CAROLINA CARVALHO RAMOS VIANA

STUDY OF BACTERIAL-ENCAPSULATING AGENT INTERACTION IN MICROENCAPSULATION OF PROBIOTICS

Thesis submitted to the Graduate Program in Food Science and Technology of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae.*

Adviser: Ítalo Tuler Perrone

Co-advisers: Antônio Fernandes de Carvalho
Rodrigo Stephani

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Assent:

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Carolina Carvalho Ramos Viana
Author

____

Italo Tuler Perrone
Adviser

I dedicate to my parents and my sun,

Carlos Eduardo.

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To my fiancé José Carlos, who has always been by my side at all times and has always supported me in all my decisions!

To my sun Carlos Eduardo, for him I try to become a better person every day and I try to be the best professional I can be!

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“Tudo posso naquele que me fortalece”

(Filipenses 4:13)

ABSTRACT

VIANA, Carolina Carvalho Ramos, D.Sc., Universidade Federal de Viçosa, February, 2022. **Study of the bacteria - encapsulating agent interaction in microencapsulation of probiotics.** Adviser: Ítalo Tuler Perrone. Co-advisers: Antônio Fernandes de Carvalho and Rodrigo Stephani.

An increasing number of microorganisms have been applied in the food industry, by incorporation in food products, in the development of probiotic ingredients and supplements, among others. This industrial use usually depends on preservation methods through dehydration, in order to ensure better metabolic activity. On the other hand, drying can cause loss of viability mainly due to cellular damage caused by this process. To avoid them, we can mention the use of microencapsulation, an important technique to improve bacterial stability during processing and application in food matrices. Thus, the objective of this thesis was to review the scientific literature on the microencapsulation of microorganisms, with a main focus on probiotics and the main encapsulating agents used by the lyophilization and spray dryer method. In this scenario, lactic acid bacteria stand out, which have been increasingly chosen in research and applications in food, especially in dairy products. Thus, in the first work, already published, a general review was carried out on microencapsulation of probiotic bacteria, focusing on spray drying and the main encapsulating agents cited in the literature for this encapsulation method. It was observed that in the literature the authors increasingly seek to optimize the production of dehydrated microbial cultures, which makes it necessary to know about the encapsulating materials and the main available and applicable methods. At the same time, they cite the need for more research, mainly to understand the protective effect, in order to guide the choice of conditions to be used in microencapsulation. Therefore, the second work brings a denser review of the literature, having as main focus the microencapsulation of lactic acid bacteria by lyophilization and spray dryer. In this article, the main hypotheses found in the literature about the negative effect of bacterial dehydration are discussed, as well as the protective action of carbohydrates, proteins and lipids used as encapsulating agents. To investigate the theories discussed, physicochemical analytical methods are suggested, in order to aid in the understanding of microencapsulation, together with microbiological methods already commonly used.

Keywords: Microorganisms. Food. Viability. Drying. *Spray dryer.*

RESUMO

VIANA, Carolina Carvalho Ramos, D.Sc., Universidade Federal de Viçosa, fevereiro, 2022. **Estudo da interação bactéria - agente encapsulante no microencapsulamento de probióticos.** Orientador: Ítalo Tuler Perrone. Co-orientadores: Antônio Fernandes de Carvalho e Rodrigo Stephani.

Um número crescente de micro-organismos vem sendo aplicados na indústria de alimentos, por incorporação em produtos alimentícios, no desenvolvimento de ingredientes e suplementos probióticos, entre outras. Esse uso industrial normalmente depende de métodos de preservação por meio da desidratação, com o intuito de garantir uma melhor atividade metabólica. Em contrapartida, a secagem pode causar perda de viabilidade principalmente devido à lesões celulares causadas por este processo. Para evitá-las, pode-se citar o uso do microencapsulamento, uma importante técnica para melhorar a estabilidade bacteriana durante o processamento e aplicação em matrizes alimentares. Assim, o objetivo desta tese foi revisar a literatura científica sobre o microencapsulamento de microrganismos, com foco principal nos probióticos e os principais agentes encapsulantes usados pelo método de liofilização e spray dryer. Neste cenário, destacam-se as bactérias láticas, que vem sendo cada vez mais escolhidas em pesquisas e aplicações nos alimentos, especialmente nos produtos lácteos. Dessa forma, no primeiro trabalho, já publicado, foi realizada uma revisão geral sobre microencapsulamento de bactérias probióticas, com foco na secagem por spray dryer e os principais agentes encapsulantes citados na literatura para este método de encapsulamento. Observou-se que na literatura os autores buscam cada vez mais otimizar a produção de culturas microbianas desidratadas, o que torna necessário o conhecimento sobre os materiais encapsulantes e os principais métodos disponíveis e aplicáveis. Ao mesmo tempo, citam a necessidade de mais pesquisas, principalmente para compreender o efeito protetor, de forma a guiar a escolha das condições a ser usadas no microencapsulamento. Por isso, o segundo trabalho traz uma revisão mais densa da literatura, tendo como foco principal o microencapsulamento de bactérias ácido láticas por liofilização e spray dryer. Neste artigo, são discutidas as principais hipóteses encontradas na literatura sobre o efeito negativo da desidratação bacteriana, bem como da ação protetora de carboidratos, proteínas e lipídios usados como agentes encapsulantes. Para investigar as teorias discutidas, métodos analíticos físico-químicos são sugeridos, de forma a auxiliar na compreensão do microencapsulamento, juntamente com métodos microbiológicos já comumente usados.

