fabricação de queijos cremosos, uma vez que ocorre modificação na rede proteica, produzindo um produto com boa espalhabilidade. Van Dender (2006) relata que a consistência cremosa é obtida quando o grande agregado proteico presente na massa sofre ruptura, apresentando um considerável aumento da área superficial das

partículas de proteína, tornando-as capazes de absorver grandes quantidades de água. Ainda de acordo com este autor, o processo de cremificação é causado por um conjunto de forças de natureza física, química e térmica, que se aplica à matéria-prima. Desta forma, a alta umidade do produto implica em significativo volume de fase aquosa com partículas de gordura e proteína não tão densamente agregadas, o que facilita a mobilidade dos constituintes durante o espalhamento (KALAB; MODLER, 1985). No entanto, os queijos cremosos apresentam uma tendência de diminuição do pH durante o seu armazenamento, com consequente aumento da acidez (BURITI et al., 2007). Singh, Drake e Cadwallader (2003) relatam que o pH é um parâmetro importante para a identidade e qualidade dos queijos, afetando diretamente a sua estrutura e propriedades físicas. Estes autores também relatam que as modificações proteolíticas e microbiológicas que contribuem para o sabor e textura dos queijos, são fortemente influenciadas pelo pH.

Referências

AKALIN, A. S.; ERSIR, D. Effects of inulin and oligofructose on the rheological characteristics and probiotic culture survival in low-fat probiotic ice cream. **Journal of Food Science**, v. 73, p.184-188, 2008.

AKIN, M. B., AKIN, M. S., KIRMACI, Z. Effects of inulin and sugar levels on the viability of yoghurt and probiotic bacteria and the physical and sensory characteristics in probiotic ice-cream. **Food Chemistry**, v. 104, p. 93–99, 2007.

ALLAN-WOJTAS, P.; TRUELSTRUP HANSEN L.; PAULSON, A. T. Microstructural studies of probiotic bacteria-loaded alginate microcapsules using standard electron microscopy techniques and

anhydrous fixation. **LWT – Food Science Technology**, v.41, p. 101-108, 2008.

ALVES, L. L.; RICHARDS, N. S. P. S.; MATTANNA, P.; ANDRADE, D. F.; REZER, A. P. S.; MILANI, L. I. G., CRUZ, A. G. ; FARIA, J. A. F. Cream cheese as a symbiotic food carrier using *Bifidobacterium animalis* Bb-12 and *Lactobacillus acidophilus* La-5 and inulin. **International Journal of Dairy Technology**, v. 66, p. 63–69, 2013.

AMERICAN PUBLIC HEALTH ASSOCIATION – APHA. **Compendium of methods of the microbiological examination of foods.** 4th. Edition. Washington D.C., 2001. 676p.

ANAL, A. K.; STEVENS, W. F. Chitosan-alginate multilayer beads for controlled release of ampicillin. **International Journal of Pharmaceutics**, v. 290, p. 45-54, 2005.

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

ANANTA, E.; VOLKERT, M.; KNORR, D. Cellular injuries and storage stability of spray-dried *Lactobacillus rhamnosus* GG. **International Dairy Journal**, v.15, p.399–409, 2005.

ANN, E. Y.; KIM, Y.; OH, S. J.; IMM J. Y.; PARK, D. J.; HAN, K. S.; KIM, S. H. Microencapsulation of *Lactobacillus acidophilus* ATCC 43121 with prebiotic substrates using a hybridisation system. **International Journal of Food Science and Technology**, v. 42, p.411-419, 2007.

ANNAN, N. T.; BORZA, A. D.; TRUELSTRUP HANSEN, L. Encapsulation in alginate coated gelatin microspheres improves survival of the probiotic *Bifidobacterium adolescentis* 15703T during exposure to simulated gastro-intestinal conditions. **Food Research International**, v.41, p.184–193, 2008.

ANNUNZIATA, A.; VECCHIO, R. Consumer perception of functional foods: A conjoint analysis with probiotics. **Food Quality and Preference**, v.28, p.348-355, 2013.

ANUKAM, K. C.; OSAZUWA, E.; OSEMENE, G. I.; EHIGIAGBE, F.; BRUCE, A. W.; REID, G. Clinical study comparing probiotic *Lactobacillus* GR-1 and RC-14 with metronidazole vaginal gel to treat symptomatic bacterial vaginosis. **Microbes and Infection**, v.8, p. 2772-2776, 2006.

ARAGON-ALEGRO, L. C.; ALEGRO, J. H. A.; CARDARELLI, H. R.; CHIU, M. C.; SAAD, S. M. I. Probiotic and synbiotic chocolate mousse. **LWT – Food Science and Technology**, v.40, p.669-675, 2007.

ARYANA, K. J.; MCGREW, P. Quality attributes of yogurt with *Lactobacillus casei*
and various prebiotics. **LWT - Food Science and Technology**, v. 40, p. 1808-1814, 2007.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS - AOAC. **Official methods of analysis of the association analytical chemists**. 18th. Edition. Maryland,USA, 2005.

AURELI, P.; CAPURSO L.; CASTELLAZZI, A. M.; CLERICI, M.; GIOVANNINI, M.; MORELLI, L.; POLI, A.; PREGLIASCO, F.; SALVINI, F.; ZUCCOTTI, G. V. Probiotics and health: An evidence-based review. **Pharmacological Research**, v. 63, p.366–376, 2011.

AWAISHEH, S. S.; HADDADIN, M. S. Y.; ROBINSON, R. K. I. Incorporation of selected nutraceuticals and probiotic bacteria into fermented milk. **International Dairy Journal**, *v.*15, *p.*1184-1190, 2005.

BARBOSA-CÁNOVAS, G.V.; JULIANO, P. **Physical and chemical properties of food powders**. In: Encapsulated and Powdered Foods. Boca Raton, FL, USA: Taylor & Francis. p. 39-71, 2005.

BARCLAY, T.; GINIC-MARKOVICA, M.; COOPER, P.; PETROVSKY, N. Inulin—A versatile polysaccharide with multiple pharmaceutical and food chemical uses. **Journal of Excipients and Food Chemicals**, v. 1, p. 27–50, 2010.

BERGAMINI, C.; HYNES, E. MEINARDI, C.; SUÁREZ, V.; QUIBERONI, A.; ZALAZAR, C. Pategrás cheese as a suitable carrier

for six probiotic cultures**. Journal of Dairy Research**, v. 77, p. 265–272, 2010.

BIEDRZYCKA, E.; BIELECKA, M. Prebiotic effectiveness of fructans of different degrees of polymerization. **Trends in Food Science & Technology**, v. 15, p. 170–175, 2004.

BIELECKA, M.; BIEDRZYCKA, E.; MAJKOWSKA, A. Selection of probiotics and prebiotics for synbiotics and confirmation of their in vivo effectiveness. **Food Research International**, v.35, p.125-131, 2002.

BLECKER, C.; CHEVALIER, J. P.; FOUGNIES, C.; VAN HERCK, J. C.; DEROANNE, C.; PAQUOT, M. Characterisation of different inulin samples by DSC: Influence of polymerisation degree on melting temperature. **Journal of Thermal Analysis and Calorimetry**, v. 71, p. 215–224, 2003.

BOHM, A.; KAISER, I.; TREBSTEIN, A.; HENLE, T. Heat-induced degradation of inulin. **European Food Research and Technology**, v. 220, p. 466-471, 2005.

BOSSCHER, D.; VAN LOO, J.; FRANK, A. Inulin and oligofructose as functional ingredients to improve bone mineralization. **International Dairy Journal**, v. 16, p. 1092-1097, 2006.

BOYLSTON, T. D.; VINDEROLA, C. G.; GHODDUSI, H. B.; REINHEIMER, J. A. Incorporation of bifidobacteria into cheeses: Challenges and rewards**. International Dairy Journal**, v.14, p.375-387, 2004.

BURGAIN, J.; GAIANI, C.; LINDER, M.; SCHER, J. Encapsulation of probiotic living cells: From laboratory scale to industrial Applications. **Journal of Food Engineering**, v. 104, p. 467-483, 2011.

BURITI, F. C. A.; CARDARELLI, H. R.; FILISETTI, T. M. C. C.; SAAD, S. M. I. Synbiotic potential of fresh cream cheese supplemented with inulin *and Lactobacillus paracasei* in co-culture with *Streptococcus thermophilus*. **Food Chemistry,** v.104, p.1605-1610, 2007.

BURITI, F. C. A.; CARDARELLI, H. R.; SAAD, S. M. I. Textura instrumental e avaliação sensorial de queijo fresco cremoso simbiótico: implicações da adição de *Lactobacillus paracasei* e inulina. **Revista Brasileira de Ciências Farmacêuticas**, v.44, p.75-84, 2008.

BURITI, F. C. A.; CASTRO, I. A.; SAAD, S. M. I. Viability of *Lactobacillus acidophilus* in synbiotic guava mousses and its survival under in vitro simulated gastrointestinal conditions. **International Journal of Food Microbiology**, v. 137, p. 121–129, 2010.

CAPELLAS, M.; MOR-MUR, M.; SENDRA, E.; GUAMIS, B. Effect of high-pressure processing on physico-chemical characteristics of fresh goats' milk cheese (*Mató*). **International Dairy Journal**, v.11, p.165-173, 2001.

CASIRAGHI, M. C.; CANZI, E.; ZANCHI, R.; DONATI, E.; VILLA, L. Effects of a synbiotic milk product on human intestinal Ecosystem. **Journal of Applied Microbiology**, v. 103, p. 499-506, 2007.

CHANDRAMOULI, V.; KAILASAPATHY, K.; PEIRIS, P.; JONES, M. An improved method of microencapsulation and its evaluation to protect *Lactobacillus* spp. in simulated gastric conditions. **Journal of Microbiological Methods**, v. 56, p. 27– 35, 2004.

CHÁVARRI, M.; MARAÑÓN, I.; ARES, R.; IBÁÑEZ, F. C.; MARZO, F.; VILLARÁN, M. D. C. Microencapsulation of a probiotic and prebiotic in alginate-chitosan capsules improves survival in simulated gastro-intestinal conditions. **International Journal of Food Microbiology**, v. 142, p. 185-189, 2010.

CHÁVEZ, B. E.; LEDEBOER, A. M. Drying of probiotics: optimization of formulation and process to enhance storage survival. **Drying Technology**, v. 25, p.193-201, 2007.

CHEN, K. N.; CHEN, M. J.; LIU, J. R.; LIN, C. W.; CHIU, H. Y. Optimization of incorporated prebiotics as coating materials for probiotic microencapsulation. **Journal of Food Science**, v.70, p.M260-M266, 2005.

CHEN, M. J.; CHEN, K. N.; KUO, Y.-T. Optimal Thermotolerance of *Bifidobacterium bifidum* in Gellan-Alginate Microparticles. **Biotechnology and Bioengineering**, v. 98, p. 411-419, 2007.

COMMANE, D.; HUGHES, R.; SHORTT, C.; ROWLAND, I. The potential mechanisms involved in the anti-carcinogenic action of probiotics. **Mutation Research**, v. 591, p. 276–289, 2005.

CONCEIÇÃO, A. C.; SILVA, M. R.; OLIVEIRA, V. S.; SOARES, B. G.; MARTINS, M. L.; MARTINS, A. D. O. Avaliação da utilização de cloreto de cálcio em substituição ao ácido lático para fabricação de ricota. **Revista do Instituto de Laticínios Cândido Tostes**, v.64, n.369, p.32-38, 2009.

COOK, M. T.; TZORTZIS, G.; CHARALAMPOPOULOS, D.; KHUTORYANSKIY, V. V. Microencapsulation of probiotics for gastrointestinal delivery**. Journal of Controlled Release**, v.162, p. 56-67, 2012.

CORBO, M. R.; ALBENZIO, M.; DE ANGELIS, M.; SEVI, A.; GOBBETTI, M. Microbiological and biochemical properties of Canestrato Pugliese hard cheese supplemented with bifidobacteria. **Journal of Dairy Science**, v.84, p.551–561, 2001.

CORCORAN, B. M.; ROSS, R. P.; FITZGERALD, G. F.; STANTON, C. Comparative survival of probiotic lactobacilli spray-dried in the presence of prebiotic substances. **Journal of Applied Microbiology**, v.96, p.1024–1039, 2004.

CORCORAN, B. M.; STANTON, C.; FITZGERALD, G.; ROSS, R. P. Life under stress: The probiotic stress response and how it may be manipulated. **Current Pharmaceutical Design**, v. 14, p. 1382-1399, 2008.

COUDRAY, C.; TRESSOL, J. C.; GUEUX, E.; RAYSSIGUIER, Y. Effects of inulin-type fructans of different chain length and type of branching on intestinal absorption and balance of calcium and magnesium in rats. **European Journal of Nutrition**, v.42, p.91–98, 2003.

CRUZ, A. G.; BURITI, F. C. A.; SOUZA, C. H. B.; FARIA, J. A. F.; SAAD, S. M. I. Probiotic cheese: health benefits, technological and stability aspects. **Trends in Food Science & Technology**, v.20, p.344-354, 2009.

DE CASTRO, F. P.; CUNHA, T. M.; BARRETO, P. L. M.; AMBONI, R. D. D. M.; PRUDÊNCIO, E. S. Effect of oligofructose incorporation on the properties of fermented probiotic lactic beverages. **International Journal of Dairy Technology**, v.62, p.68–74, 2009.

DE VOS, P.; FAAS, M. M.; SPASOJEVIC, M.; SIKKEMA, J. Encapsulation for preservation of functionality and targeted delivery of bioactive food components. **International Dairy Journal**, v. 20, p. 292-302, 2010.

DESAI, K. G. H.; PARK, H. J. Recent developments in microencapsulation of food ingredients. **Drying Technology**, v.23, p.1361-1394, 2005.

DESMOND, C.; ROSS, R. P.; O'CALLAGHAN, E.; FITZGERALD, G.; STANTON, C. Improved survival *of Lactobacillus paracasei* NFBC 338 in spray-dried powders containing gum acacia. **Journal of Applied Microbiology**, v. 93, p. 1003–1011, 2002.

DIMITRELI, G.; THOMAREIS, A.S. Effect of temperature and chemical composition on processed cheese apparent viscosity. **Journal of Food Engineering**, v. 64, p.265–271, 2004.

DING, W. K.; SHAH, N. P. Acid, Bile, and Heat Tolerance of Free and Microencapsulated Probiotic Bacteria. **Journal of Food Science**, v. 72, p. M446- M450, 2007.

DING, W. K.; SHAH, N. P. An improved method of microencapsulation of probiotic bacteria for their stability in acidic and bile conditions during storage. **Journal of Food Science**, v.74, p.53-61, 2009.

EL-TINAY, A. H.; ISMAIL, I. A. Effect of some additives and processes on the characteristics of agglomerated and granulated spray-dried Roselle powder. **Acta Alimentaria Hungaricae**, v. 14, p. 283–295, 1985.

FANG, Z. X.; BHANDARI, B. Encapsulation of polyphenols - a review. **Trends in Food Science & Technology**, v. 21, p. 510-523, 2010.

FAO/WHO. **Probióticos en los alimentos**. Propiedades saludables y nutricionales y directrices para la evaluación: Estudio FAO Alimentación y Nutrición, Roma, 2006. 45p. Disponível em: ftp://ftp.fao.org/docrep/fao/009/a0512s/a0512s00.pdf. Acesso em 11 de junho de 2013.

FARIA, C. P.; BENEDET, H. D.; GUERROUE, J. L. Parâmetros de produção de leite de búfala fermentado por *Lactobacillus casei*. **Pesquisa Agropecuária Brasileira**, v.41, p.511-516, 2006.

FÁVARO-TRINDADE, C. S.; PINHO, S. C.; ROCHA, G. A. Revisão: Microencapsulação de ingredientes alimentícios. **Brazilian Journal of Food Technology**, v. 11, p. 103-112, 2008.

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

FRITZEN-FREIRE, C. B.; MÜLLER, C. M. O.; LAURINDO, J. B.; PRUDÊNCIO E. S. The influence of *Bifidobacterium* Bb-12 and lactic acid incorporatin on the properties of Minas Frescal cheese. **Journal of Food Engineering**, v. 96, p. 621-627, 2010.

FRITZEN-FREIRE, C. B.; PRUDÊNCIO, E. S.; AMBONI, R. D. M. C.; PINTO, S. S.; NEGRÃO-MURAKAMI, A. N.; MURAKAMI, F. S. Microencapsulation of bifidobacteria by spray drying in the presence of prebiotics. **Food Research International**, v. 45, p. 306-312, 2012.

FU, N.; CHEN, X. D. Towards a maximal cell survival in convective thermal drying processes. **Food Research International**, v. 44, p.1127–1149, 2011.

GHARSALLAOUI, A.; ROUDAUT, G.; CHAMBIN, O.; VOILLEY, A.; SAUREL, R. Applications of spray-drying in microencapsulation of food ingredients. **Food Research International**, v. 40, p. 1107-1121, 2007.

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.

GIBSON, G. R.; PROBERT, H. M.; VAN LOO, J.; RASTALL, R. A.; ROBERFROID, M. B. Dietary modulation of the human colonic microbiota: Updating the concept of prebiotics. **Nutrition Research Review**, v. 17, p. 259–275, 2004.

GOUIN, S. Microencapsulation: industrial appraisal of existing technologies and
trends. **Food Science and Technology**, v. 15, p. 330-347, 2004.

GRANATO, D.; BRANCO, G. F.; CRUZ, A. G.; FARIA, J. A. F.; SHAH, N. P. Probiotic dairy products as functional foods. **Comprehensive Reviews in Food Science and Food Safety**, v. 9, p. 455–470, 2010.

GUARNER, F.; MALAGELADA, J. R. Gut flora in health and disease. **Lancet**, v.360, p.512-518, 2003.

GUSSO, A. P.; MATTANNA, P.; PELLEGRINI, L. G.; CASSANEGO, D. B.; RICHARDS, N. S. P. S.; RIBEIRO, A. S. Comparação de diferentes métodos analíticos para quantificação de lipídios em creme de ricota. **Revista do Instituto de Laticínios Cândido Tostes**, v.67, p. 51-55, 2012.

HOMAYOUNI, A.; AZIZI, A.; EHSANI M. R.; YARMAND M. S.; RAZAVI S. H. Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of synbiotic ice cream. **Food Chemistry**, v.111, p.50-55, 2008.

INTERNATIONAL DAIRY FEDERATION- IDF. **Dried milk and dried cream: Determination of water content**. International Dairy

Federation standard 26A. Brussels, Belgium: International Dairy Federation. 1993.

INSTITUTO ADOLFO LUTZ- IAL. **Normas Analíticas do Instituto Adolfo Lutz***:*Métodos físico-químicos para análise de alimentos (4 ed.), São Paulo, Brasil, 2005.

IONASHIRO, M., **Giolito: Fundamentos da Termogravimetria e Análise Térmica Diferencial/ Calorimetria Exploratória Diferencial**, São Paulo: Giz editorial, 2004.

IYER, C.; KAILASAPATHY, K. Effect of co-encapsulation of probiotics with prebiotics on increasing the viability of encapsulated bacteria in simulated gastrointestinal conditions and in yoghurt. **Journal of Food Science**, v. 70, p. M18-M23, 2005.

JOBIN, G.; GRONDIN, G.; COUTURE, G.; BEAULIEU, C. Microscopic examination of chitosan-polyphosphate beads with entrapped spores of the biocontrol agent *Streptomyces melanosporofaciens* EF-76. **Microscopy and Microanalysis**, v. 11, p.154–165, 2005.

KAILASAPATHY, K.; HARMSTORF, I.; PHILLIPS, M. Survival of *L. acidophilus* and *B. animalis* ssp. *Lactis* in stirred fruit yogurts. **LWT – Food Science and Technology**, v. 41, p. 1317-1322, 2008.

KALAB, M. Microstructure of dairy foods. 2. Milk products based on fat. **Journal of Dairy Science**, v. 68, p.3234-3241, 1985.

KALAB, M.; MODLER, H. W. Milk gel structure. XV. Electron microscopy of whey protein-based cream cheese spread. **Milchwissenschaft**, v. 40, p. 193-196, 1985.

KARIMI, R.; SOHRABVANDI, S.; MORTAZAVIAN, A. M. Review Article: Sensory Characteristics of Probiotic Cheese. **Comprehensive Reviews in Food Science and Food Safety**, v.11, p. 437-452, 2012.

KIM, S. J.; CHO, S.Y.; KIM, S.H .; SONG, O. J.; SHIN, S.; CHA, D. S; PARK, H. J. Effect of microencapsulation on viability and other

characteristics in *Lactobacillus acidophilus* ATCC 43121. **LWT – Food Science and Technology**, v. 41, p. 493–500, 2008.

KIP, P.; MEYER, D.; JELLEMA, R. H. Inulins improve sensoric and textural properties of low-fat yoghurts. **International Dairy Journal**, v.16, p.1098-1103, 2006.

KOLIDA, S.; TUOHY, K.; GIBSON, G. R. Prebiotic effects of inulin and oligofructose. **British Journal of Nutrition**, v.87, p.193-197, 2002.

KOMATSU, T. R.; BURITI, F. C. A.; SAAD, S. M. I. Inovação, persistência e criatividade superando barreiras no desenvolvimento de alimentos probióticos. **Revista Brasileira de Ciências Farmacêuticas**, v. 44, p. 329-347, 2008.

KOTZAMPASSI, K.; GIAMARELLOS-BOURBOULIS, E. J. Probiotics for infectious diseases: more drugs, less dietary supplementation. **International Journal of Antimicrobial Agents**, v. 40, p. 288-296, 2012.

KRASAEKOOPT, W.; BHANDARI, B.; DEETH, H. The influence of coating materials on some properties of alginate beads and survivability of microencapsulated probiotic bacteria. **International Dairy Journal**, v. 14, p. 737–743, 2004.

LIAN, W. C.; HSIAO, H. C.; CHOU, C. C. Survival of bifidobacteria after spray-drying. **International Journal of Food Microbiology**, v. 74, p. 79-86, 2002.

MACÊDO, R. O.; MOURA, O. M.; SOUZA, A. G.; MACÊDO, A. M. C. Comparative studies on some analytical methods: Thermal decomposition of powder milk. **Journal of Thermal Analysis**, v.49, p.857–862, 1997.

MADENE, A.; MURIEL, J.; SCHER, J.; DESOBRY, S. Flavour encapsulation and controlled release – a review. **International Journal of Food Science and Technology**, v. 41, p. 1-21, 2006.

MANDAL, S.; PUNIYA, A. K; SINGH, K. Effect of alginate concentration on survival of encapsulated *Lactobacillus casei* NCDC-298. **International Dairy Journal**, v.16, p. 1190-1195, 2006.

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

MARTÍNEZ-CERVERA, S.; SALVADOR, A.; MUGUERZA, B.; MOULAY, L.; FISZMAN, S. M. Cocoa fibre and its application as a fat replacer in chocolate muffins. **LWT - Food Science and Technology**, v. 44, p. 729–736, 2011.

MARTÍÑEZ-VILLALUENGA, C.; FRÍAS, J.; GÓMEZ, R.; VIDAL-VALVERDE, C. Influence of addition of raffinose family oligosaccharides on probiotic survival in fermented milk during refrigerated storage. **International Dairy Journal**, v.16, p.768-774, 2006.

MASCO, L.*;* HUYS, G.*;* DE BRANDT, E.*;* TEMMERMAN, R.; SWINGS, J. Culture-dependent and culture-independent qualitative analysis of probiotic products claimed to contain bifidobacteria. **International Journal of Food Microbiology,** *v.*102, *p.*221–230, 2005.

MEILGAARD, M.; CIVILLE, G. V.; CARR, B. T. **Sensory evaluation techniques**. 4.ed., Boca Raton, FL.: CRC Press, 2007. 448p.

MENG, X. C.; STANTON, C.; FITZGERALD, G. F.; DALY, C.; ROSS, R. P. Anhydrobiotics: the challenges of drying probiotic cultures. **Food Chemistry**, v. 106, p. 1406–1416, 2008.

NAGPAL, R.; KUMAR, A.; KUMAR, M.; BEHARE, P. V.; JAIN, S.; YADAV, H. Probiotics, their health benefits and applications for developing healthier foods: a review. **Fems Microbiology Letters,** v. 334, p. 1–15, 2012.

NASKAR, B.; DAN, A.; GHOSH, S.; MOULIK, S. P. Viscosity and solubility behavior of the polysaccharide inulin in water,water + dimethyl sulfoxide, andwater+isopropanol media. **Journal of Chemical & Engineering Data**, v. 55, p. 2424–2427, 2010.

NITSCHKE, M.; UMBELINO, D. C. Frutooligossacarídeos: novos ingredientes funcionais. **Boletim da Sociedade Brasileira de Ciência e Tecnologia de Alimentos**, v.36, n.1, p.27-34, 2002.

NOZIÈRE, P.; GRAULET, B.; LUCAS, A.; MARTIN, B.; GROLIER, P.; DOREAU, M. Carotenoids for ruminants: From forages to dairy products. **Animal Feed Science and Technology**, v.131, p.418–450, 2006.

O'HAGAN, P.; HASAPIDIS, K.; CODER, A.; HELSING, H.; POKRAJA, G. **Particle size analysis of food powders**. In: Encapsulated and Powdered Foods. Boca Raton, FL, USA: Taylor & Francis. p. 215–245, 2005.

OLIVEIRA, M. N.; SIVIERI, K.; ALEGRO, J. H. A; SAAD, S. M. I. Aspectos tecnológicos de alimentos funcionais contendo probióticos. **Revista Brasileira Ciências Farmacêuticas**, v.38, p.1-21, 2002.

ÖZER, B.; AVNI KIRMACI, H.; SHENEL, E.; ATAMER, M.; HAYALOĞLU, A. Improving the viability of *Bifidobacterium bifidum* BB-12 and *Lactobacillus acidophilus* LA-5 in white-brined cheese by microencapsulation. **International Dairy Journal**, v. 19, p. 22–29, 2009.

PASEEPHOL, T.; SMALL, D. M.; SHERKAT, F. Rheology and texture of set yogurt as affected by inulin addition. **Journal of Texture Studies**, v.39, p.617–634, 2008.

PASEEPHOL, T.; SHERKAT, F. Probiotic stability of yoghurts containing Jerusalem artichoke inulins during refrigerated storage. **Journal of Functinal Foods**, v. 1, p. 311-318, 2009.

PÉREZ-ALONSO, C.; BÁEZ-GONZÁLEZ, J.; BERISTAIN, C.; VERNON-CARTER, E.; VIZCARRA-MENDONZA, M. Estimation of the activation energy of carbohydrate polymer blends as selection criteria for their use as wall material for spray-dried microcapsules. **Carbohydrate Polymers**, v.53, p.197-203, 2003.

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, p. 505-515, 2004.

PINTADO, M. E.; MACEDO, A. C.; MALCATA, F. X. Review: Technology, Chemistry and Microbiolgy of Whey Cheeses. **Food Science Technology International**, v.7, p.105-116, 2001.

RÉ, M. I. Microencapsulation by spray drying. **Drying Techcology**, v.16, p.1195-1236, 1998.

RÉ, M. I. Formulating drug delivery systems by spray drying. **Drying Techcology**, v. 24, p.433-446, 2006.

REDDY, K. B. P. K.; MADHU, A. N.; PRAPULLA, S. G. Comparative survival and evaluation of functional probiotic properties of spray-dried lactic acid bacteria. **International Journal of Dairy Technology**, v. 62, p.240-248, 2009.

RIBEIRO, A. C.; MARQUES, S. C.; SODRÉ, A. F.; ABREU, L. R.; PICCOLI, R. H. Controle microbiológico da vida de prateleira de ricota cremosa. **Ciência agrotecnológica**, v. 29, p. 113-117, 2005.

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

ROBERFROID, M. B.; VAN LOO, J.; GIBSON, G. R.The Bifidogenic nature of chicory inulin and its hydrolysis products. **The Journal of Nutrition**, v.128 p. 11-19, 1998.

ROBERFROID, M. B. Inulin-Type Fructans: Functional Food Ingredients. **The Journal of Nutrition**, v. 137, p. 2493-2502, 2007.

ROCHA, G.A.; FÁVARO-TRINDADE, C. S.; GROSSO, C. R. F. Microencapsulation of lycopene by spray drying: Characterization, stability and application of microcapsules. **Food and Bioproducts Processing**, v.90, p. 37-42, 2012.

RODRÍGUEZ-HUEZO, M. E.; DURAN-LUGO, R.; PRADO-BARRAGAN, L. A.; CRUZ-SOSA, F.; LOBATO- CALLEROS, C.; ALVAREZ-RAMÍREZ, J.; VERNON-CARTER, E. J. Pre-selection of

protective colloids for enhanced viability of *Bifidobacterium bifidum* following spray-drying and storage, and evaluation of aguamiel as thermoprotective prebiotic. **Food Research International**, v.40, p.1299–1306, 2007.

RONKART, S. N.; PAQUOT, M.; FOUGNIES, C.; DEROANNE, C.; BLECKER, C. S. Effect of water uptake on amorphous inulin properties. **Food Hydrocolloids**, v. 23, p. 922–927, 2009.

ROKKA, S.; RANTAMÄKI, P. Protecting probiotic bacteria by microencapsulation: challenges for industrial applications. **European Food Research and Technology**, v. 231, p. 1-12, 2010.

ROY, D. Technological aspects related to the use of bifidobacteria in dairy products. **Lait**, v. 85, p. 39–56, 2005.

SAAD, S. M. I. Probióticos e Prebióticos: o estado da arte. **Revista Brasileira de Ciências Farmacêuticas**, v.42, p.1-16, 2006.

SAAD, N.; DELATTRE, C.; URDACI, M.; SCHMITTER, J. M.; BRESSOLLIE, P. An overview of the last advances in probiotic and prebiotic field. **LWT - Food Science and Technology**, v. 50, p. 1-16, 2013.

SABIKHI, L.; BABU, R.; THOMPKINSON, D. K.; KAPILA, S. Resistance of microencapsulated *Lactobacillus acidophilus* LA1 processing treatments and simulated gut conditions**. Food Bioprocess Technology**, v. 3, p. 586-593, 2010.

SAÉNZ, C.; TAPIA, S.; CHÁVEZ, J.; ROBERT, P. Microencapsulation by spray drying of bioactive compounds from cactus pear (*Opuntia ficus-indica*). **Food Chemistry**, v. 114, p. 616–622, 2009.

SANDOVAL-CASTILLA, O.; LOBATO-CALLEROS, C.; CARCIA-GALINDO, H. S.; ALVAREZ-RAMIREZ, J.; VERNON-CARTER, E. J. Textural properties of alginate-pectin beads and survivability of entrapped *Lb. casei* in simulated gastrointestinal conditions and in yoghurt. **Food Research International**, v. 43, p.111–117, 2010.

SEMYONOV, D.; RAMON, O.; KAPLUN, Z.; LEVIN-BRENER, L.; GUREVICH, N.; SHIMONI, E. Microencapsulation of *Lactobacillus paracasei* by Spray Freeze Drying. **Food Research International**, v.43, p.193–202, 2010.

SHAH, N. P. Functional cultures and health benefits. **International Dairy Journal**, v. 17, p. 1262-1277, 2007.

SHEU, T. Y.; MARSHALL, R. T.; HEYMANN, H. Improving survival of culture bacteria in frozen desserts by microentrapment. **Journal of Dairy Science**, v. 76, p. 1902–1907, 1993.

SILVA, J.; FREIXO, R.; GIBBS, P.; TEIXEIRA, P. Spray-drying for the production of dried cultures.**International Journal of Dairy Technology**, v. 64, p. 321-335, 2011.

SIMPSON, P. J.; STANTON, C.; FITZGERALD, G. F.; ROSS, R. P. Intrinsic tolerance of *Bifidobacterium* species to heat and oxygen and survival following spray drying and storage. **Journal of Applied Microbiology**, v. 99, p. 493–501, 2005.

SINGH, T.; DRAKE, M. A; CADWALLADER, K. R. Flavor of Cheddar cheese: a chemical and sensory perspective. **Comprehensive Reviews in Food Science and Food Safety**, v. 2, p.139–162, 2003.

STANTON, C.; ROSS, R. P.; FITZGERALD, G. F.; VAN SINDEREN, D. Fermented functional foods based on probiotics and their biogenic metabolites. **Current Opinion in Biotechnology**, v.16, p.198-203, 2005.

SULTANA, K.; GODWARD, G.; REYNOLDS, N.; ARUMUGASWAMY, R.; PEIRIS, P.; KAILASAPATHY, K. Encapsulation of probiotic bacteria with alginate-strach and evaluation of survival in simulated gastrointestinal conditions and in yogurt. **International Journal of Food Microbiology**, v. 62, p. 47–55, 2000.

TAMIME, A.Y. Microbiology of starter cultures. **Dairy microbiology handbook.** 3. ed., NY: Wiley, 2002.

TÁRREGA, A.; ROCAFULL, A.; COSTELL, E. Effect of blends of short and long-chain inulin on the rheological and sensory properties of prebiotic low-fat custards. **LWT-Food Science and Technology**, v. 43, p. 556–562, 2010.

TONON, R. V.; BRABET, C.; PALLET, D.; BRAT, P.; HUBINGER, M. D. Physicochemical and morphological characterisation of açai (*Euterpe oleraceae* Mart.) powder produced with different carrier agents. **International Journal of Food Science and Technology**, v.44, p.1950–1958, 2009.

TRUELSTRUP HANSEN, L.; ALLAN-WOJTAS, P.M.; JIN, Y.L.; PAULSON, A.T. Survival of Ca-alginate microencapsulated *Bifidobacterium* spp. in milk and simulated gastrointestinal conditions. **Food Microbiology**, v. 19, p. 35-45, 2002.

TURGUT, T; CAKMAKCI, S. Investigation of the possible use of probiotics in ice cream manufacture. **International Journal of Dairy Technology**, v. 62, p.444–451, 2009.

URALA, N.; LÄHTEENMÄKI, L. Consumers‘ changing attitudes towards functional foods. **Food Quality and Preference**, v. 18, p.1-12, 2007.

VAN DENDER, A. G. F. **Requeijão cremoso e outros queijos fundidos**: tecnologia de fabricação, controle do processo e aspectos de mercado. Campinas: Fonte Comunicações e Editora Ltda, 2006. 392p.

VILLEGAS, B.; TÁRREGA, A.; CARBONELL, I.; COSTELL, E. Optimising acceptability of new prebiotic low-fat milk beverages. **Food Quality and Preference**, v.21, p. 234-242, 2010.

VINDEROLA, C. G.; REINHEIMER, J. A. Culture media for the enumeration of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in the presence of yoghurt bacteria. **International Dairy Journal**, v. 9, p. 497–505, 1999.

VINDEROLA, G.; MOLINARI, F.; PROSELLO,W.; GHIBERTO, D.; REINHEIMER, J. Growth of *Lactobacillus paracasei* A13 in Argentinian probiotic cheese and its impact on the characteristics of the

product. **International Journal of Food Microbiology**, v. 135, p. 171–174, 2009.

VULEVIC, J.; RASTALL, R. A.; GIBSON, G. R. Developing a quantitative approach for determining the in vitro prebiotic potential of dietary oligosaccharides. **FEMS Microbiology Letters**, v. 236, p.153–159, 2004.

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

ZOMORODI, S.; ASL, A. K.; ROHANI, S. M. R.; MIRAGHAEI, S. Survival of *Lactobacillus casei*, *Lactobacillus plantarum* and *Bifidobacterium bifidum* in free and microencapsulated forms on Iranian white cheese produced by ultrafiltration. **International Journal of Dairy Technology**, v.64, p.84-91, 2011.

CAPÍTULO 2

Microencapsulação de bifidobactéria por *spray drying* na presença de prebióticos

Artigo publicado:
FRITZEN-FREIRE, C. B.; PRUDÊNCIO, E. S.; AMBONI, R. D. M. C.; PINTO, S. S.; NEGRÃO-MURAKAMI, A. N.; MURAKAMI, F. S. Microencapsulation of bifidobacteria by spray drying in the presence of prebiotics**. Food Research International**, v. 45, p.306-312, 2012.

Microencapsulação de bifidobactéria por *spray drying* na presença de prebióticos

Resumo

Este estudo foi realizado para avaliar a viabilidade e as propriedades físicas de *Bifidobacterium* BB-12 microencapsulada por *spray drying* com substituição parcial do leite desnatado reconstituído (LDR), como agente encapsulante, pelos prebióticos inulina, inulina enriquecida com oligofrutose e oligofrutose (a uma razão de 1:1, concentração total de 200 g L^{-1}). As contagens das células viáveis das microcápsulas foram determinadas durante o armazenamento por 180 dias a 4 °C e a -18 °C. A caracterização física incluiu as análises de morfologia, tamanho de partícula, umidade, atividade de água, solubilidade, higroscopicidade, cor, e propriedades térmicas. Todas as microcápsulas produzidas neste estudo mostraram uma elevada taxa de sobrevivência da bifidobactéria durante o armazenamento, em ambas as temperaturas avaliadas. As microcápsulas produzidas com inulina e aquelas produzidas com inulina enriquecida com oligofrutose apresentaram as maiores contagens iniciais. No entanto, a mistura de LDR tanto com inulina enriquecida com oligofrutose, como com oligofrutose, resultou em uma melhor proteção para a bifidobactéria durante o armazenamento. Todas as microcápsulas apresentaram morfologia similar com tamanho de partícula entre 14,45 e 18,78 μm. A substituição parcial com prebióticos diminui o conteúdo de umidade e a atividade de água das microcápsulas. O tempo de solubilização em água foi maior para as microcápsulas produzidas com inulina, enquanto que as microcápsulas produzidas com oligofrutose foram as mais higroscópicas. O valor de a* aumentou nas microcápsulas produzidas com prebióticos. Os resultados das análises térmicas sugerem uma maior estabilidade para as microcápsulas produzidas com prebióticos do que para aquelas produzidas apenas com LDR.

Palavras-chave: Microencapsulação, *Spray Drying*, Bifidobactéria, Probióticos, Prebióticos, Propriedades físicas.

Microencapsulation of bifidobacteria by spray drying in the presence of prebiotics

Abstract

This study was conducted to evaluate the viability and the physical properties of *Bifidobacterium* BB-12 microencapsulated by spray drying with partial replacement of reconstituted skim milk (RSM), as encapsulating agent, with the prebiotics inulin, oligofructose, and oligofructose-enriched inulin (at a ratio of 1:1, 200 g L^{-1} total concentrations). The viable cell counts of the microcapsules were determined during storage for 180 days at 4 °C and at -18 °C. The physical characterization included analysis of morphology, particle size, moisture content, water activity, dissolution, hygroscopicity, color, and thermal properties. All the microcapsules produced in this study showed a high survival rate of bifidobacteria during storage at the temperatures evaluated. The microcapsules produced with inulin and those produced with oligofructose-enriched inulin showed higher initial counts. However, blending oligofructose-enriched inulin with RSM and blending oligofructose with RSM resulted in better protection of bifidobacteria during storage. All microcapsules showed similar morphologies and particle sizes, between 14.45 and 18.78 μm. The partial replacement with prebiotics decreased moisture content and water activity of the microcapsules. The time of dissolution in water was higher for the microcapsules produced with inulin, while the microcapsules produced with oligofructose were more hygroscopic. The value of a* increased in the microcapsules produced with prebiotics. The results of the thermal analysis suggest a higher stability of the microcapsules produced with prebiotics than those produced with RSM only.

Keywords: Microencapsulation, Spray drying, Bifidobacteria, Probiotic, Prebiotic, Physical properties.

1 Introduction

Bifidobacteria are considered to be probiotic and have been incorporated into various dairy products such as yogurts (AWAISHEH; HADDADIN; ROBINSON, 2005; KAILASAPATHY; HARMSTORF; PHILLIPS, 2008; RAMCHANDRAN; SHAH, 2010), ice creams (AKYN; AKYN; KIRMACI, 2007; TURGUT; CAKMAKCI, 2009) and cheeses (ONG; HENRIKSSON; SHAH, 2006; FRITZEN-FREIRE et al., 2010). These microorganisms benefit human health by improving the balance of intestinal microbiota and by strengthening mucosal defenses against pathogens (BOYLSTON et al., 2004). However, for probiotics to be therapeutically effective, it has been suggested that products should contain at least 6 log CFU g^{-1} of bacteria until the end of their shelf life (TALWALKAR et al., 2004). Although bifidobacteria are being increasingly recognized as probiotics that have advantageous properties, they are also fastidious, obligate anaerobes and, therefore, pose a technological challenge for the food industry (HANSEN et al., 2002). Several factors have been claimed to affect the viability of bifidobacteria, including acidity, pH, time and temperature of storage, and oxygen content (PICOT; LACROIX, 2004; RANADHEERA; BAINES; ADAMS, 2010).

Within this context, microencapsulation of probiotic bacteria is currently drawing more and more attention for being a method to improve the stability of probiotic organisms in functional food products (ANAL; SINGH, 2007; SEMYONOV et al., 2010). Moreover, according to Ding and Shah (2009), microencapsulation may improve the survival of these microorganisms, during both processing and

storage, and also during passage through the human gastrointestinal tract. Spray drying is regarded as a microencapsulation method and it has been investigated as a means of stabilizing probiotic bacteria in a number of food matrices, most often composed of proteins, polysaccharides, sugars, and combinations thereof (CHÁVEZ; LEDEBOER, 2007). The survival rate of the culture during spray drying and subsequent storage depends upon a number of factors, which may include the species and strain of the culture, the drying conditions and also the use of encapsulating agents (DESMOND et al., 2002).

Reconstituted skim milk (RSM) is an encapsulating agent that has shown a favorable effect on the improvement of cell survival during the spray drying process (FU; CHEN, 2011). Another approach to increase the viability of bifidobacteria is the use of prebiotics (RODRÍGUEZ-HUEZO et al., 2007), which are nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of bacteria in the colon (WANG, 2009). Inulin is a prebiotic whose degree of polymerization (DP) ranges between 10 and 60. It is extracted from chicory roots and consists of chains of fructose units (BIEDRZYCKA; BIELECKA, 2004). Oligofructose is obtained through partial hydrolysis of inulin and therefore has a lower DP, which ranges from 2 to 8 (FRANCK, 2002). A mixture of oligofructose and inulin is known as oligofructose-enriched inulin (ROBERFROID, 2007). According to Corcoran et al. (2004), these prebiotics may potentially be exploited as carrier media for spray drying and may be useful for enhancing probiotic survival during processing. However, the use of different encapsulating agents for production of microcapsules can result in different physical properties, depending on the structure and the

characteristics of each agent (TONON et al., 2009), and it can also modify functional properties of microcapsules (CHEN et al., 2005). Therefore, the objective of this study was to evaluate the effect of partial replacement of RSM with prebiotic agents (inulin, oligofructose, and oligofructose-enriched inulin) on the viability of *Bifidobacterium* BB-12, microencapsulated by spray drying, during storage for 180 days at 4 °C and at -18 °C, as well as to characterize the microcapsules in relation to their physical properties.

2 Materials and methods

2.1 Materials

A probiotic culture composed of *Bifidobacterium* BB-12 (BB-12®, Chr. Hansen, Hónsholm, Denmark) was used as the active material for the microcapsules. The encapsulating agents used were commercial skim milk powder (Molico®, Nestlé, São Paulo, Brazil) and the prebiotic agents inulin (Orafti® HPX, Orafti, Tienen, Belgium) with DP ≥ 23; oligofructose-enriched inulin (Orafti® Synergy1, Orafti, Tienen, Belgium), which is a 1:1 mixture of oligofructose (DP 2 - 8) and long-chain inulin fraction (DP 10 - 60); and oligofructose (Orafti® P95, Orafti, Tienen, Belgium) with DP 2 - 8. MRS agar (Difco, Sparks, USA), lithium chloride (Vetec, Rio de Janeiro, Brazil), sodium propionate (Fluka, Neu-Ulm, Germany) and AnaeroGen® (Oxoid, Hampshire, UK) were used for the microbiological analysis. All the chemicals used were of analytical grade.

2.2 Microencapsulation by spray drying

2.2.1 Preparation of drying media

Four feed solutions were prepared following the procedures described by Ananta, Volkert, and Knorr (2005), with modifications. Reconstituted skim milk (RSM) at a concentration of 200 g L^{-1} was used as the control medium. The prebiotic media used for spray drying consisted of an equal ratio of RSM and each of the three prebiotics (ratio of 1:1, 200 g L^{-1} total concentration). All the media were homogenized into sterile distilled water and heat treated at 80 °C for 30 min.

2.2.2 Preparation of bacterial suspension

Freeze-dried probiotic cells of *Bifidobacterium* BB-12 were rehydrated at 25 g L^{-1} using a 120 g L^{-1} solution of RSM and frozen as stock solution at -18 °C into sterile glass bottles. Before spray drying, this probiotic stock solution was incubated at 37 °C for 2 hours and then inoculated (100 mL L^{-1}) into the four feed solutions.