Palavras-chave: Microrganismos. Alimentos. Viabilidade. Secagem. *Spray dryer.*

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LIST OF ACRONYMS AND ABBREVIATIONS

ATR Attenuated Total Reflection

BAL Bactérias Ácido Láticas

CD-F Cyclodextrin Fiber

cfu colony-forming unit

DNA Deoxyribonucleic acid

DSC Differential Scanning Calorimetry

DSC Differential scanning calorimetry

DTA Differential Thermal Analysis

DWPI Denatured Whey Protein Isolate

FD Freeze Drying

Fig. Figure

FOS Fructooligosaccharide

FTIR Fourier Transform Infrared Spectroscopy

GA Gum Arabic

GOS Galactooligosaccharides

GRAS Generally Recognised as Safe

i.e id est

IMOS Isomaltooligosaccharide

ITC Isothermal Thermodynamic Calorimetry

LAB Lactic Acid Bacteria

LMF Low melting point fat

MD Maltodextrin

RNA Ribonucleic acid

RSM Reconstituted Skim Milk Powder

RSW Reconstituted Sweet Whey Powder

RSW PReconstituted Sweet Whey Permeate Powder

SD Spray Drying

SERS Surface Enhanced Raman Scattering

Tg Glass Transition Temperature

TGA Thermogravimetry Analysis

WPC Whey Protein Concentrate

WPI Whey Proteins Isolate

α-LA Alpha-lactalbumin

β-LG Beta-lactoglobulin

LIST OF SYMBOLS

%	Percent
()	Parenthesis
$	Dollar sign
μ	Micro
ºC	Degrees Celsius
α	Alpha
β	Beta
*	Asterisk
Na^+	Sodium ion
K^+	Potassium ion
Δ	Delta
®	Registered trademark symbol

SUMMARY

1. GENERAL INTRODUCTION

In recent years, an increasing number of microorganisms have been investigated for their potential to confer health benefits on the host when administered in appropriate amounts. As a result, its applications in the food industry range from its incorporation into food products, as well as the development of probiotic ingredients and supplements (Abd El-Salam; El-Shibiny, 2015).

In the global economic scenario, the market for probiotics has been one of the main beneficiaries of recent trends in the functional food industry, moving around US$ 36.6 billion in 2015 and with an expected annual growth of more than 7% for the coming years. It is also estimated that the market demand for probiotic ingredients for food supplement applications may exceed US$ 260 million by 2024 (Ahuja & Deb, 2018).

The great challenge in the application of microorganisms in food is to maintain the viability during processing, storage and shelf life of products, in the face of oxidative stress, temperature and acid-base changes to which microorganisms are subjected in food matrices (Abd El-Salam; El-Shibiny, 2015).

In this context, the microencapsulation of probiotic bacteria is seen as an important technique to improve bacterial stability and viability in food matrices, especially when they are produced through dehydration (Dianawati; Mishra; Shah, 2015).

Since water contributes to the stability of molecules, especially the membrane and wall, drying by freeze drying or spray drying can cause irreversible changes in the structural and functional integrity of bacterial membranes and proteins. Thus, microencapsulation may have a protective effect, preventing dehydration damage (Ananta; Volkert; Knorr, 2005; Crowe et al, 1987; Wolfe, 1987).

The success of this technology is due, among other factors, to the correct choice of encapsulating agent and encapsulation method (Silva *et al*., 2014). Therefore, knowledge about the interaction between microorganisms and capsular materials is important to understand and improve encapsulation yield and survival rate (Abd El-Salam; El-Shibiny, 2015).

Several methods have been suggested to measure the efficiency of bacterial microencapsulation, however the criteria typically addressed in these methods are viability (number of viable cells/total cell count) of the entrapped cells and cell load (cell count/capsule) in the microcapsules (Heidebach; Först; Kulozik, 2012).

Evaluating the available literature, one sees many hypotheses that discuss the likely effect of microbial microencapsulation in relation to encapsulating agents and their benefits. However, few studies evaluate the effectiveness of this encapsulation.

In this sense, to advance this technique and direct it to probiotics, it is important to investigate the specificities of the interaction of bacterial surface molecules with the encapsulating matrices, which will allow, among other advantages, to increase yield, stability and cell viability.

In view of the above considerations, this thesis has the following objectives, divided in two papers:

- A general review on microencapsulation of probiotic bacteria, focusing on spray drying and the main encapsulating agents cited in the literature. **PAPER 1:** Microencapsulation of probiotic bacteria: a brief review.