2.2.3 Spray drying

The spray drying process was performed with a Buchi B-290 mini spray dryer (Buchi, Flawil, Switzerland) at constant air inlet temperature of 150 ± 2 °C and outlet temperature of 55 ± 3 °C. The feed solutions containing *Bifidobacterium* BB-12 were kept under magnetic agitation at room temperature and fed into the main chamber through a

peristaltic pump, with feed flow of 6 mL min^{-1}, drying air flow rate of 35 m^3 h^{-1}, and compressor air pressure of 0.7 MPa. The dried powder samples were collected from the base of the cyclone and thoroughly mixed with a spatula. The samples were placed in sterile plastic bottles and stored at 4 °C and at -18 °C. Thus, four different microcapsules were obtained through the following combination of encapsulating agents: M1 (with 200 g L^{-1} of RSM), M2 (with 100 g L^{-1} of RSM and 100 g L^{-1} of inulin), M3 (with 100 g L^{-1} of RSM and 100 g L^{-1} of oligofructose-enriched inulin) and M4 (with 100 g L^{-1} of RSM and 100 g L^{-1} of oligofructose).

To understand the effect of prebiotics on the microencapsulated bifidobacteria during storage, the viable cell counts of the microcapsules were determined on the day when the different powders were manufactured and during their storage for 180 days at 4 °C and at -18 °C. The microcapsules were also evaluated in relation to their morphology, particle size, moisture content, water activity, dissolution, hygroscopicity, color, and thermal properties on the day of their manufacture. All the experiments were performed in triplicate, except those for morphology and particle size.

2.3 Survival of microencapsulated bifidobacteria after spray drying and viability during storage time

To determine the viable cell counts, the entrapped bacteria were released from the microcapsules according to the method proposed by Sheu, Marshall, and Heymann (1993). One gram of the microcapsules

was re-suspended in 9 mL of phosphate buffer (0.1 M, pH 7.0) followed by homogenization in a magnetic stirrer for 10 min.

To enumerate the bifidobacteria, samples were serially diluted with peptone water (0.1 g 100 g^{-1}) and plated on MRS agar modified with the addition of lithium chloride (0.2 g 100 g^{-1}) and sodium propionate (0.3 g 100 g^{-1}), as proposed by Vinderola and Reinheimer (1999). The plates were incubated in anaerobic jars containing AnaeroGen® at 37 ± 1 °C for 72 h. After the incubation period, the count of viable probiotic cells was carried out and expressed as log colony-forming units per gram (log CFU g^{-1}).

2.4 Physical properties of the microcapsules

2.4.1 Morphology and particle size

The morphology and particle size of the microcapsules were observed with a Jeol scanning electron microscope model JSM 6390 LV (Jeol, Tokyo, Japan) at an accelerating voltage of 10 and 15 kV. Before using the scanning electron microscope (SEM), the samples were placed on a piece of adhesive paper and were coated with gold with a vacuum sputtering coater (Leica, model EM SCD 500, Wetzlar**,** Germany), as described by Lian, Hsiao, and Chou (2002). To calculate their diameter, at least 120 particles from each of the different formulations of microcapsules were measured (KRASAEKOOPT; BHANDARI; DEETH, 2004).

2.4.2 Moisture

The moisture content of the spray dried powders was determined through oven drying at 102 °C until reaching constant weight, according to the International Dairy Federation (IDF, 1993).

2.4.3 Water activity

Water activity was measured using an Etec analyzer model Aw 43 (Etec, São Paulo, Brazil) after the samples were stabilized at 25 °C for 30 min.

2.4.4 Dissolution

The dissolution was carried out by adding 2 g of the powders into 50 mL of distilled water (EL-TINAY; ISMAIL, 1985). The mixture was agitated in a 100 mL low form glass beaker with a magnetic stirrer (Dist, model DI 03, Florianópolis, Brazil) at 892 rpm and a stirring bar measuring 2 mm x 7 mm (at room temperature). The time required for the material to completely dissolve was recorded.

2.4.5 Hygroscopicity

The hygroscopicity of the spray dried powders was determined according to the method proposed by Cai and Corke (2000), with some modifications. Samples of each powder (approximately 1 g) were placed at 25 °C in an airtight glass container with NaCl saturated solution

(75.3% RH). After one week, the samples were weighed and their hygroscopicity was expressed as g of adsorbed moisture per 100 g of dry solids (g 100 g^{-1}).

2.4.6 Color analysis

The color analyses were performed with a Minolta Chroma Meter CR-400 (Konica Minolta, Osaka, Japan) colorimeter, adjusted to operate with D65 lightning and 10° of observation angle. The CIELab color scale was used to measure the L*, a* and b* parameters. In the CIELab color scale, the L* parameter ranges from 0 to 100, indicating the color variation from black to white; the a* axis shows the variation from red (+a*) to green (-a*); whilst the b* axis shows the variation from yellow (+b*) to blue (-b*). The total color difference (ΔE*) between the control powder (M1) and each of the powder samples produced with prebiotics (M2, M3, and M4) (Eq. (1)) was calculated as proposed by Capellas et al. (2001).

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (1)$$

where ΔL* is the lightness difference between the control powder and the powders with prebiotics; Δa* is the intensity of the color red; Δb* is the intensity of the color yellow.

2.4.7 Differential scanning calorimetry analysis (DSC)

The DSC curves of the microcapsules were obtained using a Shimadzu DSC-60 (Shimadzu, Kyoto, Japan). Samples of approximately 2 mg of powder were placed in aluminum sealed pans, under a dynamic synthetic air atmosphere (100 mL min^{-1}) and heated from 30 °C to 300 °C at a heating rate of 10 °C min^{-1}. The DSC equipment was preliminarily calibrated with a standard reference of indium.

2.4.8 Thermogravimetric analysis (TGA/DrTGA)

Thermogravimetry/derivative thermogravimetry (TGA/DrTGA) curves were obtained using a DTG-60 thermobalance (Shimadzu, model DTG-60, Kyoto, Japan). Approximately 7 mg of samples were placed in alumina pans and heated from 30 ºC to 300 ºC at a rate of 10 ºC min^{-1} under a dynamic synthetic air atmosphere (100 mL min^{-1}). The equipment was preliminarily calibrated with a standard reference of calcium oxalate.

2.5 Statistical analysis

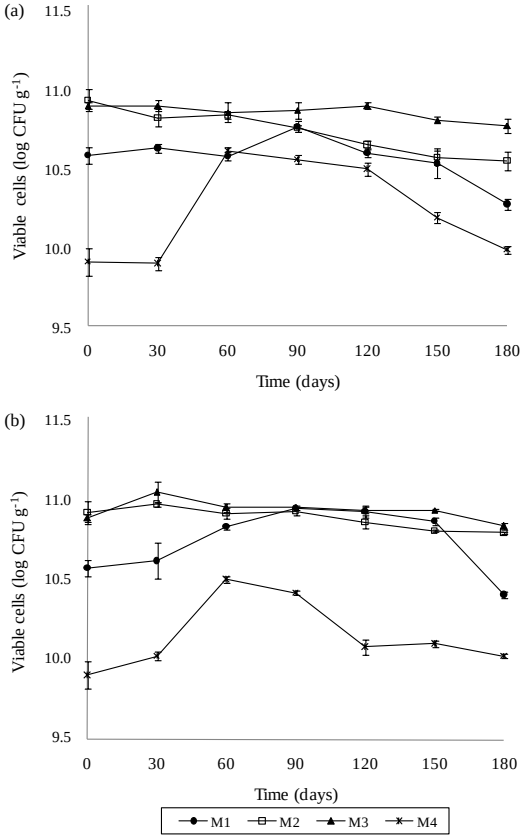
The data analysis was carried out using STATISTICA 7.0 software (StatSoft Inc., Tulsa, USA). Analysis of variance (ANOVA) was used to determine significant differences ($P < 0.05$) amongst the microcapsules. Differences between means were detected using Tukey's test.

3 Results and discussion

3.1 Survival of microencapsulated bifidobacteria after spray drying and viability during storage time

The effect of different encapsulating agents on the viability of *Bifidobacterium* BB-12 microcapsules throughout storage at 4 °C and at -18 °C is shown in Fig.1 (a) and (b), respectively.

Figure 1 - Effect of different encapsulating agents on the viability of *Bifidobacterium* BB-12 microcapsules during storage time, at 4 °C (a) and at -18 °C (b).



(M1): microcapsules with reconstituted skim milk (RSM), (M2): microcapsules with RSM and inulin, (M3): microcapsules with RSM and oligofructose-enriched inulin, (M4): microcapsules with RSM and oligofructose. Error bars represent standard deviations of the mean of experiment.

All spray dried microcapsules containing bifidobacteria showed high survival for a period of up to 180 days of storage at both

temperatures. In addition, the count of viable probiotic cells was above the recommended levels for probiotic food throughout the whole storage time, i.e., equal to or greater than 6 log CFU g^{-1} of the product, according to Roy (2005). The microcapsules produced with RSM and inulin (M2) and those produced with RSM and oligofructose-enriched inulin (M3) showed higher ($P < 0.05$) initial count (after spray drying), when compared to the microcapsules produced only with RSM (M1) and with oligofructose (M4). This fact suggests that inulin had a positive effect on the protection of bifidobacteria during the encapsulation process, probably because it acted as a thermoprotector for the cells undergoing the drying process. Lian et al. (2002) reported that besides difference in chemical characteristics, the encapsulating agents have different physical properties. Therefore, it is reasonable to expect that these agents tested in this study may exert different degrees of protective effect on the entrapped cells of a test organism when subjected to heat inactivation during spray drying and, thus, take survival of bifidobacteria to a different level. Moreover, according to Ananta, Volkert, and Knorr (2005), since the effectiveness of probiotic consumption on human health is related to their viability, it is of utmost importance to not only minimize cell death during the spray drying process but also to ensure minimal loss of viability of the microencapsulated bacteria during storage.

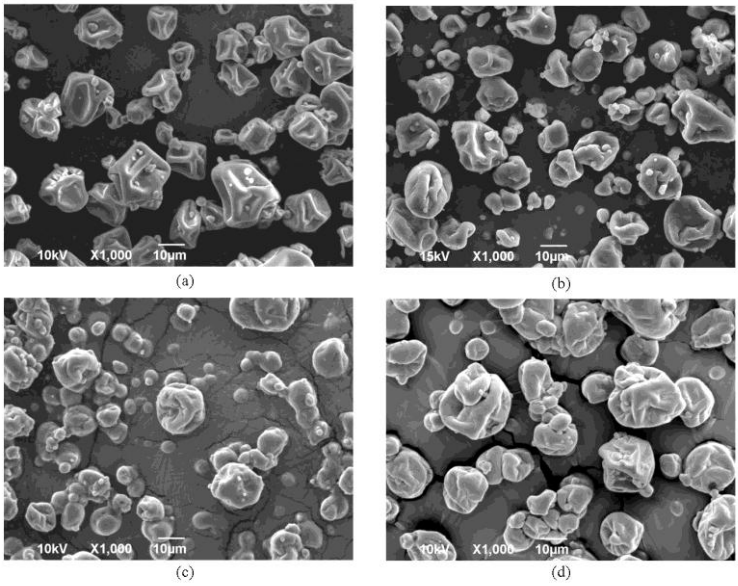
The results of the bifidobacteria counts showed a similar behavior between each of the different microcapsules stored for 180 days at both temperatures used in this study. The microcapsules M1 showed an increase in the viability within 90 days of storage, with a significant decrease ($P < 0.05$) after this period. The viability of the bifidobacteria

decreased ($P < 0.05$) according as the period of storage time increased for the microcapsules M2, while the microcapsules M3 showed constant ($P > 0.05$) viability throughout the storage period. On the other hand, microcapsules M4 showed an increase ($P < 0.05$) in the viability of *Bifidobacterium* BB-12 during up to 60 days of storage, with a gradual decrease until the end of the 180 days' storage period. However, the final counts of the microcapsules M4 did not differ ($P > 0.05$) from the initial. Thus, blending oligofructose-enriched inulin with RSM and blending oligofructose with RSM resulted in better protection for the microencapsulated bifidobacteria during storage. According to Corcoran et al. (2004), this indicates that good storage survival may not depend on selection of the best survivors following spray drying, as was observed in this study. However, at the end of the storage period, the microcapsules kept under freezing conditions (-18 °C) showed higher counts of bifidobacteria than those stored under refrigeration (4 °C). Finally, the counts of probiotics obtained in this work are in accordance with those obtained by Simpson et al. (2005), who verified a high initial survival rate following spray drying and maintained viability during storage (90 days) at refrigerated temperatures in the commercial probiotic strain of *Bifidobacterium* BB-12 encapsulated with RSM. Also according to these authors, the viability may depend on the induction of sub-lethal damage that is converted into lethal forms during storage through processes influenced by the physical characteristics of the microcapsules.

3.2 Physical properties of the microcapsules

Fig. 2 shows the SEM micrographs of the *Bifidobacterium* BB-12 microcapsules produced with different encapsulating agents. The SEM revealed the absence of free bacteria confirming the formation of microcapsules, for all the encapsulating agents.

Figure 2 - Micrographs of *Bifidobacterium* BB-12 microcapsules produced with: (a) reconstituted skim milk (RSM) (M1), (b) RSM and inulin (M2), (c) RSM and oligofructose-enriched inulin (M3) and (d) RSM and oligofructose (M4).



The particles showed a spherical shape and various sizes, with concavities typical of materials produced by spray drying. Saénz et al. (2009) reported that the formation of concavities in the surface of

atomized particles can be attributed to the shrinkage of the particles during the drying process because of the rapid evaporation of the liquid drops.

The control microcapsules (M1), produced with RSM, and the microcapsules produced with prebiotics (M2, M3, and M4) showed similar morphologies, thus indicating that the encapsulating agents did not affect morphology.

The external surfaces showed walls free of fissures or disruptions, which is fundamental for guaranteeing higher protection and lower permeability of gases. Similar results were noted by Desmond et al. (2002) and Rodríguez- Huezo et al. (2007) in *Lactobacillus paracasei* spray dried with RSM and in *Bifidobacterium bifidum* spray dried with aguamiel (a kind of prebiotic), respectively. The microcapsules were of assorted sizes, between 14.45 and 18.78 μm (Table 1). Such values are expected for microcapsules obtained through spray drying, which may vary from 10 to 100 μm, according to Fang and Bhandari (2010). However, the capsules produced with RSM and oligofructose (M4) showed a smaller particle size ($P < 0.05$) than the other microcapsules. This difference occurred probably because, as described by Bosscher, Van Loo, and Franck (2006), the molar mass of oligofructose is lower, since it is obtained through partial hydrolysis of inulin, and thus has a small DP, ranging from 2 to 8 and, therefore, showing shorter chains.

Table 1 - Properties of the *Bifidobacterium* BB-12 microcapsules produced with different encapsulating agents.

Properties	***Microcapsules***			
	M1	**M2**	**M3**	**M4**
Particles size (μm)	18.78 ± 7.25^{a}	17.79 ± 6.15^{ab}	16.74 ± 5.81^{b}	14.45 ± 3.96^{c}
Moisture (g 100g^{-1})	3.24 ± 0.12^{a}	2.90 ± 0.04^{b}	2.81 ± 0.04^{b}	2.84 ± 0.08^{b}
Water activity	0.274 ± 0.002^{a}	0.227 ± 0.002^{b}	0.211 ± 0.007^{c}	0.263 ± 0.003^{d}
Dissolution in water (s)	220.07 ± 15.68^{a}	332.37 ± 20.27^{b}	278.90 ± 14.62^{c}	190.77 ± 9.59^{a}
Hygroscopicity (g 100g^{-1})	18.82 ± 0.32^{ab}	17.56 ± 0.54^{a}	19.55 ± 0.49^{b}	23.41 ± 0.67^{c}

[a-d] Means ± standard deviation with different superscript letters in the same line indicate significant differences ($P < 0.05$) between the microcapsules.
M1: microcapsules with reconstituted skim milk (RSM)
M2: microcapsules with RSM and inulin
M3: microcapsules with RSM and oligofructose-enriched inulin
M4: microcapsules with RSM and oligofructose

The microcapsules produced only with RSM (M1) showed higher ($P < 0.05$) moisture content than the microcapsules produced with the prebiotics (Table 1). Similar results were observed by Corcoran et al. (2004) in microcapsules of *Lactobacillus rhamnosus* GG spray dried with the same encapsulating agents as those used in this study. Moreover, the moisture content of all the encapsulated samples is within the values described by Simpson et al. (2005) to guarantee microbiological stability, i.e., equal to or smaller than 4 g 100 g^{-1}.

All the microcapsules showed water activity values below 0.3 (Table 1), which, according to Tonon et al. (2009), is very positive for powder stability since it represents less free water available for biochemical reactions and hence longer shelf life.

The time required for the powders to dissolve in water was higher ($P < 0.05$) for microcapsules M2, followed by microcapsules M3 and M4, respectively (Table 1). According to Barclay et al. (2010), the solubility of inulin is closely related to the chain length of the polymer, and thus shorter oligomers are much more soluble than long chain polymers, as was noted in this present study. Moreover, as reported by Naskar et al. (2010), the solubility of inulin decreases according as temperature decreases and thus the process is weakly endothermic. These facts may be attributable for the long dissolution time noted in the microcapsules containing inulin since the experiment was performed at room temperature. Regarding the dissolution of the microcapsules produced only with RSM (M1), the short dissolution time noted can be because of the spray drying process. Gharsallaoui et al. (2007) suggest that when milk is spray dried the hydrophilic groups (a mix of lactose and milk proteins) are more exposed, therefore increasing moisture,

water activity, and also solubility. Moreover, the absence of fat on the particles' surfaces renders the skimmed milk powder more hydrophilic, such that its solubility in water is increased.

As shown in Table 1, the microcapsules produced with RSM and oligofructose (M4) were the most hygroscopic ($P < 0.05$), while the microcapsules produced with RSM and inulin (M2) were the least hygroscopic. The differences in hygroscopicity of the samples can be attributed to the size of the particles produced with each of the different encapsulating agents, which has been previously discussed. As reported by Tonon et al. (2009), the bigger the particle size, the smaller the exposed surface area and, consequently, the lower the water adsorption from the ambient air. These authors also reported the increase in hygroscopicity in carrier agents with lower molar mass and therefore with shorter chains, as was noted in this study.

The color attributes of the *Bifidobacterium* BB-12 microcapsules produced with different encapsulating agents are shown in Table 2. In relation to the L* and b* parameters, there were no significant ($P > 0.05$) differences between the microcapsules. According to Aryana and McGrew (2007), a factor influencing product color is the color of the ingredients used. This factor explains the high luminosity noted and a tendency to yellow, since the used prebiotics were whitish and the skimmed milk powder were light cream color. However, the a* parameter values differed ($P < 0.05$) between the microcapsules produced only with RSM and the microcapsules produced with addition of prebiotics. The low a* values obtained for microcapsules M1 indicated a tendency to green. This result occurred probably because of

the presence of riboflavin in milk, since it is attributable for its slightly green coloration, as described by Nozière et al. (2006).

Table 2 - The color attributes of the *Bifidobacterium* BB-12 microcapsules produced with different encapsulating agents.

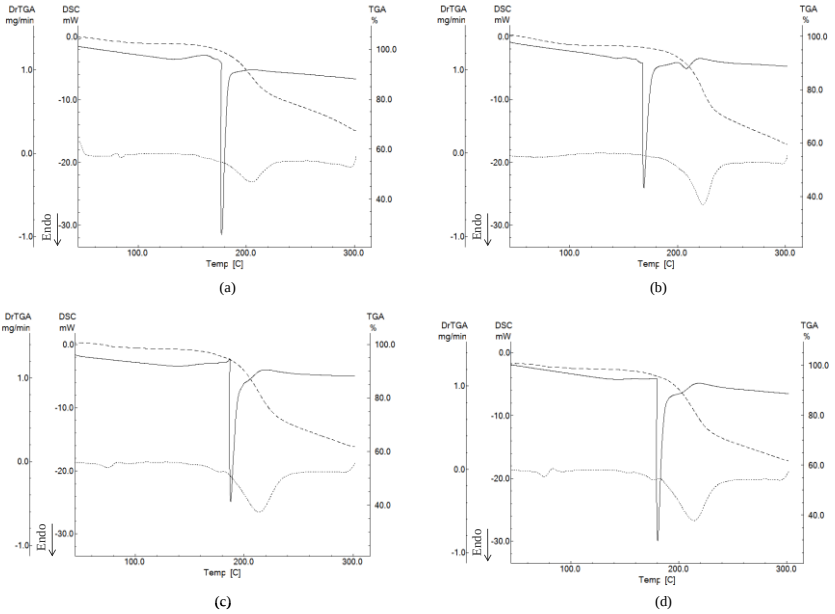
Microcapsules	***L****	***a****	***b****	**ΔE***
M1	95.73 ± 0.55^{a}	-0.47 ± 0.05^{a}	8.97 ± 0.66^{a}	-
M2	94.68 ± 0.23^{a}	0.16 ± 0.05^{bc}	8.01 ± 0.55^{a}	1.46
M3	94.89 ± 0.60^{a}	0.18 ± 0.03^{b}	8.07 ± 0.65^{a}	1.26
M4	95.06 ± 0.61^{a}	0.08 ± 0.02^{c}	8.74 ± 0.43^{a}	0.81

[a-c] Means ± standard deviation with different superscript letters in the same column indicate significant differences ($P < 0.05$) between the microcapsules.
M1: microcapsules with reconstituted skim milk (RSM)
M2: microcapsules with RSM and inulin
M3: microcapsules with RSM and oligofructose-enriched inulin
M4: microcapsules with RSM and oligofructose

The total color difference (ΔE*) noted between the control microcapsule (M1) and each of the formulations of microcapsules containing prebiotics (M2, M3, and M4) was lower than 3, which is considered not obvious to the human eye, as reported by Martínez-Cervera et al. (2011). This result probably occurred because of the stability of the L* and b* parameters.

The thermal behavior of the microcapsules can be noted in the DSC and TGA/DrTGA curves shown in Fig. 3.

Figure 3 - DSC (———) (solid line) and TGA (- - - -) (thick dashed line) / DrTGA (·····) (thin dashed line) curves of *Bifidobacterium* BB-12 microcapsules produced with: (a) reconstituted skim milk (RSM) (M1), (b) RSM and inulin (M2), (c) RSM and oligofructose-enriched inulin (M3) and (d) RSM and oligofructose (M4).



For all the microcapsules, the DSC curves show one well-defined thermal event (an endothermic peak), which probably corresponds to the melting point of RSM, which in turn was noted at temperatures between 168 °C and 188 °C. An additional slight endothermic peak was noted (though not very clearly) in the microcapsules produced with prebiotics (M2, M3, and M4) at the temperature range between 200 °C and 210 °C, and such peak can be attributed to the melting of the prebiotics. A

similar behavior was noted by Blecker et al. (2003) in samples of inulin with different degrees of polymerization (DP). These authors also verified that as DP of the prebiotics increased, the temperature of the melting point of the samples also increased.

Two representative stages were differentiated by using the analysis of the thermogravimetric curves. The first mass loss of the TGA/DrTGA curves refers to moisture loss (between 30 °C and 100 °C). Above this temperature range, the second stage of mass loss corresponds to the decomposition process. According to Macêdo et al. (1997), in this step there can be occurrence of decomposition reactions in the constituents of the microcapsules, i.e., proteins and carbohydrates. Moreover, Bohm et al. (2005) reported that thermal degradation of inulin has been described as being a consequence of the breakdown of the fructose chains and, as was noted in this study, the breakdown of the fructose chains in microcapsules M2, M3, and M4 may have occurred between 213 °C and 223 °C.

4 Conclusions

The partial replacement of RSM with inulin and the partial replacement of RSM with oligofructose-enriched inulin increased the initial count of bifidobacteria in the microcapsules. On the other hand, the microcapsules produced with oligofructose-enriched inulin and those produced with oligofructose showed better protection for the bifidobacteria during storage.

The use of prebiotics did not affect the morphology of the microcapsules. However, the capsules produced with oligofructose

showed a smaller particle size. The inclusion of prebiotics decreased the moisture content and water activity in the microcapsules. The microcapsules produced with inulin showed the lowest dissolution in water, while the microcapsules produced with oligofructose were the most hygroscopic. The total color difference of the microcapsules was not considered obvious to the human eye. The results of the thermoanalyses suggest an increase in the stability of the microcapsules produced with prebiotics. Finally, the results showed that the oligofructose-enriched inulin is the most appropriate prebiotic to be used as partial replacement of RSM to microencapsulate *Bifidobacterium* BB-12 by spray drying, with a great potential as a functional ingredient to be applied in dairy foods.

Acknowledgments

The authors gratefully thank the Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC) for their financial support, and also Clariant for providing the prebiotics.

References

AKYN, M. B.; AKYN, M. S.; KIRMACI, Z. Effects of inulin and sugar levels on the viability of yoghurt and probiotic bacteria and the physical and sensory characteristics in probiotic ice-cream. **Food Chemistry**, v. 104, p. 93-99, 2007.

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

ANANTA, E.; VOLKERT, M.; KNORR, D. Cellular injuries and storage stability of spray-dried *Lactobacillus rhamnosus* GG. **International Dairy Journal**, v.15, p.399-409, 2005.

ARYANA, K. J.; MCGREW, P. Quality attributes of yogurt with *Lactobacillus casei* and various prebiotics. **LWT - Food Science and Technology**, v.40, p.1808-1814, 2007.

AWAISHEH, S. S.*;* HADDADIN, M. S. Y.; ROBINSON, R. K. I. Incorporation of selected nutraceuticals and probiotic bacteria into fermented milk. **International Dairy Journal**, *v.*15, *p.*1184-1190, 2005.

BARCLAY, T.; GINIC-MARKOVICA, M.; COOPER, P.; PETROVSKY, N. Inulin - a versatile polysaccharide with multiple pharmaceutical and food chemical uses. **Journal of Excipients and Food Chemicals**, v.1, p.27-50, 2010.

BIEDRZYCKA, E.; BIELECKA, M. Prebiotic effectiveness of fructans of different degrees of polymerization. **Trends in Food Science & Technology**, v.15, p.170-175, 2004.

BLECKER, C.; CHEVALIER, J. P.; FOUGNIES, C.; VAN HERCK, J. C.; DEROANNE, C.; PAQUOT, M. Characterisation of Different Inulin Samples by DSC: Influence of polymerisation degree on melting temperature. **Journal of Thermal Analysis and Calorimetry**, v.71, p.215-224, 2003.

BOHM, A.; KAISER, I.; TREBSTEIN, A.; HENLE, T. Heat-induced degradation of inulin. **European Food Research and Technology**, v.220, p.466–471, 2005.

BOSSCHER, D.; VAN LOO, J.; FRANK, A. Inulin and oligofructose as functional ingredients to improve bone mineralization. **International Dairy Journal**, v.16, p.1092-1097, 2006.

BOYLSTON, T. D.; VINDEROLA, C. G.; GHODDUSI, H. B.; REINHEIMER, J. A. Incorporation of bifidobacteria into cheeses: challenges and rewards. **International Dairy Journal**, v.14, p.375-387, 2004.

CAI, Y. Z.; CORKE, H. Production and properties of spray-dried *Amaranthus* betacyanin pigments. **Journal of Food Science**, v.65, p.1248-1252, 2000.

CAPELLAS, M.; MOR-MUR, M.; SENDRA, E.; GUAMIS, B. Effect of high-pressure processing on physico-chemical characteristics of fresh goats' milk cheese (Mató). **International Dairy Journal**, v.11, p.165-173, 2001.

CHÁVEZ, B. E.; LEDEBOER, A. M. Drying of probiotics: optimization of formulation and process to enhance storage survival. **Drying Technology**, v. 25, p.1193-1201, 2007.

CHEN, K. N.; CHEN, M. J.; LIU, J. R.; LIN, C. W.; CHIU, H. Y. Optimization of incorporated prebiotics as coating materials for probiotic microencapsulation. **Journal of Food Science**, v.70, p. M260-M266, 2005.

CORCORAN, B. M.; ROSS, R. P.; FITZGERALD, G. F.; STANTON, C. Comparative survival of probiotic lactobacilli spray-dried in the presence of prebiotic substances. **Journal of Applied Microbiology**, v. 96, p.1024-1039, 2004.

DESMOND, C.; ROSS, R. P.; O'CALLAGHAN, E.; FITZGERALD, G.; STANTON, C. Improved survival of *Lactobacillus paracasei* NFBC 338 in spray-dried powders containing gum acacia. **Journal of Applied Microbiology**, v.93, p.1003-1011, 2002.

DING, W. K.; SHAH, N. P. Effect of Various Encapsulating Materials on the Stability of Probiotic Bacteria. **Journal of Food Science**, v.74, p.M100-M107, 2009.

EL-TINAY, A. H.; ISMAIL, I. A. Effect of some additives and processes on the characteristics of agglomerated and granulated spray-dried Roselle powder. **Acta Alimentaria Hungaricae**, v.14, p.283-295, 1985.

FANG, Z. X.; BHANDARI, B. Encapsulation of polyphenols - a review. **Trends in Food Science & Technology**, v.21, p.510-523, 2010.

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

FRITZEN-FREIRE, C. B.; MÜLLER, C. M. O.; LAURINDO, J. B.; PRUDÊNCIO E. S. The influence of *Bifidobacterium* Bb-12 and lactic acid incorporation on the properties of Minas Frescal cheese. **Journal of Food Engineering**, v.96, p.621-627, 2010.

FU, N.; CHEN, X. D. Towards a maximal cell survival in convective thermal drying processes. **Food Research International**, v.44, p.1127-1149, 2011.

GHARSALLAOUI, A. ; ROUDAUT, G. ; CHAMBIN, O. ; VOILLEY, A. ; SAUREL, R. Applications of spray-drying in microencapsulation of food ingredients: an overview. **Food Research International**, v.40, p.1107-1121, 2007.

HANSEN, L. T.; ALLAN-WOJTAS, P. M.; JIN, Y. L.; PAULSON, A. T. Survival of Ca-alginate microencapsulated *Bifidobacterium* spp. in milk and simulated gastrointestinal conditions. **Food Microbiology**, v.19, p.35-45, 2002.

INTERNATIONAL DAIRY FEDERATION- IDF. **International Dairy Federation standard 26A**. Dried milk and dried cream: Determination of water content. Brussels, Belgium: International Dairy Federation, 1993.

KAILASAPATHY, K.; HARMSTORF, I.; PHILLIPS, M. Survival of *L. acidophilus* and *B. animalis* ssp. *lactis* in stirred fruit yogurts. **LWT – Food Science and Technology**, v.41, p.1317-1322, 2008.

KRASAEKOOPT, W.; BHANDARI, B.; DEETH, H. The influence of coating materials on some properties of alginate beads and survivability of microencapsulated probiotic bacteria. **International Dairy Journal**, v.14, p.737-743, 2004.

LIAN, W. C.; HSIAO, H. C.; CHOU, C. C. Survival of bifidobacteria after spray-drying. **International Journal of Food Microbiology**, v.74, p.79-86, 2002.

MACÊDO, R. O.; MOURA, O. M.; SOUZA, A. G.; MACÊDO, A. M. C. Comparative Studies on some Analytical Methods: Thermal decomposition of powder milk. **Journal of Thermal Analysis**, v.49, p.857-862, 1997.

MARTÍNEZ-CERVERA, S.; SALVADOR, A.; MUGUERZA, B., MOULAY, L.; FISZMAN, S. M. Cocoa fibre and its application as a fat replacer in chocolate muffins. **LWT - Food Science and Technology**, v.44, p.729-736, 2011.

NASKAR, B.; DAN, A.; GHOSH, S.; MOULIK, S. P. Viscosity and Solubility Behavior of the Polysaccharide Inulin in Water, Water + Dimethyl Sulfoxide, and Water + Isopropanol Media. **Journal of Chemical & Engineering Data**, v.55, p.2424-2427, 2010.

NOZIERE, P.; GRAULET, B.; LUCAS, A.; MARTIN, B., GROLIER, P.; DOREAU, M. Carotenoids for ruminants: From forages to dairy products. **Animal Feed Science and Technology**, v.131, p.418-450, 2006.

ONG, L.; HENRIKSSON, A.; SHAH, N. P. Development of probiotic Cheeddar cheese containing *Lactobacillus acidophilus*, *L. casei*, *L. paracasei* and *Bifidobacterium* spp. and the influence of these bacteria on proteolytic patterns and production of organic acid. **International Dairy Journal**, v.16, p.446-456, 2006.

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, p.505-515, 2004.

RAMCHANDRAN, L.; SHAH, N. P. Characterization of functional, biochemical and textural properties of symbiotic low-fat yogurts during refrigerated storage. **LWT - Food Science and Technology**, v.43, p.819–827, 2010.

RANADHEERA, R. D. C. S.; BAINES, S. K.; ADAMS, M. C. Importance of food in probiotic efficacy. **Food Research International**, v.43, p.1-7, 2010.

ROBERFROID, M. B. Inulin-Type Fructans: Functional Food Ingredients. **The Journal of Nutrition**, v.137, p 2493-2502, 2007.

RODRÍGUEZ-HUEZO, M. E.; DURÁN-LUGO, R.; PRADO-BARRAGÁN, L. A.; CRUZ-SOSA, F.; LOBATO-CALLEROS, C.; ALVAREZ-RAMÍREZ, J. Pre-selection of protective colloids for enhanced viability of *Bifidobacterium bifidum* following spray-drying and storage, and evaluation of aguamiel as thermoprotective prebiotic. **Food Research International**, v.40, p.1299-1306, 2007.

ROY, D. Technological aspects related to the use of bifidobacteria in dairy products. **Lait**, v.85, p.39-56, 2005.

SAÉNZ, C.; TAPIA, S.; CHÁVEZ, J.; ROBERT, P. Microencapsulation by spray drying of bioactive compounds from cactus pear (*Opuntia ficus-indica*). **Food Chemistry**, v.114, p.616-622, 2009.

SEMYONOV, D.; RAMON, O.; KAPLUN, Z.; LEVIN-BRENER, L.; GUREVICH, N.; SHIMONI, E. Microencapsulation of *Lactobacillus paracasei* by spray freeze drying. **Food Research International**, v.43, p.193-202, 2010.

SHEU, T. Y.; MARSHALL, R. T.; HEYMANN, H. Improving Survival of Culture Bacteria in Frozen Desserts by Microentrapment. **Journal of Dairy Science**, v.76, p.1902-1907, 1993.

SIMPSON, P. J.; STANTON, C.; FITZGERALD, G. F.; ROSS, R. P. Intrinsic tolerance of *Bifidobacterium* species to heat and oxygen and survival following spray drying and storage. **Journal of Applied Microbiology**, v.99, p.493-501, 2005.

TALWALKAR, A.; MILLER, C. W.; KAILASAPATHY, K.; NGUYEN, M. H. Effect of packaging materials and dissolved oxygen on the survival of probiotic bacteria in yoghurt. **International Journal of Food Science and Technology**, v.39, p.605-611, 2004.

TONON, R. V.; BRABET, C.; PALLET, D.; BRAT, P.; HUBINGER, M. D. Physicochemical and morphological characterisation of açai (*Euterpe oleraceae* Mart.) powder produced with different carrier

agents. **International Journal of Food Science and Technology**, v.44, p.1950-1958, 2009.

TURGUT, T.; CAKMAKCI, S. Investigation of the possible use of probiotics in ice cream manufacture. **International Journal of Dairy Technology**, v.62, p.444-451, 2009.

VINDEROLA, C. G.; REINHEIMER, J. A. Culture media for the enumeration of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in the presence of yoghurt bacteria. **International Dairy Journal**, v.9, p.497-505, 1999.

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

CAPÍTULO 3

Efeito da microencapsulação na sobrevivência de *Bifidobacterium* BB-12 exposta a condições gastrintestinais simuladas e ao tratamento térmico

Artigo publicado:
FRITZEN-FREIRE, C. B.; PRUDÊNCIO, E. S.; PINTO, S. S.; MUÑOZ, I. B.; AMBONI, R. D. M. C. Effect of microencapsulation on survival of *Bifidobacterium* BB-12 exposed to simulated gastrointestinal conditions and heat treatments. **LWT – Food Science and Technology**, v. 50, p.39-44, 2013.

Efeito da microencapsulação na sobrevivência de *Bifidobacterium* BB-12 exposta a condições gastrintestinais simuladas e ao tratamento térmico

Resumo
O objetivo deste estudo foi avaliar a sobrevivência de *Bifidobacterium* BB-12 microencapsulada por *spray drying* com substituição parcial (1:1) de leite desnatado reconstituído (LDR) por prebióticos (inulina, inulina enriquecida com oligofrutose e oligofrutose) durante o processo de encapsulação (rendimento) e em condições de estresse, como em condições gastrintestinais simuladas e após tratamentos térmicos. As microcápsulas produzidas com LDR e inulina e aquelas produzidas com LDR e inulina enriquecida com oligofrutose apresentaram maior viabilidade após o processo de *spray drying* e maior rendimento da encapsulação. Todas as microcápsulas mostraram uma elevada taxa de sobrevivência da bifidobactéria após os testes com os fluidos ácidos e sais de bile, quando comparadas às células livres. Além disso, as microcápsulas produzidas com LDR e inulina e aquelas produzidas com LDR e inulina enriquecida com oligofrutose também conferiram maior proteção para a bifidobactéria sumetida às condições gastrintestinais simuladas. Da mesma forma, estas microcápsulas apresentaram relevantes contagens de bifidobactéria em condições extremas de temperatura.

Palavras-chave: Microencapsulação, *Spray drying*, *Bifidobacterium* BB-12, Prebióticos, Condições de estresse.

Effect of microencapsulation on survival of *Bifidobacterium* BB-12 exposed to simulated gastrointestinal conditions and heat treatments

Abstract

The aim of this study was to evaluate the survival of *Bifidobacterium* BB-12 microencapsulated by spray drying with the partial (1:1) replacement of reconstituted skim milk (RSM) with prebiotics (inulin, oligofructose-enriched inulin, and oligofructose) during the encapsulation process (yield), and under stress conditions, such as simulated gastrointestinal conditions and heat treatments. The microcapsules produced with RSM and inulin and those produced with RSM and oligofructose-enriched inulin showed a higher viability after spray drying and encapsulation yield. All the microcapsules produced in this present study showed the highest survival rate of bifidobacteria after gastric acid fluid and bile salts tests when compared with the free cells. The microcapsules produced with RSM and inulin and those produced with RSM and oligofructose-enriched inulin also showed higher protection for bifidobacteria under simulated gastrointestinal conditions. Similarly, these microcapsules showed relevant counts of bifidobacteria under extreme heat conditions.

Keywords: Microencapsulation, Spray drying, *Bifidobacterium* BB-12, Prebiotics, Stress conditions.

1 Introduction

To exert health benefits, the concentration of live probiotic bacteria needs to be of approximately 6 log CFU g^{-1} of the product at the time of consumption (ROY, 2005). Some of the major benefits of probiotics include control of serum cholesterol levels and intestinal infection, which beneficially influence the immune system, improving lactose utilization and anticarcinogenic activity (NOMOTO, 2005; SHAH, 2007). Thus, probiotic bacteria, such as bifidobacteria, have been incorporated into various food products (FRITZEN-FREIRE et al., 2010; PASEEPHOL; SHERKAT, 2009; RIVERA-ESPINOZA; GALLARDO-NAVARRO, 2010). However, a major challenge in relation to the application of probiotic cultures in functional foods is the maintenance of viability during processing (GRANATO et al., 2010). Moreover, since viable and biologically active micro-organisms are usually required at the target site in the host, it is essential that probiotics withstand the host's natural barriers such as gastrointestinal transit (CHEN, CHEN; KUO, 2007; KIM et al.., 2008).

Microencapsulation technologies are a promising prospect for introducing viable probiotic bacteria in foods because the encapsulation matrix can provide a physical barrier against stress conditions (CHÁVARRI et al., 2010; NAZZARO et al., 2009). The capsules should also be able to maintain their integrity during passage through the gastrointestinal tract until they reach their target destination (colon), where they should break down and release the probiotic bacteria (DING; SHAH, 2009). These technologies are also often used to protect the cells

from heat treatment in food processing, thus potentially reducing cell injury and death (DING; SHAH, 2007).

Spray drying is a commonly used method for probiotic encapsulation (DE VOS et al., 2010). This method involves atomization of an emulsion or a suspension of probiotics and encapsulating agents in a hot air drying chamber, resulting in rapid evaporation of water (GHARSALLAOUI et al., 2007). The advantages of spray drying are its rapidity and relatively low cost. The technique is highly reproducible and its most important feature is that it is suitable for industrial applications (BURGAIN et al., 2011). Despite the advantages claimed for this method, and the short residence time in the drying chamber, significant inactivation of the cells can occur during the process. Thus, the choice of an appropriate drying medium is crucial because it might enhance the survival rate of bacteria throughout stressful treatments (SILVA et al., 2011). Reconstituted skim milk (RSM) is widely used as a protective agent in spray drying (ROKKA; RANTAMÄKI, 2010), but only few studies have been reported on the application of prebiotics as a source of coating materials. According to Corcoran et al. (2004), prebiotics, as inulin and oligofructose, may potentially be exploited as carrier media for the purposes of spray drying and may be useful for enhancing probiotic survival during processing and storage. The effect of prebiotics is related to selective stimulation, for example, of *Bifidobacterium* in the gut, thereby increasing the host's natural resistance to invading pathogens (COUDRAY et al., 2003). Bielecka, Biedrzycka, and Majkowska (2002) confirmed the appropriateness of combining prebiotics and probiotics, demonstrating greater effectiveness when compared with probiotics alone.

The aim of this study was to evaluate the survival of *Bifidobacterium* BB-12 microencapsulated by spray drying with partial (1:1) replacement of RSM with prebiotic agents (inulin, oligofructose-enriched inulin, and oligofructose), during the encapsulation process (yield), and under stress conditions, such as simulated gastrointestinal conditions and heat treatments.

2 Materials and methods

2.1 Materials

Bifidobacterium BB-12 (BB-12®, Chr. Hansen, Hónsholm, Denmark) was used as the active material for the microcapsules. The encapsulating agents used were commercial skim milk powder (Molico®, Nestlé, São Paulo, Brazil) and the prebiotic agents inulin (Orafti® HPX, Orafti, Tienen, Belgium) with degree of polymerization (DP) ≥ 23; oligofructose-enriched inulin (Orafti® Synergy1, Orafti, Tienen, Belgium), which is a mixture of oligofructose (DP 2 - 8) and long-chain inulin fraction (DP 10 - 60); and oligofructose (Orafti® P95, Orafti, Tienen, Belgium) with DP 2 - 8. MRS agar (Difco, Sparks, USA), lithium chloride (Vetec, Rio de Janeiro, Brazil), sodium propionate (Fluka, Neu-Ulm, Germany), AnaeroGen® (Oxoid, Hampshire, UK) and bile salts (Oxgall, Difco, Sparks, USA) were used for the microbiological analysis. All the chemicals used were of analytical grade.

2.2 Sample preparation and microencapsulation

The feed solutions were prepared as described by Ananta, Volkert, and Knorr (2005), with modifications. Reconstituted skim milk (RSM) at a concentration of 200 g L^{-1} was used as the control medium. The prebiotic media used for spray drying consisted of an equal ratio of RSM and each of the three prebiotics (ratio of 1:1, 200 g L^{-1} total concentration). All the media were homogenized in sterile distilled water and heat treated at 80 °C for 30 min. Freeze-dried probiotic cells of *Bifidobacterium* BB-12 were rehydrated at 25 g L^{-1} using a 120 g L^{-1} solution of RSM and frozen as stock solution at -18 °C into sterile glass bottles. This probiotic stock solution was incubated at 37 °C for 2 hours and after that it was inoculated (100 mL L^{-1}) into the four feed solutions and then submitted to spray drying.

The microencapsulation process was performed with a laboratory scale spray dryer (Buchi B-290, Flawil, Switzerland) at constant air inlet temperature of 150 ± 2 °C and outlet temperature of 55 ± 3 °C, as described by Fritzen-Freire et al. (2012). The microcapsules containing *Bifidobacterium* BB-12 were collected from the base of the cyclone and placed in sterile plastic bottles. Thus, four different microcapsules were obtained through the following combination of encapsulating agents: M1 (with 200 g L^{-1} of RSM), M2 (with 100 g L^{-1} of RSM and 100 g L^{-1} of inulin), M3 (with 100 g L^{-1} of RSM and 100 g L^{-1} of oligofructose-enriched inulin) and M4 (with 100 g L^{-1} of RSM and 100 g L^{-1} of oligofructose).

To evaluate the survival rate of bifidobacteria during spray drying, the enumeration was determined before (in the feed solutions)

and after (microcapsules M1, M2, M3 and M4) the process. The viable cell counts of the bifidobacteria exposed to simulated gastrointestinal conditions and different heat treatments were performed in the free (control) and in the microencapsulated forms.

2.3 Enumeration of bifidobacteria

To enumerate the bifidobacteria, the samples were serially diluted with peptone water (0.1 g 100 g^{-1}) and plated on MRS agar modified with the addition of lithium chloride (0.2 g 100 g^{-1}) and sodium propionate (0.3 g 100 g^{-1}), as proposed by Vinderola and Reinheimer (1999). The plates were incubated in anaerobic jars containing AnaeroGen® at 37 ± 1 °C for 72 h. After the incubation period, the count of viable probiotic cells was carried out and expressed as log colony-forming units per gram (log CFU g^{-1}). However, the entrapped bifidobacteria (1 g) was first re-suspended in 9 mL of phosphate buffer (0.1 mol L^{-1}, pH 7.0) followed by homogenization in a magnetic stirrer for 10 min, according to the method proposed by Sheu, Marshall, and Heymann (1993). This treatment ensured the complete release of the entrapped bifidobacteria from inside the dried particles. All the experiments were performed in triplicate.