- A dense review of the microencapsulation of lactic acid bacteria, which stand out in their use as probiotics in foods. Also highlighted are the main hypotheses cited in the literature of the interaction between encapsulating agents and this group of bacteria, by the spray dryer and lyophilization method, in addition to the suggestion of analytical methods to verify such hypotheses. **PAPER 2:** Microencapsulation of Lactic Acid Bacteria by drying techniques: the protective action of the encapsulant agent

**The papers presented below are in the format requested by the different journals in which the works were submitted.

REFERENCES

Abd El-Salam, M. H., & El-Shibiny, S. (2015). Preparation and properties of milk proteins-based encapsulated probiotics: a review. *Dairy Science and Technology*, 95(4), 393–412. https://doi.org/10.1007/s13594-015-0223-8

Ahuja, K., & Deb, S. (2018). Probiotics Market Size By Ingredients Bifidobacterium, Streptococcus, Bacillus), By End-Use (Human, Animal), By Application (Food and Beverages {Dairy Products, Non-Dairy Products, Cereals, Baked Food, Fermented Meat Products, Dry Food}, Die. *Food, Nutricion and Animal Feed*, 260. https://www.gminsights.com/industry-reports/food-nutrition-and-animal-feed

Ananta, E., Volkert, M., & Knorr, D. (2005). Cellular injuries and storage stability of spray-dried Lactobacillus rhamnosus GG. *International Dairy Journal*, 15(4), 399–409. https://doi.org/10.1016/j.idairyj.2004.08.004

Crowe, J. H., Crowe, L. M., Carpenter, J. F., & Aurell Wistrom, C. (1987). Stabilization of dry phospholipid bilayers and proteins by sugars. *The Biochemical Journal*, 242(1), 1–10. https://doi.org/10.1042/bj2420001

Dianawati, D., Mishra, V., & Shah, N. P. (2015). Survival of Microencapsulated Probiotic Bacteria after Processing and during Storage: A Review. *Critical Reviews in Food Science and Nutrition*, 56(10), 1685–1716. https://doi.org/10.1080/10408398.2013.798779

Heidebach, T., Först, P., & Kulozik, U. (2012). Microencapsulation of Probiotic Cells for Food Applications. *Critical Reviews in Food Science and Nutrition*, 52(4), 291–311. https://doi.org/10.1080/10408398.2010.499801

Wolfe, J. (1987). Lateral Stresses in Membranes at Low Water Potential. *Functional Plant Biology*, 14(3), 311. https://doi.org/10.1071/pp9870311

2. PAPER 1

Viana, C. C. R., Renhe, I. R. T., de Bessa, M. E., Martins, E., Stephani, R., de Carvalho, A. F., & Perrone, Í. T. (2021). Microencapsulamento de bactérias probióticas: uma breve revisão. **Research, Society and Development**, 10(13), e242101320814-e242101320814..

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Microencapsulamento de bactérias probióticas: uma breve revisão

Microencapsulation of probiotic bacteria: a brief review

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Carolina Carvalho Ramos Viana
ORCID: https://orcid.org/0000-0002-1542-1612
EPAMIG - Instituto de Laticínios Cândido Tostes, Brasil
E-mail:carolcrviana@yahoo.com.br
Isis Rodrigues Toledo Renhe
ORCID: https://orcid.org/0000-0003-3724-4060
EPAMIG - Instituto de Laticínios Cândido Tostes, Brasil
E-mail: isis@epamig.br
Martha Eunice de Bessa
ORCID: https://orcid.org/0000-0002-8198-1773
Universidade Federal de Juiz de Fora, Brasil
E-mail: marthaebessa@hotmail.com
Evandro Martins
ORCID: https://orcid.org/0000-0002-5448-8385
Universidade Federal de Viçosa, Brasil
E-mail: evandromartins@ufv.br
Rodrigo Stephani
ORCID: https://orcid.org/0000-0003-0237-8325
Universidade Federal de Juiz de Fora, Brasil
E-mail: rodrigo.stephani@ufjf.edu.br
Antônio Fernandes de Carvalho
ORCID: https://orcid.org/0000-0002-3238-936X
Universidade Federal de Viçosa, Brasil
E-mail: antoniofernandes@ufv.br
Ítalo Tuler Perrone
ORCID: https://orcid.org/0000-0002-3393-4876
Universidade Federal de Juiz de Fora, Brasil
E-mail: italotulerperrone@gmail.com

Resumo
No cenário econômico global, os probióticos têm sido responsáveis por uma expressiva movimentação financeira, e com crescimento progressivo esperado para os próximos anos. Busca-se cada vez mais otimizar a produção de culturas microbianas desidratadas, o que torna necessário o conhecimento sobre as técnicas disponíveis e já estudadas. O microencapsulamento se destaca por viabilizar a desidratação de microrganismos, de forma a proteger as células dos efeitos negativos da secagem. O objetivo deste artigo foi compilar dados da literatura sobre o microencapsulamento de probióticos, os principais agentes encapsulantes e métodos já estudados, com foco na secagem por atomização. Pode-se concluir com os trabalhos citados, que a estabilidade e viabilidade das culturas produzidas têm íntima relação com o agente encapsulante e o método utilizado para o microencapsulamento. Todavia, faz-se necessário a realização de mais pesquisas que tentem explicar o efeito protetor das condições utilizadas, de forma a direcionar para técnicas otimizadas na produção de culturas probióticas que sejam adequadas ao uso industrial.
Palavras-chave: Secagem de micro-organismos; Probióticos; Agente encapsulante; Cultura industrial; *Spray dryer*.

Abstract
In the global economic scenario, probiotics have been responsible for a significant financial movement, with progressive growth expected for the coming years. Therefore, the production of dehydrated microbial cultures needs to be optimized, making it necessary to know about the available and already studied techniques. Microencapsulation stands out for enabling the dehydration of microorganisms, protecting cells from the negative effects of drying. This article aimed to compile the literature on the microencapsulation of probiotics, the main encapsulating agents and methods already studied, focusing on drying by atomization. It can be concluded with the consulted literature that the stability and viability of the produced cultures are closely related to the encapsulating agent and the method used for microencapsulation. Further research is needed to explain the protective effect of the conditions used, to direct the optimization of techniques in the production of probiotic cultures that are appropriate for industrial use.
Keywords: Drying of microorganisms; Probiotics; Encapsulating agent; Industrial culture; Spray dryer.

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Carolina Carvalho Ramos Viana

ORCID: https://orcid.org/0000-0002-1542-1612

EPAMIG/ Instituto de Laticínios Cândido Tostes, Brasil

E-mail:carolcrviana@yahoo.com.br

Isis Rodrigues Toledo Renhe

ORCID: https://orcid.org/0000-0003-3724-4060

EPAMIG/ Instituto de Laticínios Cândido Tostes, Brasil

E-mail: isis@epamig.br

Martha Eunice de Bessa

ORCID: https://orcid.org/0000-0002-8198-1773

Universidade Federal de Juiz de Fora, Brasil

E-mail:marthaebessa@hotmail.com

Evandro Martins

ORCID: https://orcid.org/0000-0002-5448-8385

Universidade Federal de Viçosa, Brasil

E-mail:evandromartins@ufv.br

Rodrigo Stephani

ORCID: https://orcid.org/0000-0003-0237-8325

Universidade Federal de Juiz de Fora, Brasil

E-mail:rodrigo.stephani@ufjf.edu.br

Antônio Fernandes de Carvalho

ORCID: https://orcid.org/0000-0002-3238-936X

Universidade Federal de Viçosa, Brasil

E-mail: antoniofernandes@ufv.br

Ítalo Tuler Perrone

ORCID: https://orcid.org/0000-0002-3393-4876

Universidade Federal de Juiz de Fora, Brasil

E-mail: italotulerperrone@gmail.com

Resumo
No cenário econômico global, os probióticos têm sido responsáveis por uma expressiva movimentação financeira, e com crescimento progressivo esperado para os próximos anos. Busca-se cada vez mais otimizar a produção de culturas microbianas desidratadas, o que torna necessário o conhecimento sobre as técnicas disponíveis e já estudadas. O microencapsulamento se destaca por viabilizar a desidratação de microrganismos, de forma a proteger as células dos efeitos negativos da secagem. O objetivo deste artigo foi compilar dados da literatura sobre o microencapsulamento de probióticos, os principais agentes encapsulantes e métodos já estudados, com foco na secagem por atomização. Pode-se concluir com os trabalhos citados, que a estabilidade e viabilidade das culturas produzidas têm íntima relação com o agente encapsulante e o método utilizado para o microencapsulamento. Todavia, faz-se necessário a realização de mais pesquisas que tentem explicar o efeito protetor das condições utilizadas, de forma a direcionar para técnicas otimizadas na produção de culturas probióticas que sejam adequadas ao uso industrial.

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Keywords: Drying of microorganisms; Probiotics; Encapsulating agent; Industrial culture; Spray dryer.

Resumen
En el escenario económico global los probióticos han sido responsables de un movimiento financiero significativo, con un crecimiento progresivo esperado para los próximos años. Se busca optimizar la producción de cultivos microbianos deshidratados, lo que hace necesario conocer las técnicas disponibles y ya estudiadas. La microencapsulación se destaca por permitir la deshidratación de microorganismos, con el fin de proteger las células de los efectos negativos del secado. El objetivo de este artículo es recopilar la

literatura relacionada con la microencapsulación de probióticos, los principales agentes encapsulantes y métodos ya estudiados, centrándose en el secado por atomización. Se puede concluir con los estudios antes mencionados que la estabilidad y viabilidad de los cultivos producidos están estrechamente relacionadas con el agente encapsulante y el método utilizado para la microencapsulación. Un enfoque para próximas investigaciones es explicar el efecto protector de las condiciones utilizadas, con el fin de dirigir a técnicas optimizadas en la producción de cultivos probióticos que sean apropiados para uso industrial.

Palabras clave: Secado de microorganismos; Probióticos; Agente encapsulante; Cultivo industrial; Secador por pulverización.

1. Introdução

Um número crescente de micro-organismos tem sido investigado quanto ao seu potencial de conferir benefícios à saúde do hospedeiro, quando administrados em quantidades apropriadas, e tem sido amplamente utilizado em vários alimentos e bebidas (FAO / WHO, 2006; Huang et al., 2017; Marques et al., 2020).

Bactérias probióticas, como algumas cepas pertencentes ao gênero *Lactobacillus*, integram a microbiota intestinal humana e contribuem para o balanço do ecossistema microbiano do hospedeiro (Goldin, 2011; Liu et al., 2017; Miranda et al., 2021). Suas aplicações na indústria de alimentos incluem uso como culturas starter ou coadjuvantes no desenvolvimento de alimentos fermentados, incorporação em produtos alimentícios e como ingrediente com apelo funcional (Crovesy et al., 2017; Freire et al., 2021; Saad et al., 2013). Os benefícios ao hospedeiro incluem maior saúde digestiva e da pele, regularidade intestinal e fortalecimento do sistema imunológico (Abd El-Salam & El-Shibiny, 2015; Chen et al., 2015; Figueiredo et al., 2020).