2.4 Encapsulation Yield

Encapsulation yield (EY) (g 100 g^{-1}), i.e., survival rate during the microencapsulation process (Eq.(1)), was calculated as proposed by Picot and Lacroix (2004):

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

where N is the number of viable cells (log CFU g^{-1}) of dry matter in the microcapsules, and N_0 is the number of viable cells (log CFU g^{-1}) of dry matter in the feed solutions (before drying).

2.5 Survival of free and microencapsulated bifidobacteria in simulated gastric conditions and bile salts

The NGYC medium (12.0 g 100 g^{-1} non-fat milk, 2.0 g 100 g^{-1} glucose, 1.0 g 100 g^{-1} yeast extract, and 0.05 g 100 g^{-1} cysteine) was used to simulate gastric conditions, as described by Iyer and Kailasapathy (2005). The free (1 mL) and microencapsulated (1 g) bifidobacteria were added to the NGYC medium that had been previously adjusted to pH 2.0, pH 3.0, or 6.5 (control) with 5 mol L^{-1} HCl or 1 mol L^{-1} NaOH in 10 mL aliquots. The samples were incubated at 37 ± 1 °C for 3 h. An aliquot of 1 mL from each treatment was taken hourly for determination of the viable cell counts.

The resistance to bile salts was determined by inoculating free and microencapsulated cells in milk-yeast extract medium (10.0 g 100 g^{-1} non-fat milk, 0.5 g 100 g^{-1} yeast extract, and 0.05 g 100 g^{-1} cysteine) (TRUELSTRUP HANSEN et al., 2002) containing 0 (control), 5.0 and 10.0 g L^{-1} of Oxgall bile salts. The samples were withdrawn after incubation at 37 ± 1 °C for 0, 3 and 6 h to carry out cell counts of the bifidobacteria.

2.6 Survival of free and microencapsulated bifidobacteria under heat treatments

The resistance of free and microencapsulated *Bifidobacterium* BB-12 (M1, M2, M3 and M4) to heat treatments (55, 65 and 75 ± 1 °C for 1 and 10 min) was investigated using sterile distilled water, as a suspending medium. One gram of microcapsules and 1mL of the free cell suspension were transferred into test tubes containing 10 mL of distilled water each, as suggested by Sabikhi et al. (2010). After the heat treatments (water bath), the content was cooled to room temperature (~25 °C) and then the cell counts of bifidobacteria were performed.

2.7 Statistical analysis

The data analysis was carried out using STATISTICA 7.0 software (StatSoft Inc., Tulsa, USA). Analysis of variance (ANOVA) was used to determine significant differences ($P < 0.05$) amongst the microcapsules. Differences between means were detected by using Tukey's test.

3 Results and discussion

3.1 Enumeration of bifidobacteria and encapsulation yield

The viable cell counts for *Bifidobacterium* BB-12 in the feed solutions (before spray drying), in the microcapsules (after microencapsulation, i. e., M1, M2, M3 and M4) produced with different

encapsulating agents and also the encapsulating yields are shown in Table 1.

Table 1 - Viable cell counts of *Bifidobacterium* BB-12 in the feed solutions and in the microcapsules produced with different encapsulating agents and the encapsulation yields (EY).

	Encapsulating agents			
	Reconstituted skim milk (RSM)	*RSM and inulin*	*RSM and oligofructose-enriched inulin*	*RSM and oligofructose*
Feed solutions (log CFU g^{-1})	14.73 ± 0.01^{a}	14.48 ± 0.18^{a}	14.37 ± 0.17^{a}	14.03 ± 0.33^{a}
Microcapsules (log CFU g^{-1})	10.57 ± 0.05^{a}	10.91 ± 0.07^{b}	10.88 ± 0.02^{b}	9.90 ± 0.08^{a}
EY (g 100 g^{-1})	71.72 ± 0.27^{a}	75.37 ± 0.43^{b}	75.68 ± 1.04^{b}	70.58 ± 1.03^{a}

[a-b] Means ± standard deviation with different superscript letters in the same line indicate significant differences ($P < 0.05$) among the studied samples.

The results showed similar ($P > 0.05$) counts of bifidobacteria in the different feed solutions. On the other hand, the counts of bifidobacteria and the encapsulation yields were higher ($P < 0.05$) for the microcapsules produced with reconstituted skim milk (RSM) and inulin (M2) and with RSM and oligofructose-enriched inulin (M3) when compared to the microcapsules produced only with RSM (M1) and with oligofructose (M4). This result occurred probably because, as described

by Fritzen-Freire et al. (2012), the inulin acts as a thermoprotector for the cells of *Bifidobacterium* BB-12 undergoing the spray drying process, showing a positive effect on their survival during the encapsulation process. Moreover, Chávez and Ledeboer (2007) reported that inlet and outlet temperatures are one of the most important causes of bacterial stress and mortality during the spray drying process and consequently affect the encapsulation yield. Therefore, in this present study low temperatures (inlet temperature of 150 ± 2 °C and outlet temperature of 55 ± 3 °C) were used in order to minimize heat treatment, while obtaining a good overall drying result. Fu and Chen (2011) also reported that the cell loss during the atomization step could be attributed to the choice of the appropriate drying medium, as well as to the high water content of the feed solution and the short residence time in the drying chamber of the equipment. Therefore, as described by those authors, the combined effect of these three factors could protect the bacterial cells from being heated up to a fatal temperature, and thus result in good encapsulation efficiency, as was verified in this present study.

In addition, the count of viable probiotic cells obtained for all the microcapsules was above the recommended levels for a probiotic food, i.e., equal to or greater than 6 log CFU g^{-1} of the product, which is in accordance with what was reported by Shah (2007).

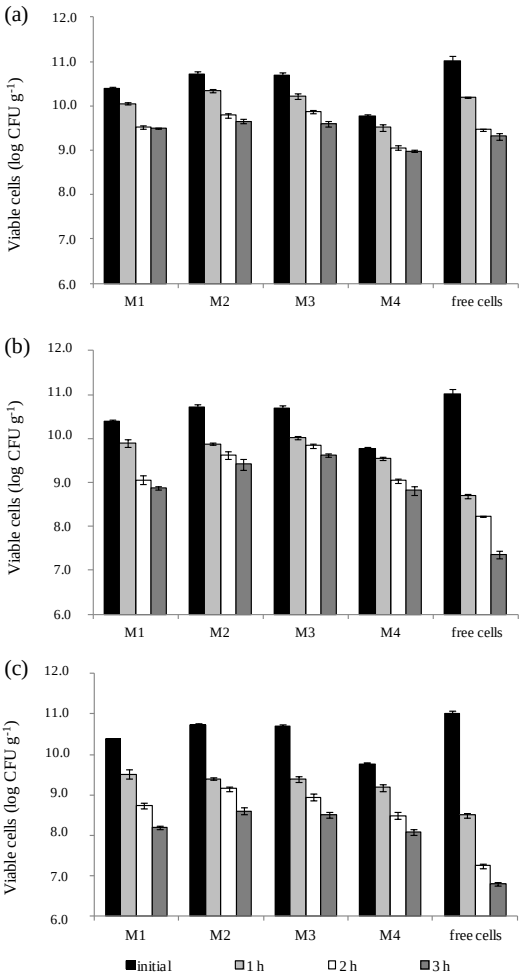
3.2 Survival of free and microencapsulated bifidobacteria in simulated gastric conditions and bile salts

The effect of acid conditions on the viability of the free and of the microencapsulated *Bifidobacterium* BB-12 is shown in Fig.1. This strain

showed a steady loss in viability when exposed to acid conditions. However, the microcapsules containing bifidobacteria survived very well ($P < 0.05$) after exposure to in vitro acid conditions when compared with the free cells. Also, a decrease of approximately 4 log was noted in the number of free cells after 3 h of incubation at pH 2, when compared to decreases of about 2 log in the all microencapsulated bifidobacteria under similar conditions.

The microcapsules produced with RSM and inulin (M2) and those produced with RSM and oligofructose-enriched inulin (M3) showed higher ($P < 0.05$) count when incubated in pH 3.0 and 2.0 after 3 h of exposure, when compared to the microcapsules produced only with RSM (M1) and with oligofructose (M4). These results suggest that inulin had a better effect on the protection of the bifidobacteria during the gastric simulation. Barclay et al. (2010) reported that inulin might be ideal to transport substances to the colon since it is stable to the range of pH and ionic strength in the human gastrointestinal tract. Moreover, Mantzouridou, Spanou, and Kiosseoglou (2012) also state that the inulin molecules are not hydrolyzed by the human gastrointestinal tract enzymes and hence provide a beneficial effect on health, acting as dietary fiber. Apart from such properties, Ann et al. (2007) claim that the use of prebiotic substrates, such as inulin, as encapsulating agents may promote more beneficial effects, including the reduction of diarrhea and the inactivation of pathogens in the gastrointestinal tracts of both humans and animals.

Figure 1 - Survival of *Bifidobacterium* BB-12 free and microencapsulated with different encapsulating agents, under in vitro acid conditions at 37 ± 1 °C, (a) pH 6.5, (b) pH 3.0 and (c) pH 2.0.

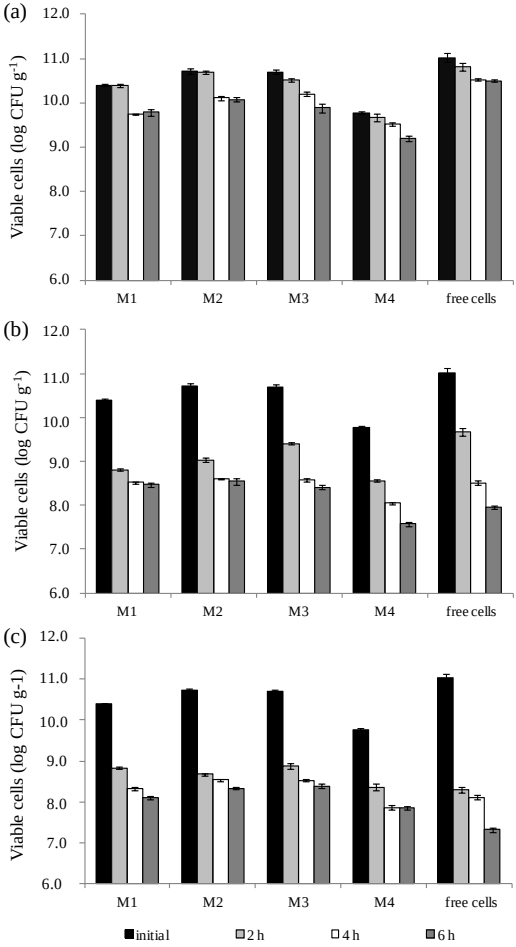


(M1): microcapsule with reconstituted skim milk (RSM); (M2): microcapsule with RSM and inulin; (M3): microcapsule with RSM and oligofructose-enriched inulin; (M4): microcapsule with RSM and oligofructose. Error bars represent the mean of the results of the experiment.

The survival of free and microencapsulated *Bifidobacterium* BB-12 was also monitored for up to 6 h after exposure to milk-yeast medium containing 5.0 and 10.0 g L^{-1} bile salts (Fig. 2). The results indicate a similar trend that was found under high acid conditions. From the initial counts of free and microencapsulated bifidobacteria, the numbers declined steadily as the bile concentration and time of incubation increased. Moreover, the decrease rate was much greater in the free cells. The microcapsules less susceptible to oxgall were those produced with RSM and inulin (M2) and those produced with RSM and oligofructose-enriched inulin (M3), with decreases of 2.39 and 2.32 log CFU g^{-1} in viability after 6 h of incubation and at 10.0 g L^{-1} bile salts, respectively. On the other hand, the free cells showed the largest ($P < 0.05$) decreases in their viability under the same conditions (3.71 log CFU g^{-1}). These results are in accordance with those obtained by Chandramouli et al. (2004) and Iyer and Kailasapathy (2005), who used the same concentrations of bile salts as those employed in this present study and noted that encapsulated probiotic bacteria can survive in higher numbers than the free probiotic cells.

Finally, the results obtained in this present study showed that microencapsulation with RSM and the prebiotics inulin and oligofructose-enriched inulin could provide good protection for bifidobacteria undergoing the gastric acid fluid and bile salts tests.

Figure 2 - Survival of *Bifidobacterium* BB-12 free and microencapsulated with different encapsulating agents in the presence of (a) 0.0 g L^{-1}, (b) 5.0 g L^{-1} and (c) 10.0 g L^{-1} bile salts, after 6 h exposure at 37 ± 1 °C.



(M1): microcapsule with reconstituted skim milk (RSM); (M2): microcapsule with RSM and inulin; (M3): microcapsule with RSM and oligofructose-enriched inulin; (M4): microcapsule with RSM and oligofructose. Error bars represent the mean of the results of the experiment.

3.3 Survival of free and microencapsulated bifidobacteria under heat treatments

The survival of free and microencapsulated bifidobacteria exposed to temperatures of 55, 65, and 75 ± 1 °C are shown in Table 2. The free cells of *Bifidobacterium* BB-12 were very sensitive to heat treatment, their numbers decreased from 11.03 log cycles to 10.00 log at 55 ± 1 °C and less than 6.00 log at 65 ± 1 °C and 75 ± 1 °C, after 10 min. Differences between the decrease in the counts of the free and of the encapsulated cells ($P < 0.05$) were noted. This result occurred probably because, as described by Corcoran et al. (2008), the excessive heat unfolds the higher order structure of macromolecules such as protein and nucleic acid of bacterial cells, breaks the linkage between monomeric units, and eventually causes the destruction of the monomers, leading to bacterial death.

The microcapsules produced only with RSM (M1) survived well at 55 ± 1 °C for 10 min, with an average loss of only 0.15 log CFU g^{-1} compared to microcapsules containing prebiotics (M2, M3 and M4), with an average loss of 0.44, 0.51 and 0.34 log CFU g^{-1}, respectively, after 10 min. However, at 65 ± 1 °C, all microcapsules showed a similar reduction ($P < 0.05$) in viable cell count. Meanwhile, none of the microcapsules showed any probiotic count (> 6.00 log CFU g^{-1}) after exposure for 10 min at 75 ± 1 °C. These results indicate that the encapsulating agents used in this present study offered little protection for the probiotic bacteria after this time/temperature combination. Barclay et al. (2010) suggest that in the presence of water an increase in temperature leads to an increase in hydrolysis of inulin that follows first-

or pseudo-first-order kinetics at both neutral and acid pHs. Those authors also reported that the inulin hydrolysis rate can be considered insignificant for processing time frames up to ~ 60 ºC, but can be more relevant at higher temperatures, as those used in this present study.

Table 2 - The effect of exposure to 55, 65 and 75 ± 1 °C for 0, 1 and 10 min on the survival of bifidobacteria free and microencapsulated with different encapsulating agents.

Temp (°C)	*Time (min)*	*Viability (log CFU g^{-1})*				
		M1	*M2*	*M3*	*M4*	*Free cells*
55	0	10.57 ± 0.05^{aA}	10.91 ± 0.07^{bA}	10.88 ± 0.02^{bA}	9.90 ± 0.08^{cA}	11.03 ± 0.11^{bA}
	1	10.46 ± 0.08^{aA}	10.80 ± 0.02^{bcA}	10.76 ± 0.04^{cA}	9.73 ± 0.04^{dAB}	10.58 ± 0.04^{acB}
	10	10.42 ± 0.07^{aA}	10.47 ± 0.02^{aB}	10.37 ± 0.04^{aB}	9.56 ± 0.04^{bB}	10.00 ± 0.05^{cC}
65	0	10.57 ± 0.05^{aA}	10.91 ± 0.07^{bA}	10.88 ± 0.02^{bA}	9.90 ± 0.08^{cA}	11.03 ± 0.11^{bA}
	1	10.30 ± 0.02^{aB}	10.61 ± 0.04^{bB}	10.51 ± 0.04^{bB}	9.23 ± 0.04^{cB}	9.09 ± 0.04^{dB}
	10	9.87 ± 0.03^{aC}	10.12 ± 0.04^{bC}	10.07 ± 0.03^{bC}	9.01 ± 0.04^{cC}	NP
75	0	10.57 ± 0.05^{aA}	10.91 ± 0.07^{bA}	10.88 ± 0.02^{bA}	9.90 ± 0.08^{cA}	11.03 ± 0.11^{b}
	1	10.07 ± 0.03^{aB}	10.42 ± 0.03^{bB}	10.48 ± 0.05^{bB}	9.18 ± 0.01^{cB}	NP
	10	NP	NP	NP	NP	NP

NP = not probiotic enumeration (minor than 6 log CFU g^{-1})

$^{a-d}$ Means ± standard deviation with different superscript letters in the same line indicate significant differences ($P < 0.05$) among the studied microcapsules in the same time and temperature.

$^{A-C}$ Means ± standard deviation with different superscript letters in the same column indicate significant differences ($P < 0.05$) among times studied for same microcapsule in the same temperature evaluated.

M1: microcapsule with reconstituted skim milk (RSM)

M2: microcapsule with RSM and inulin

M3: microcapsule with RSM and oligofructose-enriched inulin

M4: microcapsule with RSM and oligofructose

Nevertheless, it was evident that the microcapsules produced with RSM and inulin (M2) and those produced with RSM and oligofructose-enriched inulin (M3) showed the highest counts ($P < 0.05$) of bifidobacteria in extreme heat conditions, i.e, at 75 ± 1 °C for 1 min. In addition, the microcapsules produced with RSM and oligofructose (M4) showed the lowest resistance to heat treatments. Lian, Hsiao, and Chou (2002) reported that besides differences in their chemical characteristics, encapsulating agents have different physical properties. Furthermore, according to Tárrega, Rocafull, and Costell (2010) long-chain inulin, with a high degree of polymerization (used in the microcapsules M2 and M3), is more thermally stable and less soluble, thus rendering higher protection against *Bifidobacterium* BB-12. The results of this present study suggest that microencapsulation using inulin and oligofructose-enriched inulin may enhance thermal resistance of the *Bifidobacterium* BB-12.

4 Conclusions

The use of the prebiotics inulin and oligofructose-enriched inulin improved both the survival of *Bifidobacterium* BB-12 after spray drying and the encapsulation yield of the microcapsules. These prebiotics also conferred better protection for the bifidobacteria submitted to simulated gastrointestinal conditions and extreme heat treatments.

This study shows that microencapsulation by spray drying can be used as an effective method for maintaining the survival of *Bifidobacterium* BB-12 exposed to stress conditions. In addition, the results showed that inulin and oligofructose-enriched inulin are the most

appropriate prebiotics to be used as partial replacement of reconstituted skim milk (RSM), providing greater resistance to microencapsulated bifidobacteria.

Acknowledgments

The authors gratefully thank the Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC) for their financial support, and also Clariant for providing the prebiotics.

References

ANANTA, E.; VOLKERT, M.; KNORR, D. Cellular injuries and storage stability of spray dried *Lactobacillus rhamnosus* GG. **International Dairy Journal**, v.15, p.399-409, 2005.

ANN, E. Y.; KIM, Y.; OH, S. J.; IMM J. Y.; PARK, D. J.; HAN, K. S.; KIM, S. H. Microencapsulation of *Lactobacillus acidophilus* ATCC 43121 with prebiotic substrates using a hybridisation system. **International Journal of Food Science and Technology,** v.42, p.411-419, 2007.

BARCLAY, T.; GINIC-MARKOVICA, M.; COOPER, P.; PETROVSKY, N. Inulin - A versatile polysaccharide with multiple pharmaceutical and food chemical uses. **Journal of Excipients and Food Chemicals**, v.1, p.27-50, 2010.

BIELECKA, M.; BIEDRZYCKA, E.; MAJKOWSKA, A. Selection of probiotics and prebiotics for synbiotics and confirmation of their in vivo effectiveness. **Food Research International**, v.35, p.125-131, 2002.

BURGAIN, J.; GAIANI, C.; LINDER, M.; SCHER, J. Encapsulation of probiotic living cells: From laboratory scale to industrial applications. **Journal of Food Engineering**, v.104, p.467-483, 2011.

CHANDRAMOULI, V.; KAILASAPATHY, K.; PEIRIS, P.; JONES, M. An improved method of microencapsulation and its evaluation to protect *Lactobacillus* spp. in simulated gastric conditions. **Journal of Microbiological Methods**, v.56, p.27-35, 2004.

CHÁVARRI, M.; MARAÑÓN, I.; ARES, R.; IBÁÑEZ, F. C.; MARZO, F.; VILLARÁN, M. D. C. Microencapsulation of a probiotic and prebiotic in alginate-chitosan capsules improves survival in simulated gastro-intestinal conditions. **International Journal of Food Microbiology**, v.142, p.185-189, 2010.

CHÁVEZ, B. E.; LEDEBOER, A. M. Drying of probiotics: optimization of formulation and process to enhance storage survival. **Drying Technology**, v..25, p.193-201, 2007.

CHEN, M. J.; CHEN, K. N.; KUO, Y.-T. Optimal Thermotolerance of *Bifidobacterium bifidum* in Gellan-Alginate Microparticles. **Biotechnology and Bioengineering**, v.98, p.411-419, 2007.

CORCORAN, B. M.; ROSS, R. P.; FITZGERALD, G. F.; STANTON, C. Comparative survival of probiotic lactobacilli spray-dried in the presence of prebiotic substances. **Journal of Applied Microbiology**, v.96, p.1024-1039, 2004.

CORCORAN, B. M.; STANTON, C.; FITZGERALD, G.; ROSS, R. P. Life under stress: The probiotic stress response and how it may be manipulated. **Current Pharmaceutical Design**, v.14, p.1382-1399, 2008.

COUDRAY, C.; TRESSOL, J. C.; GUEUX, E.; RAYSSIGUIER, Y. Effects of inulin-type fructans of different chain length and type of branching or intestinal absorption and balance of calcium and magnesium in rats. **European Journal of Nutrition**, v.42, p.91-98, 2003.

DE VOS, P.; FAAS, M. M.; SPASOJEVIC, M.; SIKKEMA, J. Encapsulation for preservation of functionality and targeted delivery of bioactive food components. **International Dairy Journal**, v.20, p.292-302, 2010.

DING, W. K.; SHAH, N. P. Acid, Bile, and Heat Tolerance of Free and Microencapsulated Probiotic Bacteria. **Journal of Food Science**, v.72, p.M446- M450, 2007.

DING, W. K.; SHAH, N. P. An improved method of microencapsulation of probiotic bacteria for their stability in acidic and bile conditions during storage. **Journal of Food Science**, v.74, p.M53-M61, 2009.

FRITZEN-FREIRE, C. B.; MÜLLER, C. M. O.; LAURINDO, J. B.; PRUDÊNCIO, E. S. The influence of *Bifidobacterium* Bb-12 and lactic acid incorporation on the properties of Minas Frescal cheese. **Journal of Food Engineering**, v.96, p.621-627, 2010.

FRITZEN-FREIRE, C. B.; PRUDÊNCIO, E. S.; AMBONI, R. D. M. C.; PINTO, S. S.; NEGRÃO-MURAKAMI, A. N.; MURAKAMI, F. S. Microencapsulation of bifidobacteria by spray drying in the presence of prebiotics. **Food Research International**, v.45, p.306-312, 2012.

FU, N.; CHEN, X. D. Towards a maximal cell survival in convective thermal drying processes. **Food Research International**, v.44, p.1127-1149, 2011.

GHARSALLAOUI, A.; ROUDAUT, G.; CHAMBIN, O.; VOILLEY, A.; SAUREL, R. Applications of spray-drying in microencapsulation of food ingredients: An overview. **Food Research International**, v.40, p.1107-1121, 2007.

GRANATO, D.; BRANCO, G. F.; CRUZ, A. G.; FARIA, J. A. F.; SHAH, N. P. Probiotic dairy products as functional foods. **Comprehensive Reviews in Food Science and Food Safety**, v.9, p.455-470, 2010.

IYER, C.; KAILASAPATHY, K. Effect of co-encapsulation of probiotics with prebiotics on increasing the viability of encapsulated bacteria in simulated gastrointestinal conditions and in yoghurt**. Journal of Food Science**, v.70, p.M18-M23, 2005.

KIM, S. J.; CHO, S. Y.; KIM, S. H.; SONG, O. J.; SHIN, I. S.; CHA, D. S. Effect of microencapsulation on viability and other characteristics in

Lactobacillus acidophilus ATCC 43121. **LWT - Food Science and Technology**, v.41, p.493-500, 2008.

LIAN, W. C.; HSIAO, H. C.; CHOU, C. C. Survival of bifidobacteria after spray drying. **International Journal of Food Microbiology**, v.74, p.79-86, 2002.

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

NAZZARO, F.; FRATIANNI, F.; COPPOLA, R.; SADA, A.; ORLANDO, P. Fermentative ability of alginate-prebiotic encapsulated *Lactobacillus acidophilus* and survival under simulated gastrointestinal conditions. **Journal of Functional Foods**, v.1, p.319-323, 2009.

NOMOTO, K. Review prevention of infections by probiotics. **Journal of Bioscience and Bioengineering**, v.100, p.583-592, 2005.

PASEEPHOL, T.; SHERKAT, F. Probiotic stability of yoghurts containing Jerusalem artichoke inulins during refrigerated storage. **Journal of Functinal Foods**, v.1, p.311-318, 2009.

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, p.505-515, 2004.

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

ROKKA, S.; RANTAMÄKI, P. Protecting probiotic bacteria by microencapsulation: challenges for industrial applications. **European Food Research Technology**, v.231, p.1-12, 2010.

ROY, D. Technological aspects related to the use of bifidobacteria in dairy products. **Lait**, v.85, p.39-56, 2005.

SABIKHI, L.; BABU, R.; THOMPKINSON, D. K.; KAPILA, S. Resistance of microencapsulated *Lactobacillus acidophilus* LA1

processing treatments and simulated gut conditions. **Food Bioprocess Technology**, v.3, p.586-593, 2010.

SHAH, N. P. Functional cultures and health benefits. **International Dairy Journal**, v.17, p.1262-1277, 2007.

SHEU, T. Y.; MARSHALL, R. T.; HEYMANN, H. Improving survival of culture bacteria in frozen desserts by microentrapment. **Journal of Dairy Science**, v.76, p.1902-1907, 1993.

SILVA, J.; FREIXO, R.; GIBBS, P.; TEIXEIRA, P. Spray-drying for the production of dried cultures. **International Journal of Dairy Technology**, v.64, p.321-335, 2011.

TÁRREGA, A.; ROCAFULL, A.; COSTELL, E. Effect of blends of short and long-chain inulin on the rheological and sensory properties of prebiotic low-fat custards. **LWT-Food Science and Technology**, v.43, p.556–562, 2010.

TRUELSTRUP HANSEN, L.; ALLAN-WOJTAS, P. M.; JIN, Y. L.; PAULSON, A. T. Survival of free and calcium-alginate microencapsulated *Bifidobacterium* spp. in simulated gastro-intestinal conditions. **Food Microbiology**, v.19, p.35-45, 2002.

VINDEROLA, C. G.; REINHEIMER, J. A. Culture media for the enumeration of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in the presence of yoghurt bacteria. **International Dairy Journal**, v.9, p.497-505, 1999.

CAPÍTULO 4

Efeito da aplicação de *Bifidobacterium* BB-12 microencapsulada com prebióticos por *spray drying* nas propriedades de creme de ricota

Artigo publicado:
FRITZEN-FREIRE, C. B.; PRUDÊNCIO, E. S.; PINTO, S. S.; MUÑOZ, I. B.; MÜLLER, C. M. O.; VIEIRA, C. R. W.; AMBONI, R. D. M. C. Effect of the application of *Bifidobacterium* BB-12 microencapsulated by spray drying with prebiotics on the properties of ricotta cream. **Food Research International**, v. 52, p. 50-55, 2013.

Efeito da aplicação de *Bifidobacterium* BB-12 microencapsulada com prebióticos por *spray drying* nas propriedades de creme de ricota

Resumo

O efeito da aplicação de *Bifidobacterium* BB-12 microencapsulada por *spray drying* com prebióticos nas propriedades microbiológicas, físico-químicas, de textura, de cor e sensoriais de creme de ricota foi avaliado. Leite desnatado reconstituído (LDR)/inulina e LDR/inulina enriquecida com oligofrutose foram utilizados como agentes encapsulantes da bifidobactéria. A viabilidade após 60 dias de armazenamento das amostras de creme de ricota contendo a bifidobactéria microencapsulada foi muito maior do que a amostra adicionada de células livres. Durante o armazenamento de todas as amostras de creme de ricota, os valores de acidez e sólidos totais aumentaram, enquanto os do pH diminuiram. A adição das microcápsulas contribuiu para o aumento da firmeza das amostras. Os valores da adesividade aumentaram durante o armazenamento das amostras de creme de ricota adicionadas de bifidobactéria livre e de bifidobactéria microencapsulada com LDR e inulina enriquecida com oligofrutose, enquanto os valores da elasticidade permaneceram estáveis. Todas as amostras apresentaram alta luminosidade e uma tendência à coloração verde e amarela durante o período de armazenamento. Os resultados da análise sensorial mostraram que a adição de bifidobactéria nas amostras de creme de ricota, tanto na forma livre quanto microencapsulada, resultou em uma boa aceitabilidade do produto.

Palavras-chave: Creme de ricota, Bifidobactéria, Microencapsulação, *Spray drying*, Prebióticos.

Effect of the application of *Bifidobacterium* BB-12 microencapsulated by spray drying with prebiotics on the properties of ricotta cream

Abstract

The effect of the application of *Bifidobacterium* BB-12 microencapsulated by spray drying with prebiotics on the microbiological, physicochemical, texture, color, and sensory properties of ricotta cream was evaluated. Reconstituted skim milk (RSM)/inulin and RSM/oligofructose-enriched inulin were used as encapsulating agents of the bifidobacteria. The viability count for the ricotta cream samples with microencapsulated bifidobacteria was much greater after 60 days of storage than for those with free cells. During storage of all the ricotta cream samples, the acidity and total solids content increased, while the pH decreased. The addition of microcapsules contributed to the increase in the firmness of the samples. The values for adhesiveness increased during storage of the ricotta cream samples with free bifidobacteria and with bifidobacteria microencapsulated with RSM and oligofructose-enriched inulin, while the values for elasticity remained stable. All the samples showed high luminosity and a tendency towards a green and yellow color during the storage period. The results of the sensory analysis showed that the addition of bifidobacteria in the ricotta cream samples, whether in the free or in the microencapsulated form, resulted in a good acceptability of the product.

Keywords: Ricotta cream, Bifidobacteria, Microencapsulation, Spray drying, Prebiotics.

1 Introduction

Microorganisms of the genus *Bifidobacterium* are considered as probiotics and have been widely used as active ingredients in functional dairy products (GONZÁLEZ-SÁNCHEZ et al., 2010). Probiotics are viable microorganisms that are beneficial to the host when administered in appropriate quantities (FAO/WHO, 2001). They are responsible for the protection of the human body from infections, especially along the colonized mucosal surfaces of the gastrointestinal tract (SANDERS, 2003). However, probiotic products must have a microbial count higher than 6 log CFU per mL or per g until the end of their shelf life in order to produce their claimed benefits (ROY, 2005). In this sense, bifidobacteria have been incorporated into a range of different types of dairy products, including cheeses (BOYLSTON et al., 2004).

Ricotta cream is a cheese obtained from the homogenization of ricotta cheese with further ingredients. It has a soft consistency and is used as bread spread. Because of their manufacturing process, fresh cream cheeses, such as ricotta cream, can potentially serve as a carrier for probiotic bacteria (BURITI et al., 2007). However, several factors have been claimed to affect the viability of probiotic cultures in cheeses. Acidity and pH have been shown to have an effect during manufacture and storage (ROY, 2005). Other factors, such as storage temperature and oxygen content, also have been presumed to affect the viability of bifidobacteria (CRUZ et al., 2009). Consequently, industrial demand for technologies that ensure bifidobacteria stability in foods remains strong, since high cell survival is important for both economical and health

effects (DOLEYRES; LACROIX, 2005). Microencapsulation of probiotic bacteria is one of the most promising of these technologies.

Microencapsulation is a technique that can be used for bacterial cell protection and several studies have been carried out to investigate the protective effect of this technique against adverse conditions to which probiotics can be exposed in dairy products (BRINQUES; AYUB, 2011; GONZÁLEZ-SÁNCHEZ et al., 2010; MIRZAEI; POURJAFAR; HOMAYOUNI, 2012; PINTO et al., 2012). Spray drying is regarded as an appropriate microencapsulation method and it has been investigated as a means of stabilizing probiotic bacteria in a number of food matrices (CHÁVEZ; LEDEBOER, 2007). Carrier agents such as reconstituted skim milk (RSM) and prebiotics have been added to the drying media prior to spray drying in order to protect the viability of probiotics (ANANTA; VOLKERT; KNORR, 2005; FRITZEN-FREIRE et al., 2012; FRITZEN-FREIRE et al., 2013). RSM has been commonly used as encapsulating agent, showing a favorable effect on the improvement of bacterial cell survival during the spray drying process (FU; CHEN, 2011). Prebiotics such as inulin and oligofructose, which are dietary fibers and have bifidogenic effect (ROBERFROID, 2007), may have functional benefits for probiotics and subsequently for consumers and the use of these prebiotics may also affect the properties of the product (CASTRO et al., 2008). Thus, researches related with microencapsulation of probiotics with prebiotics and the incorporation of microcapsules into food products are relevant from both food technology and nutritional perspectives, which may be mutually complementary. However, the application of probiotic microencapsulated by spray drying with prebiotics could change the

properties of dairy products. Therefore, the aim of this study was to evaluate the effect of the application of *Bifidobacterium* BB-12 microencapsulated with RSM and prebiotics (inulin and oligofructose-enriched inulin) on the microbiological, physicochemical, texture, color and sensory properties of ricotta cream during 60 days of storage.

2 Materials and methods

2.1 Materials

A probiotic culture composed of *Bifidobacterium* BB-12 (BB-12®, Chr. Hansen, Hónsholm, Denmark) was used as the active material for the microcapsules. The encapsulating agents used were commercial skim milk powder (Molico®, Nestlé, São Paulo, Brazil) and the prebiotics inulin (Orafti® HPX, Orafti, Tienen, Belgium) with degree of polymerization (DP) ≥ 23, and oligofructose-enriched inulin (Orafti® Synergy1, Orafti, Tienen, Belgium) which is a mixture of oligofructose (DP 2–8) and long-chain inulin fraction (DP 10–60). MRS agar (Difco, Sparks, USA), lithium chloride (Vetec, Rio de Janeiro, Brazil), sodium propionate (Fluka, Neu-Ulm, Germany) and AnaeroGen® (Oxoid, Hampshire, UK) were used for the microbiological analysis. The commercial ingredients used in the manufacture of ricotta cream were: skimmed milk UHT (Tirol®, Santa Catarina, Brazil), butter (Elegê®, Rio Grande do Sul, Brazil), sodium chloride (Cisne®, Rio de Janeiro, Brazil) and potassium sorbate (Vetec®, Rio de Janeiro, Brazil). All the chemicals used were of analytical grade.

2.2 Microencapsulation by spray drying

2.2.1 Preparation of bacterial suspension and drying media

The bacterial suspension was prepared following the procedures described by Fritzen-Freire et al. (2012). This probiotic stock solution was used as free cells (ricotta cream control) and inoculated into two feed solutions for microencapsulation by spray drying. The feed solutions were prepared following the procedures described by Ananta, Volkert and Knorr (2005), with modifications. RSM and inulin (ratio of 1:1, 200 g L^{-1} total concentration) and RSM and oligofructose-enriched inulin (ratio of 1:1, 200 g L^{-1} total concentration) were used as drying media. The media were homogenized into sterile distilled water and heat treated at 80 °C for 30 min. Then, the bacterial suspension (14 log CFU g^{-1}) was added to two feed solutions at a concentration of 100 mL L^{-1}.

2.2.2 Spray Drying

The microencapsulation process was performed with a laboratory scale spray dryer (Buchi B-290, Flawil, Switzerland) at constant air inlet temperature of 150 ± 2 °C and outlet temperature of 55 ± 3 °C. The feed solutions containing *Bifidobacterium* BB-12 were kept under magnetic agitation at room temperature and fed into the main chamber through a peristaltic pump, with feed flow of 6 mL min^{-1}, drying air flow rate of 35 m^3 h^{-1}, and compressor air pressure of 0.7 MPa. The microcapsules were collected from the base of the cyclone and placed in sterile plastic bottles.

2.3 Manufacture of ricotta cheese

The ricotta cheese was manufactured from the acid coagulation of cheese whey (obtained from the manufacture of Minas Frescal cheese) by adding lactic acid (1 mL L^{-1} of 85 g per 100 g of food-grade solution) in the whey, at 90 ± 2 °C, as suggested by Pizzillo et al. (2005), with modifications. The precipitation of proteins occurred rapidly at this temperature and after approximately 2 minutes the precipitate was gently stirred and then completely drained. The ricotta cheese obtained was packaged and stored at 5 ± 1 °C. The ricotta cheese was the main ingredient for the manufacture of the ricotta cream, and it showed the following characteristics: 10.19 g $100g^{-1}$ protein content and 3.08 g 100 g^{-1} fat content.

2.4 Manufacture of ricotta cream

The ricotta cream was manufactured through intense mixing, with a blender (LiqFaz, Walita, Varginha, Brazil), of the following ingredients: ricotta cheese (56 g 100 g^{-1}), butter (9 g 100 g^{-1}), and skimmed milk UHT (34 g $100g^{-1}$) heated to 100 ± 1 °C. Sodium chloride (0.9 g $100g^{-1}$) and potassium sorbate (0.1 g $100g^{-1}$) were then added to the base cream. After all the ingredients were mixed and the cream cooled down (~ 45 °C), the free and the microencapsulated bifidobacteria were added to make different types of ricotta cream. Two batches were prepared for each of the three types of ricotta cream, denoted C1 (control), C2, and C3. C1 was produced with addition of bifidobacteria (0.2 g 100 g^{-1}) in their free form. C2 was prepared with

addition of bifidobacteria microencapsulated with RSM and inulin (1 g 100 g^{-1}), while C3 was added with bifidobacteria microencapsulated with RSM and oligofructose-enriched inulin (1 g 100 g^{-1}). The products obtained were packaged in plastic cups of 100 mL capacity, sealed with aluminum foil lids, and stored at 5 ± 1 ºC.

Evaluation of the ricotta cream samples in relation to their physicochemical composition was performed on day 1 of storage, whereas the analyses for total solids content, acidity and pH levels, and microbiological properties, as well as for texture and color, were performed on days 1, 15, 30, 45, and 60 of storage. Sensory analysis was carried out after one week of storage, according to the time required to guarantee the safety of panelists.

2.5 Microbiological analysis

To evaluate the survival of the microencapsulated bifidobacteria in the ricotta cream samples (C2 and C3), the entrapped bacteria were released from the microcapsules according to the methodology proposed by Sheu, Marshall, and Heymann (1993), where 25 g portions of ricotta cream were re-suspended in 225 mL of phosphate buffer (0.1 M, pH 7.0) followed by homogenization using a magnetic stirrer for 10 min. The ricotta cream sample that contained the free bifidobacteria (C1) was treated in the identical way in order to maintain the same treatment conditions as those for the samples added with microcapsules. To enumerate the bifidobacteria, samples were serially diluted with peptone water (0.1 g 100 g^{-1}) and plated on MRS agar modified with the addition of lithium chloride (0.2 g 100 g^{-1}) and sodium propionate (0.3 g 100 g^{-1}),

as proposed by Vinderola and Reinheimer (1999). The plates were incubated in anaerobic jars containing AnaeroGen® at 37 ± 1 °C for 72 h. After the incubation period, the count of viable probiotic cells was carried out and expressed as log colony forming units per gram (log CFU g^{-1}).

Prior to the sensory analysis, to ensure the microbiological safety of the ricotta cream samples, the counts of coliforms at 45 ºC, Coagulase-Positive Staphylococci**,** *Salmonella* sp. and *Listeria monocytogenes* were carried according to the methodology proposed by APHA (2001). All analyses were determined in triplicate.

2.6 Physicochemical analysis

The ricotta cream samples were analyzed for fat content (g 100 g^{-1}) by Soxhlet extraction with ether and for total solids (g 100 g^{-1}), obtained by drying to constant weight at 105 ºC, as described by the Analytical Norms of the Adolfo Lutz Institute (IAL, 2005). The proteic level (g 100 g^{-1}) was estimated trough the determination of the total nitrogen level by Kjeldahl method (N x 6. 38) and the ash (g 100 g^{-1}) according to AOAC (2005). Titratable acidity (g 100 g^{-1} lactic acid) was determined according to the AOAC (2005) while pH values were determined with a pH meter (MP220, Metler- Toledo, Greinfensee, Switzerland) by the potentiometric method. All the analyses were carried out in triplicate.

2.7 Texture analysis

For the texture analysis of the samples, it was used a texturometer model TA-XT plus (Stable Micro Systems, Texture Exponent software, Surrey, UK). An aluminum probe of 25 mm of diameter was used to compress the ricotta cream samples (50 mm diameter and 20 mm height). The measurements were made at 5 ± 1 ºC, with test speed of 1.0 mm s^{-1} and distance of 10.0 mm, according to the methodology described by Buriti, Cardarelli, and Saad (2008). The data for force as a function of time were obtained for the two compression-decompression cycles, using the TA-XT plus software. The following parameters were obtained: firmness (N), adhesiveness (N s) and elasticity. All determinations were carried out in quadruplicate.

2.8 Color analysis

The color analyses were performed with a Minolta Chroma Meter CR-400 (Konica Minolta, Osaka, Japan) colorimeter, adjusted to operate with D65 lightning and 10° of observation angle. The CIELab color scale was used to measure the L*, a* and b* parameters. In the CIELab color scale, the L* parameter ranges from 0 to 100, indicating the color variation from black to white; the a* axis shows the variation from red (+ a*) to green (- a*); whilst the b* axis shows the variation from yellow (+ b*) to blue (- b*). For each ricotta cream sample the analysis was made in triplicate.

2.9 Sensory analysis

Prior to sensory analysis, this study was approved by the Ethics Committee on Human Research of the Federal University of Santa Catarina (number 31668). Sensory acceptance was analysed according to the methodology described by Meilgaard, Civille, and Carr (2007). The acceptability test was carried out using a group of not trained panelists (n = 45) and a nine-point structured hedonic scale (1-dislike extremely; 9-like extremely), for the attributes spreadability, flavor and overall acceptability. About 20 g of each sample were monadically presented, at 5 ± 1 ºC, in plastic cups (50 mL) coded with random three digit numbers. Plastic knifes and pieces of bread were offered together with the samples for the evaluation of spreadability and flavor.

2.10 Statistical analysis

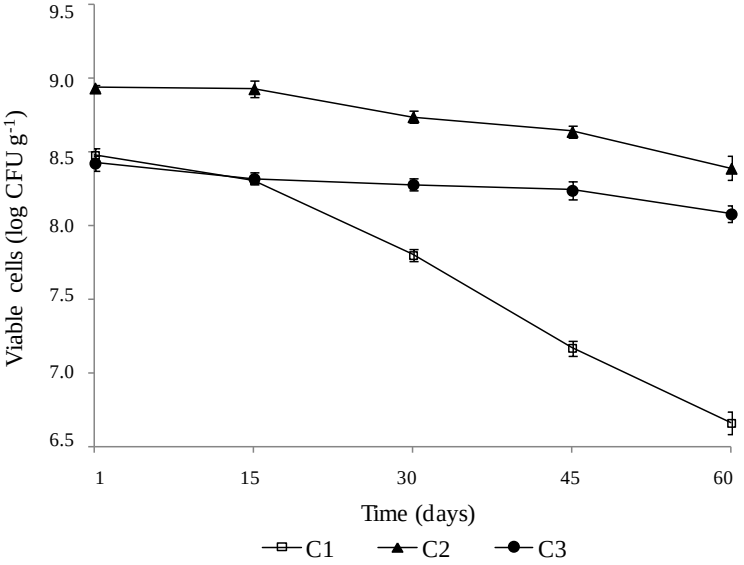
The experimental treatments constituted a randomized complete block design replicated three times. The data analysis was carried out using STATISTICA 7.0 software (StatSoft Inc., Tulsa, USA). Analysis of variance (ANOVA) was used to determine significant differences ($P < 0.05$) for every parameter amongst the different types of the ricotta cream manufactured. Differences between means were detected using Tukey's test.

3 Results and discussion

3.1 Microbiological analysis

The viable cell counts for *Bifidobacterium* BB-12 in the ricotta cream samples, in either free or microencapsulated form, during the storage period of 60 days are shown in the Fig. 1.

Figure 1 - Survival of *Bifidobacterium* BB-12 in the free form (C1), microencapsulated with reconstituted skim milk and inulin (C2), and microencapsulated with RSM and oligofructose-enriched inulin (C3), in the ricotta cream samples during 60 days of storage.



Error bars represent standard deviations of the mean of experiment. Mean ± standard deviation (n=3).