Para produzir tais efeitos benéficos na saúde, o probiótico deve ser capaz de sobreviver e se multiplicar no hospedeiro; deve ser metabolicamente estável e ativo no produto; resistir ao ambiente adverso durante a passagem gastrointestinal; e atingir o intestino em grande quantidade (Brasil, 2018; Martín et al., 2015; Sarao & Arora, 2017).

A eficácia dos probióticos está relacionada à dose e, por isso, os alimentos probióticos devem conter pelo menos 10^6 a 10^7 unidades formadoras de colônias viáveis por grama no momento de seu consumo (Abd El-Salam & El-Shibiny, 2015; Brasil, 2018). O desafio é manter a viabilidade durante o processamento, armazenamento, vida de prateleira dos produtos, além de resistir aos estresses oxidativos, de temperatura e às alterações ácido-base a que os micro-organismos são submetidos nas matrizes alimentares.

Na maioria dos casos, o uso industrial de probióticos depende de métodos de preservação por meio da desidratação, com o intuito de garantir uma melhor viabilidade e atividade metabólica celular durante as aplicações alimentícias, processamento e armazenamento (Martins et al., 2019; Silva et al., 2014).

Várias tecnologias estão disponíveis para melhorar a conservação dos micro-organismos de interesse e produzir culturas industrialmente viáveis. A secagem é um dos processos aplicados, e que viabiliza uma vida útil estável e estendida. Entretanto, tem como fator limitante a perda de viabilidade principalmente devido à ruptura das células durante o processo (Dianawati et al., 2015; Huang et al., 2017; Rosolen et al., 2019).

De forma a proteger os micro-organismos dos efeitos negativos da secagem, a técnica de microencapsulamento pode ser aplicada. Esta tecnologia melhora a estabilidade bacteriana no processamento industrial, nos alimentos, bem como pode contribuir na redução da perda de viabilidade durante a passagem por condições enzimáticas e de pH adversos no trato gastrointestinal (Barroso et al., 2021; Dianawati et al., 2015; Panghal et al., 2018; Silva et al., 2014).

Por ser uma técnica que vem se destacando na produção de culturas para uso na indústria de alimentos, o objetivo deste trabalho foi compilar, por meio de uma breve revisão, as principais informações científicas sobre o microencapsulamento de probióticos, tendo como foco o uso de *spray drying* como método de produção e descrição dos principais agentes encapsulantes empregados nos estudos.

2. Metodologia

O trabalho trata-se de uma revisão narrativa, de caráter qualitativo (Pereira et al., 2018). Baseia-se em pesquisas de bibliográficas em diferentes bases de indexação, em temas relacionados ao microencapsulamento de microrganismos probióticos, com foco no método por *spray dryer*. Buscaram-se artigos científicos adicionando as palavras-chave “microencapsulamento de microrganismos”, “microencapsulamento de bactérias”, “microencapsulamento de probióticos”, “agentes encapsulantes, "*spray dryer*", “probióticos”, “métodos de microencapsulamento”.

Para a seleção dos artigos foram incluídas publicações em língua inglesa e portuguesa, majoritariamente entre os anos de 2011 à 2021.

3. Referencial teórico

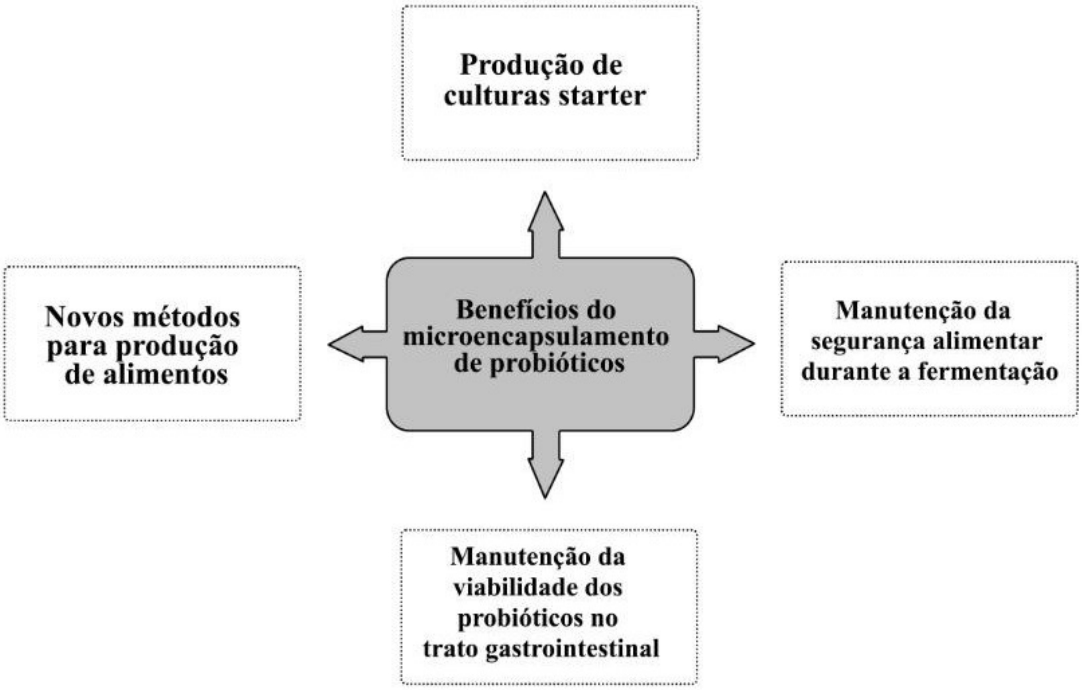
O microencapsulamento é uma técnica relativamente recente. Configura-se como uma tecnologia que promove o revestimento fino de partículas sólidas, gotas de líquidos, micro-organismos e dispersões (Barroso et al., 2021; Food Ingredients Brasil, 2013). O microencapsulamento é um processo de imobilização ou compartimentalização por cápsulas cujo tamanho está entre 1 µm e 1000 µm. É uma tecnologia que vem sendo empregada na indústria de cosméticos, produtos farmacêuticos, agrotóxicos e alimentos e, nesta, é usado em aromas, ácidos, óleos, vitaminas, micro-organismos, entre outros (Martins et al., 2017).