The viable cell counts for all the ricotta cream samples remained above 6 log CFU g^{-1} throughout the whole storage period, which, according to Shah (2007), is the recommended content of probiotic microorganisms in food products at the time of consumption. However, from the initial counts of free and microencapsulated bifidobacteria, the numbers declined steadily for all the samples as the period of storage time increased. Moreover, the decrease rate was much greater in the free cells, which showed the largest ($P < 0.05$) decreases in their viability after 60 days (1.82 log CFU g^{-1}). This decrease in the bacterial counts occurred probably due to cell injuries, caused by storage stress, which eventually lead to their death. Moreover, some factors such as high concentration of acid and oxygen toxicity have been shown to be detrimental to the survival of probiotics in dairy products during processing and storage, as described by De Vos et al. (2010). The counts for free cells of *Bifidobacterium* BB-12 obtained in this present study are in accordance with those obtained by Özer et al. (2009) for white-brined cheese.

The results for the counts of the microencapsulated bifidobacteria showed a higher ($P < 0.05$) viability during storage in sample C2 (containing bifidobacteria microencapsulated with RSM and inulin), when compared to sample C3 (containing bifidobacteria microencapsulated with RSM and oligofructose-enriched inulin). These results suggest that inulin had a better protective effect on the bifidobacteria during the processing and storage of the ricotta cream samples than oligofructose-enriched inulin. Lian, Hsiao and Chou (2002) reported that besides the differences in their chemical characteristics, encapsulating agents have different physical properties.

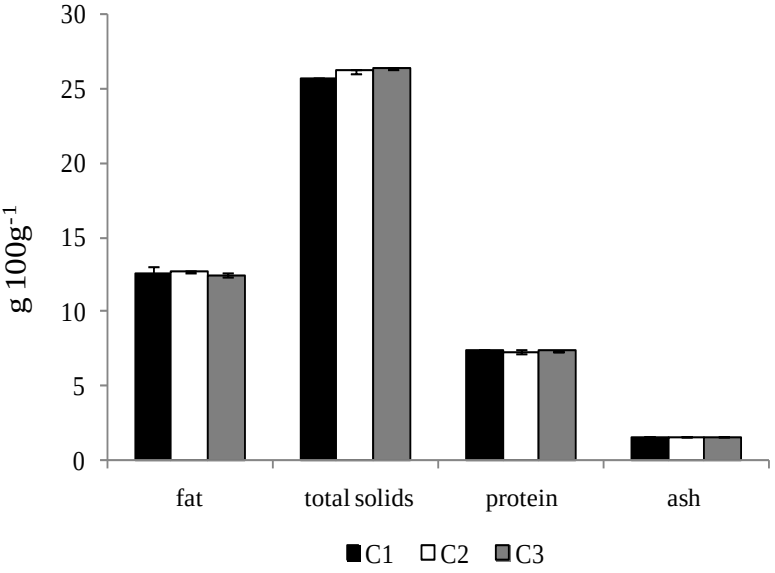
Barclay et al. (2010) stated that the solubility of inulin-type fructans is closely related to the chain length of the polymer, and thus shorter oligomers such as oligofructose-enriched inulin are more soluble than long chain polymers, such as inulin. This difference in solubility was noted by Fritzen-Freire et al. (2012) for the same microcapsules used in this present study, and it may be related to the different levels of survival of microencapsulated bifidobacteria in samples C2 and C3. However, the results obtained in the present study showed that microencapsulation with RSM and with both prebiotics, inulin and oligofructose-enriched inulin, could provide good protection for bifidobacteria added in the ricotta cream samples.

The results for the counts of coliforms at 45 ºC and for Coagulase-Positive Staphylococci in the ricotta cream samples were < 3 NMP g^{-1} and < 2 log CFU g^{-1}, respectively; while the *Salmonella* sp. and the *Listeria monocytogenes* counts showed absence of these bactertia in the ricotta cream samples, confirming the safety of the products investigated.

3.2 Physicochemical analysis

The results for the physicochemical composition of the ricotta cream samples are shown in Fig. 2.

Figure 2 - Physicochemical composition of ricotta cream samples with *Bifidobacterium* BB-12 in the free form (C1), microencapsulated with reconstituted skim milk and inulin (C2), and microencapsulated with reconstituted skim milk and oligofructose-enriched inulin (C3), on day 1 of storage.



Error bars represent standard deviations of the mean of experiment.
Mean ± standard deviation (n=3).

There were no differences ($P > 0.05$) between all the samples in relation to fat, protein, and ash content. Moreover, the values obtained for all the ricotta cream samples for fat were lower than those found in other types of cream cheese, as noted by Cunha, Dias and Viotto (2010) for spreadable processed cheese (~23 g 100 g^{-1}) and by Monteiro et al. (2009) for traditional cream cheese (~36 g 100 g^{-1}). Nevertheless, the ricotta cream samples added with microcapsules (C2 and C3) showed

higher total solids content ($P < 0.05$) than the sample added with free bacteria (C1). A similar behavior was noted by Pinto et al. (2012) in samples of frozen yogurts added with microcapsules of bifidobacteria produced with RSM and/or inulin.

The results for titratable acidity, pH, and total solids content during storage of the ricotta cream samples with bifidobacteria are shown in Table 1.

Table 1 - Mean values ± standard deviation of titratable acidity, pH, and total solids content during storage of the ricotta cream samples with bifidobacteria.

Samples	Days	Acidity (g 100 g^{-1} lactic acid)	pH	Total solids (g 100 g^{-1})
C1	1	0.27 ± 0.00^{Aa}	6.16 ± 0.01^{Aa}	25.71 ± 0.04^{ABa}
	15	0.29 ± 0.02^{Aa}	6.15 ± 0.05^{Aa}	25.41 ± 0.47^{Ba}
	30	0.31 ± 0.00^{Aa}	5.83 ± 0.01^{Ba}	26.41 ± 0.13^{ABa}
	45	0.47 ± 0.02^{Ba}	5.47 ± 0.14^{Ca}	26.71 ± 0.28^{Aa}
	60	0.63 ± 0.06^{Ca}	5.16 ± 0.01^{Da}	28.04 ± 0.43^{Ca}
C2	1	0.26 ± 0.00^{Aa}	6.14 ± 0.06^{Aa}	26.21 ± 0.10^{Ab}
	15	0.31 ± 0.01^{Aa}	6.04 ± 0.01^{ABa}	26.11 ± 0.05^{Aa}
	30	0.37 ± 0.00^{Aa}	5.91 ± 0.01^{Bb}	26.63 ± 0.04^{ABa}
	45	0.51 ± 0.07^{Ba}	5.38 ± 0.09^{Ca}	26.75 ± 0.01^{ABa}
	60	0.64 ± 0.01^{Ba}	5.06 ± 0.01^{Dba}	27.28 ± 0.42^{Ba}
C3	1	0.27 ± 0.00^{Aa}	6.22 ± 0.03^{Aa}	26.37 ± 0.09^{Ab}
	15	0.31 ± 0.01^{Aa}	6.08 ± 0.01^{Aa}	26.58 ± 0.34^{ABa}
	30	0.34 ± 0.00^{Aba}	5.82 ± 0.02^{Ba}	26.62 ± 0.09^{ABa}
	45	0.45 ± 0.01^{Ba}	5.53 ± 0.10^{Ca}	26.80 ± 0.40^{ABa}
	60	0.68 ± 0.06^{Ca}	5.22 ± 0.05^{Dca}	27.76 ± 0.41^{Ba}

C1: ricotta cream added with *Bifidobacterium* BB-12 in the free form; C2: ricotta cream added with bifidobacteria microencapsulated with reconstituted skim milk and inulin; and C3: ricotta cream added with bifidobacteria microencapsulated with reconstituted skim milk and oligofructose-enriched inulin.

^{A,B,C,D} Within a column, different superscript uppercase letters denote significant differences ($P < 0.05$) among the different periods of storage for each studied sample.

^{a,b,c} Within a column, different superscript lowercase letters denote significant differences ($P < 0.05$) among the different studied samples for the same period of storage.

Mean ± standard deviation (n=3).

The acidity levels increased ($P < 0.05$) and consequently pH decreased ($P < 0.05$) for ricotta cream samples C1, C2, and C3, throughout 60 days of storage. According to Buriti et al. (2007), the decrease in pH values observed during storage of fresh cream cheeses is a natural process caused by the continuous production of lactic acid and other organic acids by lactic and/or probiotic cultures. The content of total solids in all the samples showed a small increase throughout the 60 days of storage ($P < 0.05$). The increase in total solids in all the samples may be related with the formation of liquid droplets on the inside of the aluminum foil lid during storage, which characterizes the occurrence of a slight syneresis.

3.3 Texture analysis

Table 2 shows the results obtained for the texture parameters of the ricotta cream samples with free or microencapsulated bifidobacteria during storage.

The addition of microcapsules in ricotta cream samples C2 and C3 contributed to increase ($P < 0.05$) the firmness of these samples on day 1 of storage. This increase in firmness may be associated with the higher total solids values noted in these samples (Fig. 2). Also, there was an increase ($P < 0.05$) in this parameter for all the samples during the 60 days of storage. Souza and Saad (2009) suggested that the increase of firmness in cheeses during storage also could be related with the increase of total solids content, as was noted in this present study (Table 1). Moreover, according to Monteiro et al. (2009), cream cheese firmness is strongly affected by pH, i. e., lower pH caused increase in

firmness, as was also noted in this present study. Also according to these authors, the protein-to-water interactions decreased as the cheese pH decreased, which gave rise to progressive whey expulsion of the casein network and a greater firmness of the product.

An increase ($P < 0.05$) in adhesiveness was observed throughout the storage period for ricotta cream samples C1 and C3, when compared to ricotta cream sample C2 (with higher inulin content). According to Franck (2002), this behavior may be attributable to the ability of inulin to form a gel particle network, when thoroughly mixed with water or another aqueous liquid, resulting in a creamy consistency, with a soft, spreadable texture. However, no differences ($P > 0.05$) were detected between the samples, neither between the days of storage, for the elasticity parameter. Gallina et al. (2008) reported that elasticity is dependent on the structure and on the rearrangement between the protein molecules, which may have undergone changes during storage, and these changes only become more perceptible after 150 days. As the ricotta cream samples evaluated in this present study were stored for 60 days, differences ($P > 0.05$) in elasticity were not noted.

Table 2 - Mean values ± standard deviation of texture parameters of the ricotta cream samples with free or microencapsulated bifidobacteria during storage.

Samples	Parameters	Days of storage				
		1	**15**	**30**	**45**	**60**
C1	Firmness (N)	0.66 ± 0.05^{Aa}	0.75 ± 0.06^{ABa}	0.97 ± 0.10^{Ba}	0.96 ± 0.07^{ABa}	0.97 ± 0.13^{Ba}
	Adhesiveness (N s)	3.03 ± 0.27^{Aa}	4.11 ± 0.60^{ABa}	4.14 ± 0.28^{ABa}	4.17 ± 0.97^{ABa}	4.85 ± 0.18^{Ba}
	Elasticity (–)	0.89 ± 0.02^{Aa}	0.89 ± 0.04^{Aa}	0.90 ± 0.01^{Aa}	0.91 ± 0.02^{Aa}	0.92 ± 0.01^{Aa}
C2	Firmness (N)	0.82 ± 0.03^{Ab}	1.00 ± 0.08^{Ab}	1.39 ± 0.09^{Bb}	1.48 ± 0.08^{Bb}	1.49 ± 0.05^{Bb}
	Adhesiveness (N s)	3.83 ± 0.49^{Aa}	4.85 ± 0.13^{Aa}	4.83 ± 1.63^{Aa}	4.86 ± 1.11^{Aa}	4.90 ± 0.59^{Aa}
	Elasticity (–)	0.91 ± 0.00^{Aa}	0.90 ± 0.01^{Aa}	0.89 ± 0.02^{Aa}	0.91 ± 0.02^{Aa}	0.92 ± 0.02^{Aa}
C3	Firmness (N)	0.78 ± 0.06^{Ab}	0.94 ± 0.12^{ABab}	1.09 ± 0.07^{BCa}	1.13 ± 0.07^{BCc}	1.29 ± 0.05^{Cb}
	Adhesiveness (N s)	3.63 ± 0.25^{Aa}	4.61 ± 0.66^{ABa}	4.49 ± 0.42^{ABa}	4.49 ± 0.25^{ABa}	5.03 ± 0.26^{Ba}
	Elasticity (–)	0.91 ± 0.01^{Aa}	0.90 ± 0.01^{Aa}	0.89 ± 0.05^{Aa}	0.91 ± 0.03^{Aa}	0.91 ± 0.01^{Aa}

C1: ricotta cream added with *Bifidobacterium* BB-12 in the free form; C2: ricotta cream added with bifidobacteria microencapsulated with reconstituted skim milk and inulin; and C3: ricotta cream added with bifidobacteria microencapsulated with reconstituted skim milk and oligofructose-enriched inulin.

^{A,B,C} Within a line, different superscript uppercase letters denote significant differences ($P < 0.05$) among the different periods of storage for each studied sample.

^{a,b,c} Within a column, different superscript lowercase letters denote significant differences ($P < 0.05$) among the different studied samples for the same period of storage.

Mean ± standard deviation (n=3).

3.4 Color analysis

The color attributes for the ricotta cream samples investigated are shown in Table 3.

It was possible to note that the luminosity parameter (L*) remained stable ($P > 0.05$), showing a high luminosity during the 60 days of storage for all the samples. Moreover, the parameter a* of ricotta cream samples C1, C2, and C3 showed no differences ($P > 0.05$) during the storage period, indicating a tendency of the samples towards a green color. This result occurred probably because of the presence of riboflavin in milk, since it is attributable to its slightly green coloration, as described by Nozière et al. (2006). The b* parameter values increased ($P < 0.05$) in all the samples throughout the 60 days of storage. This parameter showed positive values, indicating a tendency towards a yellow color. An increase in yellowness (b*) up to the end of the storage time (90 days) was also noted by Fresno and Álvarez (2012) for the majorero goat cheese. Also according to these authors, there is a correlation between total solids content and yellowness in color. They observed that with the increase of total solids, the b* value also increased, which is similar to the results obtained in this present study.

Table 3 - Mean values ± standard deviation of color attributes of the ricotta cream samples during 60 days of storage at 5 ± 1 ºC.

Samples	Days	L*	a*	b*
C1	1	89.23 ± 0.64 ^{Aa}	-2.04 ± 0.18 ^{Aa}	12.77 ± 0.22 ^{Aa}
	15	87.88 ± 1.35 ^{Aa}	-2.10 ± 0.12 ^{Aa}	13.65 ± 0.67 ^{Aa}
	30	87.83 ± 0.31 ^{Aa}	-1.81 ± 0.34 ^{Aa}	14.17 ± 0.66 ^{Aa}
	45	87.57 ± 1.12 ^{Aa}	-1.98 ± 0.21 ^{Aa}	14.88 ± 1.67 ^{ABa}
	60	87.61 ± 1.19 ^{Aa}	-1.65 ± 0.09 ^{Aa}	16.58 ± 0.48 ^{Ba}
C2	1	89.01 ± 0.50 ^{Aa}	-2.05 ± 0.16 ^{Aa}	12.66 ± 0.21 ^{Aa}
	15	87.30 ± 0.66 ^{Aa}	-1.97 ± 0.08 ^{Aa}	13.53 ± 0.80 ^{Aa}
	30	87.19 ± 1.30 ^{Aa}	-1.80 ± 0.69 ^{Aa}	14.64 ± 1.45 ^{ABa}
	45	86.73 ± 1.12 ^{Aa}	-1.97 ± 0.10 ^{Aa}	14.80 ± 0.67 ^{ABa}
	60	86.69 ± 0.33 ^{Aa}	-1.78 ± 0.54 ^{Aa}	16.82 ± 0.29 ^{Ba}
C3	1	88.32 ± 0.16 ^{Aa}	-1.78 ± 0.07 ^{Aa}	12.92 ± 0.33 ^{Aa}
	15	88.46 ± 0.23 ^{Aa}	-1.74 ± 0.31 ^{Aa}	13.32 ± 0.46 ^{Aa}
	30	87.39 ± 0.90 ^{Aa}	-1.30 ± 0.39 ^{Aa}	13.80 ± 0.62 ^{ABa}
	45	87.27 ± 0.91 ^{Aa}	-1.22 ± 0.15 ^{Ab}	13.77 ± 0.47 ^{ABa}
	60	87.24 ± 0.06 ^{Aa}	-1.21 ± 0.09 ^{Ab}	15.67 ± 1.40 ^{Ba}

C1: ricotta cream added with *Bifidobacterium* BB-12 in the free form; C2: ricotta cream added with bifidobacteria microencapsulated with reconstituted skim milk and inulin; and C3: ricotta cream added with bifidobacteria microencapsulated with reconstituted skim milk and oligofructose-enriched inulin.

^{A,B} Within a column, different superscript uppercase letters denote significant differences ($P < 0.05$) among the different periods of storage for each studied sample.

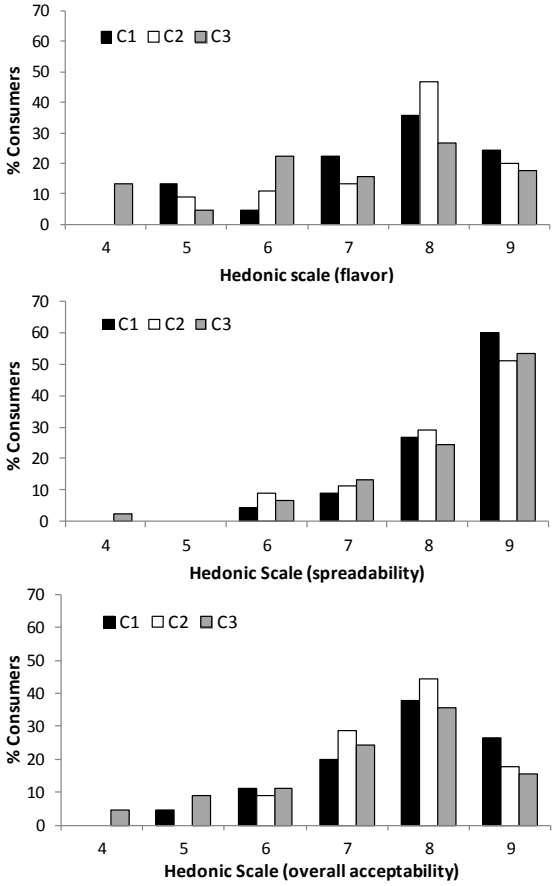
^{a,b} Within a column, different superscript lowercase letters denote significant differences ($P < 0.05$) among the different studied samples for the same period of storage.

Mean ± standard deviation (n=3).

3.5 Sensory analysis

Fig. 3 shows the scores (% consumers) for spreadability, flavor, and overall acceptability of the ricotta cream samples. For all the samples, the majority of the panelists classified the flavor and overall acceptability attributes with score 8, which, in the structured hedonic scale, means “like very much”. The score for spreadability was 9, which means “like extremely”. There were no differences ($P > 0.05$) for all the attributes between all the ricotta cream samples (C1, C2, and C3). The same behavior was verified in kasar cheese by Özer, Uzun and Kirmaci (2008), who noted that none of the sensorial attributes evaluated was affected by addition of probiotic microcapsules. Therefore, such result indicates that the microcapsules did not affect the acceptability of the product.

Figure 3 - Distribution of scores (% consumers) for the sensory analysis of the ricotta cream samples according to a nine-point scale, where: 1- dislike extremely; 2- dislike very much; 3-dislike moderately; 4- dislike slightly; 5- neither like nor dislike; 6- like slightly; 7- like moderately; 8- like very much; 9-like extremely.



C1: ricotta cream added with *Bifidobacterium* BB-12 in the free form; C2: ricotta cream added with bifidobacteria microencapsulated with reconstituted skim milk and inulin; and C3: ricotta cream added with bifidobacteria microencapsulated with reconstituted skim milk and oligofructose-enriched inulin.

4 Conclusions

The viable cell counts of *Bifidobacterium* BB-12 for all the ricotta cream samples decreased steadily during the 60 days of storage; however, it is noteworthy that all the samples were considered as potentially probiotics. In addition, the counts for the ricotta cream samples with microencapsulated bifidobacteria were much greater than the one with free cells during the storage. The bifidobacteria microencapsulated with RSM and inulin showed to be an effective alternative for protection of probiotic bacteria for their application in ricotta cream. As expected, in the samples added with the microcapsules, the total solids content and the values for firmness were higher than in the sample without microcapsules. Besides, all the ricotta cream samples showed a similar profile for pH and acidity levels, total solids content, and color parameters (luminosity, greenness, and yellowness) during the storage. According to the panelists, all the ricotta cream samples showed good spreadability, good flavor, and good overall acceptability.

Acknowledgments

The authors gratefully thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC), Brazil, for their financial support, and also Clariant for providing the prebiotics.

References

ANANTA, E.; VOLKERT, M.; KNORR, D. Cellular injuries and storage stability of spray-dried *Lactobacillus rhamnosus* GG. **International Dairy Journal**, v.15, p.399-409, 2005.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS - AOAC. **Official methods of analysis of the association analytical chemists**. 18th. Edition. Maryland, USA, 2005.

AMERICAN PUBLIC HEALTH ASSOCIATION- APHA. **Compendium of methods of the microbiological examination of foods**. 4th Edition. Washington, DC, USA, 2001.

BARCLAY, T.; GINIC-MARKOVICA, M.; COOPER, P.; PETROVSKY, N. Inulin - a versatile polysaccharide with multiple pharmaceutical and food chemical uses. **Journal of Excipients and Food Chemicals**, v.1, p.27-50, 2010.

BOYLSTON, T. D.; VINDEROLA, C. G.; GHODDUSI, H. B.; REINHEIMER, J. A. Incorporation of bifidobacteria into cheeses: challenges and rewards. **International Dairy Journal**, v.14, p.375-387, 2004.

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, p.123-128, 2011.

BURITI, F. C. A.; CARDARELLI, H. R.; FILISETTI, T. M. C. C.; SAAD, S. M. I. Synbiotic potential of fresh cream cheese supplemented with inulin and *Lactobacillus paracasei* in co-culture with *Streptococcus thermophilus*. **Food Chemistry**, v.104, p.1605-1610, 2007.

BURITI, F. C. A.; CARDARELLI, H. R.; SAAD, S. M. I. Influence of *Lactobacillus paracasei* and inulin on instrumental texture and sensory evaluation of fresh cream cheese. **Revista Brasileira de Ciências Farmacêuticas**, v.44, p.75-84, 2008.

CASTRO, F. P.; CUNHA, T. M.; BARRETO, P. L. M.; AMBONI, R. D. M. C.; PRUDÊNCIO, E. S. Effect of oligofructose incorporation on the properties of fermented probiotic lactic beverages. **International Journal of Dairy Technology**, v.62, p.68-74, 2008.

CHÁVEZ, B. E.; LEDEBOER, A. M. Drying of probiotics: optimization of formulation and process to enhance storage survival. **Drying Technology**, v.25, p.1193-1201, 2007.