Um possível design comumente usado para o encapsulamento de células microbianas envolve a microesfera, em que células vivas são incorporadas e imobilizadas aleatoriamente em uma matriz contínua. Portanto, os termos “imobilização” e "encapsulamento" são muitas vezes usados como sinônimo na maioria dos trabalhos relatados sobre a microencapsulamento de probióticos (Heidebach et al., 2012).

O microencapsulamento ajuda a proteger a célula microbiana do ambiente, melhorando assim sua estabilidade. A estrutura formada pelo agente de microencapsulamento em torno do núcleo é composta por um material semipermeável, ao mesmo tempo fino e relativamente forte (Kailasapathy, 2002). Além disso diminui as interações da substância encapsulada em relação aos fatores ambientais. Dessa forma preserva as características sensoriais e nutricionais alimentos, facilita a incorporação da substância encapsulada em sistemas secos e permite a liberação da substância encapsulada de forma lenta (Burgain et al., 2011; Kuang et al., 2010)

Conforme ilustrado na Figura 1, inúmeros são os benefícios do microencapsulamento microbiano, incluindo a proteção contra fatores prejudiciais para aumentar a resistência à secagem e armazenamento, e convertê-las em pó, de forma a facilitar o uso, pois melhoram a sua distribuição homogênea em todo o produto.

Figura 1 Benefícios do microencapsulamento de probióticos



Fonte: Elaborado pelos autores, adaptado de Kavitake et al. (2017)

Na indústria alimentícia, essa técnica tem solucionado limitações no emprego de ingredientes, visto que pode suprimir ou atenuar flavors indesejáveis, reduzir a volatilidade e aumentar a estabilidade destes em condições ambientais adversas, como na presença de luz, oxigênio e pH extremos (Barroso et al., 2021).

A viabilidade dos probióticos encapsulados, bem como características das cápsulas formadas são afetadas grandemente pelo processo de encapsulamento usado (Abd El-Salam & El-Shibiny, 2015). Vários métodos de microencapsulamento são estudados, e há uma grande tendência de crescimento à medida que vão surgindo novos materiais encapsulantes e princípios ativos que requeiram processamentos específicos (Desai & Park, 2005; Madene et al., 2006; Martín et al., 2015).

3.1. Métodos de microencapsulamento de probióticos

Geralmente, a tecnologia de microencapsulamento é realizada incorporando-se células em uma matriz líquida. Após isso, a estabilização é realizada por um processo que pode ser físico (evaporação, solidificação), químico (polimerização) ou físico-químico (gelificação), dependendo do material encapsulante utilizado (Barroso et al., 2021; Rokka & Rantamäki, 2010; Sarao & Arora, 2017).

Várias técnicas estão disponíveis para a desidratação e conservação de microrganismos. Dentre os métodos convencionais usados para o microencapsulamento de probióticos, destacam-se o método de extrusão, emulsificação e métodos de secagem, como *spray* drying e liofilização (Tabela 1) (Barroso et al., 2021; Chen et al., 2015; Silva et al., 2014).

A escolha do método mais adequado depende de características do material ativo, da aplicação e do mecanismo de liberação desejado para a sua ação. A diferença básica entre os métodos existentes está no tipo de envolvimento ou aprisionamento do material ativo pelo agente encapsulante, visto que a combinação entre o material e o agente ativo pode ser de natureza física, química ou físico-química (Food Ingredients Brasil, 2013; Rodrigues et al., 2020)

A extrusão é comumente empregada em função de baixo custo e simplicidade. Configura-se pela preparação de uma solução hidrocoloide adicionada dos probióticos, que é gotejada sob o material de revestimento por meio de um bico em alta pressão. Como agentes encapsulantes usados, pode-se citar o alginato, k-carragena e proteínas do leite (Huq et al., 2013; Kailasapathy, 2002; Silva et al., 2014; Vaniski et al., 2017).

A técnica de emulsão tem sido aplicada com sucesso para a microencapsulação de bactérias ácido láticas (BAL). Se comparado à extrusão, este método requer maior custo para desempenho (Huq et al., 2013). Realiza-se a dispersão de uma fase aquosa contendo as células bacterianas e suspensão em uma fase orgânica, como óleo, resultando em uma emulsão de água/óleo. As cápsulas formadas solidificam-se por meio de resfriamento ou adição de um agente gelificante (Kailasapathy, 2002; Martín et al., 2015; Vaniski et al., 2017).

O microencapsulamento de probióticos pode ser também feito por meio da secagem do concentrado de células. Dentre os mais importantes, observa-se a secagem em leito fluidizado, a liofilização e a secagem por *spray dryer* ou pulverização/atomização(Huq et al., 2013).

Na secagem em leito fluidizado, a suspensão de células é pulverizada e seca em transportadores inertes. Antes da secagem, é necessário que a cultura probiótica seja encapsulada em material de suporte, como alginato de cálcio, leite desnatado ou lipídeos (Martín et al., 2015).

A liofilização é um método que envolve a desidratação do material congelado sob um processo de sublimação a vácuo. É uma técnica comumente usada para preservar bactérias probióticas, uma vez que promove menor estresse às células e resulta em fermentos com maior viabilidade. Entretanto, produção de bactérias liofilizadas é limitada pelo longo tempo de processo, necessidade de armazenamento a baixas temperaturas e pelo alto custos energético demandado. Com isso, o emprego da técnica de *spray drying* vem se destacando (Martín et al., 2015; Martins et al., 2019; Silva et al., 2014).

Tabela 1 Exemplos de processos de secagem comumente utilizados para microencapsulamento

Processo	Padrão de produção		Consumo específico de energia (kJ·kg^{-1} de água)	Produtividade (ton·ano^{-1})	Vantagens	Limitações
Secagem por Leito Fluidizado	Sólido → Sólido	Contínuo	11400	n/d	Tratamento térmico baixo; produção de pó abaixo da T_g	Consumo de energia muito alto; alto tempo de permanência e investimentos
Liofilização	Líquido → Sólido	Lote	18000	~10000 *	Tratamento térmico muito baixo; melhora da reidratação	Alto tempo de residência (24h a 48h)
Secagem por *spray dryer*	Líquido → Sólido	Contínuo	5300	50000 *	Baixo Tratamento térmico e tempo de permanência	Altos invetimentos

n/d: Não descrito; Tg: Temperatura de transição vítrea

* valor aproximado para um secador em larga escala (a produtividade depende do equipamento e escala de produção).

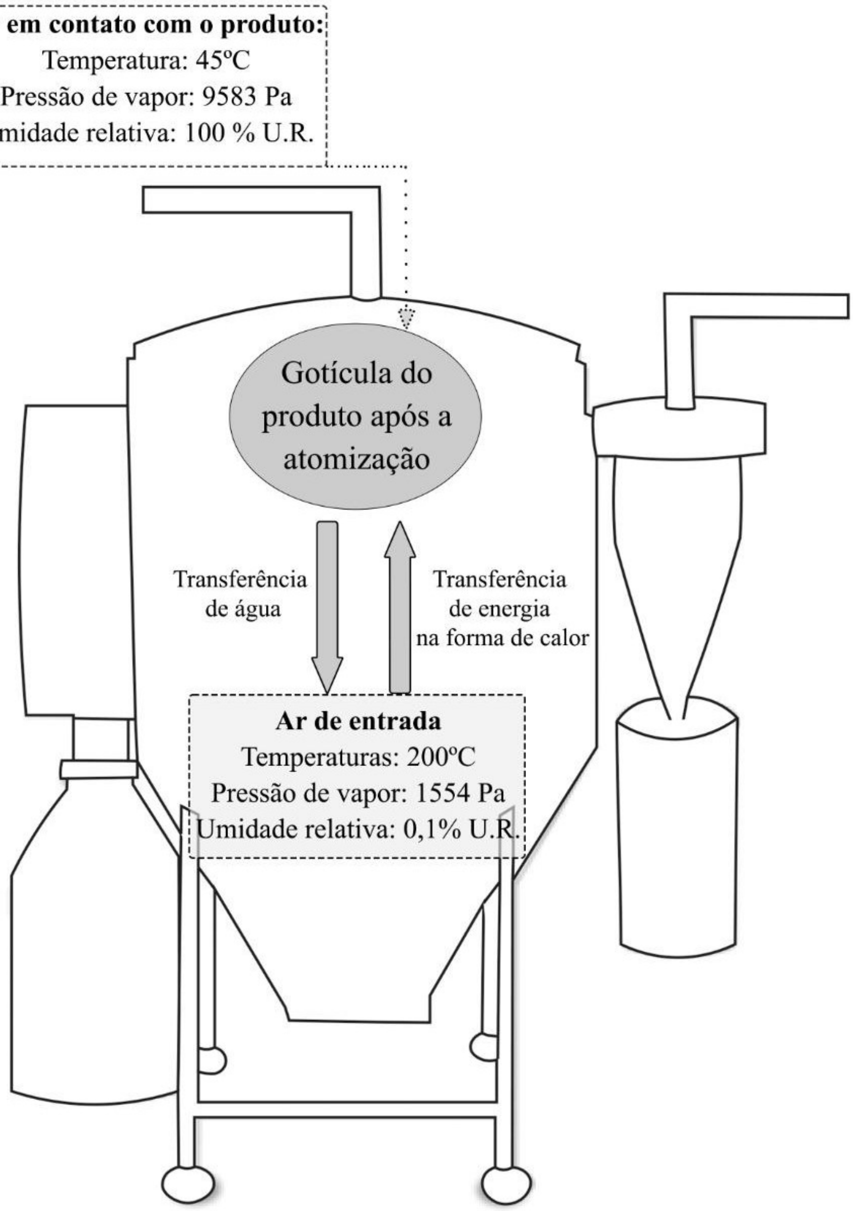
Fonte: Elaborado pelos autores, adaptado de Huang et al., (2017)