CRUZ, A. G.; BURITI, F. C. A.; SOUZA, C. H. B.; FARIA, J. A. F.; SAAD, S. M. I. Probiotic cheese: health benefits, technological and stability aspects. **Trends in Food Science and Technology**, v.20, p.344-354, 2009.

CUNHA, C. R.; DIAS, A. I.; VIOTTO, W. H. Microstructure, texture, colour and sensory evaluation of a spreadable processed cheese analogue made with vegetable fat. **Food Research International**, v.43, p.723–729, 2010.

DE VOS, P.; FAAS, M. M.; SPASOJEVIC, M.; SIKKEMA, J. Encapsulation for preservation of functionality and targeted delivery of bioactive food components. **International Dairy Journal**, v.20, p.292-302, 2010.

DOLEYRES, Y.; LACROIX, C. Technologies with free and immobilised cells for probiotic bifidobacteria production and protection. **International Dairy Journal**, v.15, p.973-988, 2005.

FAO/WHO. **Evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria***:* Report of a Joint FAO/WHO Expert Consultation, Córdoba, 2001. 34p. Disponível em: http://www.who.int/foodsafety/publications/fs_management/en/probiotics.pdf. Acesso em 06 novembro de 2010.

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

FRESNO, M.; ÁLVAREZ, S. Chemical, Textural and Sensorial Changes during the Ripening of Majorero Goat Cheese. **International Journal of Dairy Technology**, v.65, p.393-400 2012.

FRITZEN-FREIRE, C. B.; PRUDÊNCIO, E. S.; AMBONI, R. D. M. C.; PINTO, S. S.; NEGRÃO-MURAKAMI, A. N.; MURAKAMI, F. S. Microencapsulation of bifidobacteria by spray drying in the presence of prebiotics. **Food Research International**, v.45, p.306-312, 2012.

FRITZEN-FREIRE, C. B.; PRUDÊNCIO, E. S.; PINTO, S. S.; MUÑOZ, I. B.; AMBONI, R. D. M. C. Effect of microencapsulation on survival of *Bifidobacterium* BB-12 exposed to simulated gastrointestinal conditions and heat treatments. **LWT - Food Science and Technology**, v.50, p.39-44, 2013.

FU, N.; CHEN, X. D. Towards a maximal cell survival in convective thermal drying processes. **Food Research International**, v.44, p.1127-1149, 2011.

GALLINA, D. A.; VAN DENDER, A. G. F.; YOTSUYANAGI, K.; RODRIGUES DE SÁ, P. B. Z. Influence of storage temperature on the texture profile and colour characteristics of UHT Requeijão cremoso. **Brazilian Journal of Food Technology**, v.11, p.169-174, 2008.

GONZÁLEZ-SÁNCHEZ, F.; AZAOLA, A.; GUTIÉRREZ-LÓPEZ, G. F.; HERNÁNDEZ-SÁNCHEZ, H. Viability of microencapsulated *Bifidobacterium animalis* ssp. *lactis* BB12 in kefir during refrigerated storage. **International Journal of Dairy Technology**, v.63, p.431-436, 2010.

INSTITUTO ADOLFO LUTZ- IAL. **Normas Analíticas do Instituto Adolfo Lutz***:*
Métodos físico-químicos para análise de alimentos (4 ed.), São Paulo, Brasil, 2005.

LIAN, W. C.; HSIAO, H. C.; CHOU, C. C. Survival of bifidobacteria after spray-drying. **International Journal of Food Microbiology**, v.74, p.79-86, 2002.

MEILGAARD, M.; CIVILLE, G. V.; CARR, B. T. **Sensory evaluation techniques**, 4th edn. Boca Raton, FL.: CRC Press, 2007.

MIRZAEI, H.; POURJAFAR, H.; HOMAYOUNI, A. Effect of calcium alginate and resistant starch microencapsulation on the survival rate of

Lactobacillus acidophilus La5 and sensory properties in Iranian white brined cheese. **Food Chemistry**, v.132, p.1966-1970, 2012.

MONTEIRO, R. R.; TAVARES, D. Q.; KINDSTEDT P. S.; GIGANTE, M. L. Effect of pH on microstructure and characteristics of cream cheese. **Journal of Food Science**, v.74, p.C112-C117, 2009.

NOZIERE, P. ; GRAULET, B. ; LUCAS, A. ; MARTIN, B. ; GROLIER, P. ; DOREAU, M. Carotenoids for ruminants: From forages to dairy products. **Animal Feed Science and Technology**, v.131, p.418-450, 2006.

ÖZER, B.; UZUN, Y. S.; KIRMACI, H. A. Effect of microencapsulation on viability of *Lactobacillus acidophilus* LA-5 and *Bifidobacterium bifidum* BB-12 during Kasar cheese ripening. **International Journal of Dairy Technology**, v.61, p.237-244, 2008.

ÖZER, B.; KIRMACI, H. A.; SHENEL, E.; ATAMER, M.; HAYALOGLU, A. Improving the viability of *Bifidobacterium bifidum* BB-12 and *Lactobacillus acidophilus* LA-5 in white brined cheese by microencapsulation. **International Dairy Journal**, v.19, p.22-29, 2009.

PINTO, S. S.; FRITZEN-FREIRE, C. B.; MUÑOZ, I. B.; PRUDÊNCIO, E. S.; AMBONI, R. D. M. C. Effects of the addition of microencapsulated *Bifidobacterium* BB-12 on the properties of frozen yogurt. **Journal of Food Engineering**, v.111, p.563-569, 2012.

PIZZILLO, M.; CLAPS, S.; CIFUNI, G. F.; FEDELE, V.; RUBINO, R. Effect of goat breed on the sensory chemical and nutritional characteristics of Ricotta. **Livestock Production Science**, v.94, p.33-40, 2005.

ROBERFROID, M. B. Inulin-Type Fructans: Functional Food Ingredients. **Journal of Nutrition**, v.137, p.2493-2502, 2007.

ROY, D. Technological aspects related to the use of bifidobacteria in dairy products. **Lait**, v. 85, p.39-56, 2005.

SANDERS, M. E. Probiotics: considerations for human health. **Nutrition Reviews**, v.61, p.91-99, 2003.

SHAH, N. P. Functional cultures and health benefits. **International Dairy Journal**, v.17, p.1262-1277, 2007.

SHEU, T. Y.; MARSHALL, R. T.; HEYMANN, H. Improving Survival of Culture Bacteria in Frozen Desserts by Microentrapment. **Journal of Dairy Science**, v.76, p.1902-1907, 1993.

SOUZA, C. H. B.; SAAD, S. M. I. Viability of *Lactobacillus acidophilus* La-5 added solely or in co-culture with a yoghurt starter culture and implications on physico-chemical and related properties of Minas fresh cheese during storage. **LWT - Food Science and Technology**, v.42, p.633- 640, 2009.

VINDEROLA, C. G.; REINHEIMER, J. A. Culture media for the enumeration of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in the presence of yoghurt bacteria. **International Dairy Journal**, v.9, p.497-505, 1999.

CONCLUSÕES

Todas as microcápsulas avaliadas, contendo a *Bifidobacterium* BB-12, foram consideradas probióticas, pois apresentaram contagem superior a 6 log UFC g^{-1} durante os 180 dias de armazenamento, tanto a 4 °C quanto a -18 °C.

A inulina enriquecida com oligofrutose foi o agente prebiótico mais apropriado para substituir parcialmente o leite desnatado reconstituído (LDR) na microencapsulação por *spray drying* da *Bifidobacterium* BB-12, apresentando maior estabilidade após o processo de microencapsulação e durante o armazenamento.

A utilização dos prebióticos inulina, inulina enriquecida com oligofrutose e oligofrutose não influenciou a morfologia das microcápsulas. Por outro lado, diminuiu o conteúdo de umidade e a atividade de água, além de aumentar o parâmetro de cor a*.

As microcápsulas produzidas com inulina mostraram menor solubilidade em água, enquanto que àquelas produzidas com oligofrutose foram as mais higroscópicas. Além disso, as microcápsulas produzidas com prebióticos foram mais estáveis às análises térmicas, quando comparadas às produzidas apenas com LDR.

A utilização dos prebióticos inulina e inulina enriquecida com oligofrutose aumentou o rendimento da encapsulação da *Bifidobacterium* BB-12, conferindo maior proteção às microcápsulas submetidas às condições gastrintestinais simuladas e aos diferentes tratamentos térmicos.

Todas as amostras de creme de ricota, tanto com bifidobactéria microencapsulada, como com células livres, foram consideradas

probióticas. No entanto, as amostras com microcápsulas apresentaram contagens muito mais elevadas ao longo dos 60 dias de armazenamento, sendo que a combinação de LDR com inulina conferiu maior proteção às microcápsulas. Já, a adição das microcápsulas contribuiu para o aumento da firmeza e dos sólidos totais das amostras de creme de ricota.

Todas as amostras de creme de ricota apresentaram alta luminosidade e uma tendência à coloração verde e amarela durante o período de armazenamento, bem como boa aceitabilidade sensorial. Finalmente, a adição de *Bifidobacterium* BB-12 microencapsulada com LDR e inulina em creme de ricota torna-se uma boa alternativa de alimento funcional, ampliando a apelo de alimento saudável e nutritivo conferido ao produto.

Anexos

Anexo A – Artigo "**Microencapsulation of bifidobacteria by spray drying in the presence of prebiotics**" publicado no "**Food Research International**" (ISSN: 0963-9969)

Food Research International 45 (2012) 306–312

Contents lists available at SciVerse ScienceDirect

Food Research International

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

Microencapsulation of bifidobacteria by spray drying in the presence of prebiotics

Carlise B. Fritzen-Freire [a], Elane S. Prudêncio [a,*], Renata D.M.C. Amboni [a], Stephanie S. Pinto [a], Aureanna N. Negrão-Murakami [a], Fabio S. Murakami [b]

[a] Universidade Federal de Santa Catarina, Departamento de Ciência e Tecnologia de Alimentos, Rod. Admar Gonzaga, 1346, Itacorubi, 88034-001, Florianópolis, SC, Brazil
[b] Universidade Federal do Paraná, Departamento de Farmácia, Av. Pref. Lothário Meissner, 632, Jardim Botânico, 80210-170, Curitiba, PR, Brazil

Acesso do artigo em:
http://www.sciencedirect.com/science/article/pii/S0963996911005539

Anexo B – Artigo "**Effect of microencapsulation on survival of *Bifidobacterium* BB-12 exposed to simulated gastrointestinal conditions and heat treatments**" publicado no "**LWT – Food Science Technology**" (ISSN: 0023-6438)

LWT - Food Science and Technology 50 (2013) 39–44

Contents lists available at SciVerse ScienceDirect

LWT - Food Science and Technology

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

Effect of microencapsulation on survival of *Bifidobacterium* BB-12 exposed to simulated gastrointestinal conditions and heat treatments

Carlise B. Fritzen-Freire, Elane S. Prudêncio*, Stephanie S. Pinto, Isabella B. Muñoz, Renata D.M.C. Amboni

Universidade Federal de Santa Catarina, Departamento de Ciência e Tecnologia de Alimentos, Rod. Admar Gonzaga, 1346, Itacorubi, 88034-001 Florianópolis, SC, Brazil

Acesso do artigo em:

http://www.sciencedirect.com/science/article/pii/S0023643812003210

Anexo C – Artigo "**Effect of the application of *Bifidobacterium* BB-12 microencapsulated by spray drying with prebiotics on the properties of ricotta cream**" publicado no "**Food Research International**" (ISSN: 0963-9969)

Food Research International 52 (2013) 50–55

Contents lists available at SciVerse ScienceDirect

Food Research International

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

Effect of the application of *Bifidobacterium* BB-12 microencapsulated by spray drying with prebiotics on the properties of ricotta cream

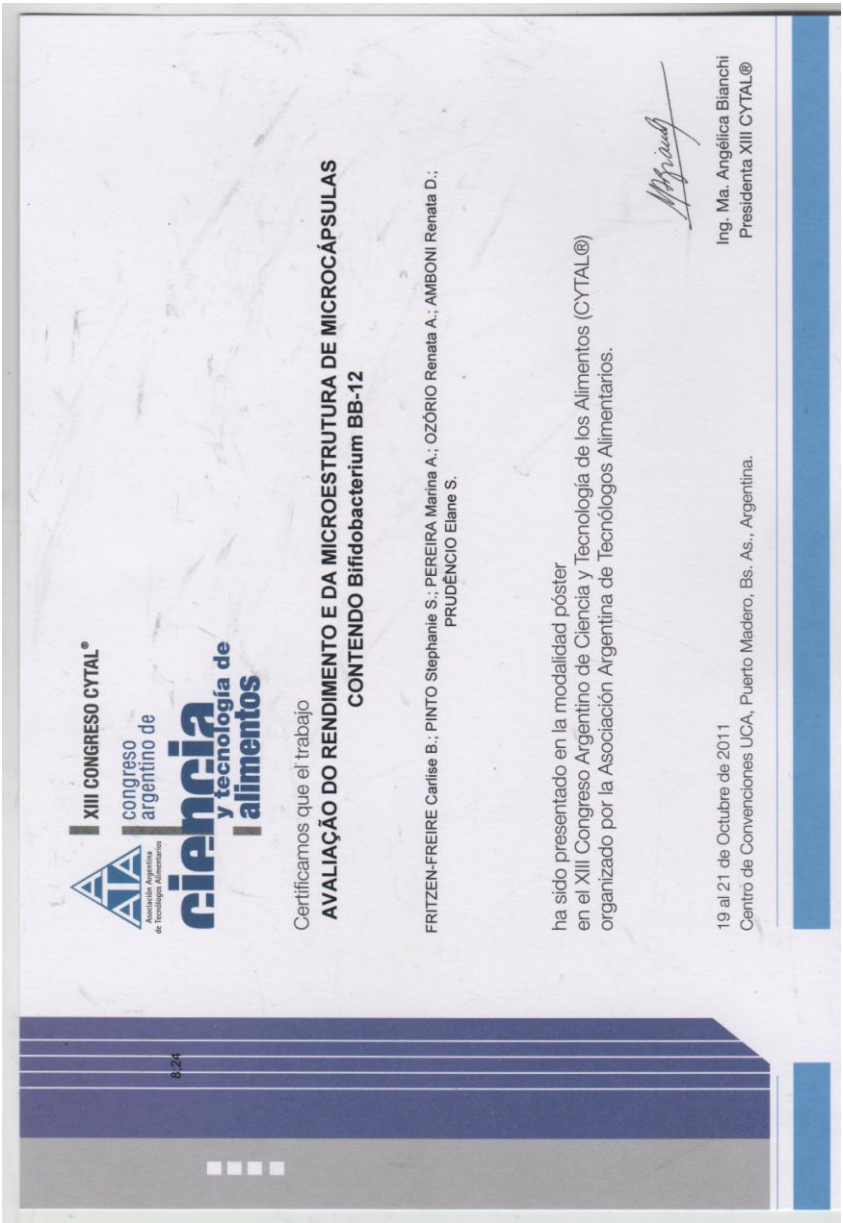
Carlise B. Fritzen-Freire, Elane S. Prudêncio *, Stephanie S. Pinto, Isabella B. Muñoz, Carmen M.O. Müller, Cleide R.W. Vieira, Renata D.M.C. Amboni

Universidade Federal de Santa Catarina, Departamento de Ciência e Tecnologia de Alimentos, Rod. Admar Gonzaga, 1346, Itacorubi, 88034-001, Florianópolis, SC, Brazil

Acesso do artigo em:

http://www.sciencedirect.com/science/article/pii/S0963996913001579

Anexo D - Trabalhos apresentados em eventos



XIII CONGRESO CYTAL®

congreso argentino de ciencia y tecnología de alimentos

Asociación Argentina de Tecnólogos Alimentarios

Certificamos que el trabajo

AVALIAÇÃO DO RENDIMENTO E DA MICROESTRUTURA DE MICROCÁPSULAS CONTENDO Bifidobacterium BB-12

FRITZEN-FREIRE Carlise B.; PINTO Stephanie S.; PEREIRA Marina A.; OZÓRIO Renata A.; AMBONI Renata D.; PRUDÊNCIO Elane S.

ha sido presentado en la modalidad póster
en el XIII Congreso Argentino de Ciencia y Tecnología de los Alimentos (CYTAL®)
organizado por la Asociación Argentina de Tecnólogos Alimentarios.

19 al 21 de Octubre de 2011
Centro de Convenciones UCA, Puerto Madero, Bs. As., Argentina.

Ing. Ma. Angélica Bianchi
Presidenta XIII CYTAL®

XIII CONGRESO CYTAL®

congreso argentino de ciencia y tecnología de alimentos

Asociación Argentina de Tecnólogos Alimentarios

8.23

Certificamos que el trabajo

INFLUÊNCIA DA TEMPERATURA E DO TEMPO DE ARMAZENAMENTO NA SOBREVIVÊNCIA DE Bifidobacterium BB-12 MICROENCAPSULADA POR SPRAY DRYER

FRITZEN-FREIRE Carlise B.; PINTO Stephanie S.; ALVES Mariane A.; MUÑOZ Isabella B.; AMBONI Renata D.; PRUDÊNCIO Elane S.

ha sido presentado en la modalidad póster
en el XIII Congreso Argentino de Ciencia y Tecnología de los Alimentos (CYTAL®)
organizado por la Asociación Argentina de Tecnólogos Alimentarios.

19 al 21 de Octubre de 2011
Centro de Convenciones UCA, Puerto Madero, Bs. As., Argentina.

Ing. Ma. Angélica Bianchi
Presidenta XIII CYTAL®

Simpósio Latino Americano de Ciência de Alimentos . Ciência de Alimentos e Qualidade de Vida: Saúde, Meio Ambiente e Sustentabilidade

Certificado

Certificamos que o trabalho intitulado **"PROPRIEDADES FÍSICAS DE MICROCÁPSULAS CONTENDO BIFIDOBACTERIA"** de autoria **FRITZEN-FREIRE,CB; PINTO,SS; MUñOZ,I.B; PRUDÊNCIO,ES; AMBONI,RDMC** foi apresentado na Sessão de Pôster no 9 SLACA - Simpósio Latino Americano de Ciência de Alimentos: *"Ciência de Alimentos e Qualidade de Vida: Saúde, Meio Ambiente e Sustentabilidade"*, realizado de 5 a 8 de Novembro de 2011, Campinas - São Paulo - Brasil.

Dr. Mario Roberto Marostica Jr
Coordenador do Comitê de Programação

Dra. Gabriela Alves Macedo
Coordenadora do Comitê Científico

Dra. Helena Teixeira Godoy

Dra. Gláucia Maria Pastore
Presidente do Evento

De 05 a 08 de novembro de 2011 . Unicamp . Campinas . São Paulo . Brasil

Certificate of Appreciation

16th IUFoST | World Congress of Food Science and Technology

We certify that the paper entitled

MORPHOLOGICAL ANALYSIS OF PROBIOTIC MICROENCAPSULATED WITH DIFFERENT ENCAPSULATING AGENTS

authored by

FRITZEN-FREIRE, C.B.; PINTO, S.S.; Renata Bongiolo Magenis; AMBONI, R.D.M.C.; PRUDÊNCIO, E.S.

was presented in the Poster Session at the **16th World Congress of Food Science and Technology** : ***"Addressing Global Food Security and Wellness through Food Science and Technology"***, held at Foz do Iguaçu, Parana, Brazil, on August 5 - 9, 2012.

Glaucia Maria Pastore
Chair

Delia B. Rodriguez-Amaya
Scientific Committee Chair

IUFoST

310a.eaa7 1f4c.ba02 341a.0824 ccab.d1fa

Certificate of Appreciation

16th IUFoST | World Congress of Food Science and Technology

We certify that the paper entitled

SURVIVAL OF BIFIDOBACTERIUM BB-12 MICROENCAPSULATED WITH PREBIOTICS DURING STORAGE

authored by FRITZEN-FREIRE, C.B.; PINTO, S.S.; MUÑOZ, I.B.; AMBONI, R.D.M.C.; PRUDÊNCIO, E.S.

was presented in the Poster Session at the **16th World Congress of Food Science and Technology** : ***"Addressing Global Food Security and Wellness through Food Science and Technology"***, held at Foz do Iguaçu, Parana, Brazil, on August 5 - 9, 2012.

Glaucia Maria Pastore
Chair

Delia B. Rodriguez-Amaya
Scientific Committee Chair

Certificate of Appreciation

16th IUFoST | World Congress of Food Science and Technology

We certify that the paper entitled

RESISTANCE OF FREE AND MICROENCAPSULATED BIFIDOBACTERIA EXPOSED TO HEAT TREATMENTS

authored by FRITZEN-FREIRE, C.B.; PINTO, S.S.; DIAS, C.O.; AMBONI, R.D.M.C.; PRUDÊNCIO, E.S.

was presented in the Poster Session at the **16th World Congress of Food Science and Technology** : "***Addressing Global Food Security and Wellness through Food Science and Technology***", held at Foz do Iguaçu, Parana, Brazil, on August 5 - 9, 2012.

Glaucia Maria Pastore
Chair

Delia B. Rodriguez-Amaya
Scientific Committee Chair

7dc2.667f824a.592c.3b52.d8b.a387.901d

VI CONGRESSO LATINOAMERICANO
E XII CONGRESSO BRASILEIRO DE

HIGIENISTAS DE ALIMENTOS

II ENCONTRO NACIONAL DE VIGILÂNCIA DAS ZOONOSES
IV ENCONTRO DO SISTEMA BRASILEIRO
DE INSPEÇÃO DE PRODUTOS DE ORIGEM ANIMAL

23 a 26 Abril de 2013 | Hotel Serrano Resort Gramado - RS

OS ALIMENTOS SOB A ÓTICA DA SUSTENTABILIDADE:
ENTRE A CONSCIÊNCIA E A PRÁTICA

Certificado

Certificamos que o trabalho: **PROPRIEDADES SENSORIAIS DE CREME DE RICOTA ADICIONADO DE BIFIDOBACTÉRIA MICROENCAPSULADA COM PREBIÓTICOS**

De autoria de: CARLISE B. FRITZEN-FREIRE, ELANE S. PRUDÊNCIO, STEPHANIE S. PINTO, ISABELLA B. MUÑOZ, CAROLINNE O. DIAS, JAQUELINE ZARPELON, CLEIDE R. W. VIEIRA, RENATA D. M. C. AMBONI foi apresentado como pôster durante o **VI CONGRESSO LATINO AMERICANO E XII CONGRESSO BRASILEIRO DE HIGIENISTAS DE ALIMENTOS,** no período de 23 a 26 de abril de 2013, na cidade de Gramado – RS – Brasil.

Gramado, 26 de abril de 2013

Realização CBMVHA

Apoio

PROFª DRª. SIBELLI PASSINI BARBOSA FERRÃO
Presidente do Congresso

PROFª DR. JOSÉ CEZAR PANETTA
Presidente da Comissão Científica