Entre as técnicas de secagem possíveis, a secagem por *spray dryer* é uma das predominantes na indústria de laticínios para desidratação de produtos lácteos. Esse processo consiste na remoção parcial da água contida no produto, viabilizando o armazenamento, conservação por períodos mais longos e redução de custos logísticos (Schuck et al., 2012).

A secagem por pulverização é uma das técnicas de microencapsulamento mais amplamente utilizadas em função da capacidade de uso de vários agentes encapsulantes, estabilidade do produto final, fácil manuseio do equipamento, rápida solubilidade da cápsula, além do baixo custo (Alves et al., 2017).

Conforme ilustrado na Figura 2, no microencapsulamento por *spray dryer* o material ativo a ser encapsulado é misturado a uma solução do composto que constitui o material encapsulante. Após isso, pulveriza-se tal mistura em gotículas finas (10 µm a 150 µm) que são direcionadas para um fluxo de ar quente e seco (geralmente 150°C a 250°C) (Martins et al., 2017; Schuck et al., 2012).

Figura 2 Princípio da secagem por atomização



Fonte: Elaborado pelos autores, adaptado de Schuck et al*.,* (2009)

Quando um produto entra em contato com uma corrente de ar tendo baixa umidade relativa (pressão de 1554 Pa) e a temperatura elevada (em média 200ºC), é formada uma diferença de pressão e temperatura da água entre o produto e o ar. Por consequência, haverá uma transferência de energia na forma de calor do ar para o alimento e uma passagem de água do produto para o ar, conforme ilustrado na Figura 2. As gotículas que são formadas no interior da câmara possuem uma grande área superficial, o que leva a uma evaporação da água mais rápida em condição de temperatura mais baixa, o que reduz danos térmicos ao produto (Schuck, 2009).

Esse processo apresenta algumas vantagens sobre os outros métodos: as propriedades e a qualidade do produto são mais eficientemente controladas; podem ser usados produtos sensíveis ao calor; há possibilidade de grandes produções em operação contínua com equipamento simples; produção de partículas relativamente uniformes e esféricas; boa eficiência e baixo custo do processo (Maciel et al., 2014)

Segundo Martin et al. (2015), a secagem por *spray dryer* é um método de microencapsulamento utilizado na indústria de alimentos por ser flexível e relativamente econômico. A vantagem do processo é que ele pode ser operado continuamente. A desvantagem é que a temperatura usada no processo pode não ser adequada para encapsular culturas bacterianas probióticas.

A fim de superar esta característica e melhorar as condições de sobrevivência de bactérias probióticas durante a secagem por atomização, vários métodos vêm sendo estudados e desenvolvidos. O ajuste dos parâmetros de secagem por pulverização, como temperatura de entrada / saída, vazão, pressão do ar de atomização e tempo de permanência na câmara do secador, configuram-se como estratégias para melhorar viabilidade celular. Normalmente, usando temperaturas mais baixas de entrada / saída promove-se maiores taxas de sobrevivência de bactérias probióticas, em função do menor grau de dano térmico nas células (Liu et al., 2017). Além disso, escolher o material encapsulante mais eficiente é primordial no emprego desta técnica (Dianawati et al., 2015).

3.2. Principais agentes encapsulantes estudados

A escolha do material a ser utilizado como agente encapsulante deve considerar uma série de fatores, destacando-se propriedades físicas e químicas de compatibilidade destes em relação ao material a ser encapsulado. Em especial, a temperatura de transição vítrea do encapsulante, a superfície do núcleo encapsulado e fatores econômicos devem ser estudados (Martín et al., 2015; Rodrigues et al., 2020).

Os encapsulantes devem atender ainda aos seguintes requisitos: boas propriedades de formação de filme; baixa higroscopicidade; baixa viscosidade a altas concentrações de sólidos (menor que 0,5 Pa.s em concentrações superiores a 45%); sabor e odor suaves; fácil reconstituição; e baixo custo. Na prática, em função da dificuldade em aliar tais características a um mesmo composto, são utilizadas misturas de materiais, de forma a obter melhores resultados em relação à viabilidade celular (Food Ingredients Brasil, 2013).

O tipo, concentração e propriedades do material capsular afetam marcadamente a viabilidade dos probióticos encapsulados. Inúmeros biomateriais, incluindo carboidratos (lactose, maltodextrina, alginato, amido, amidos modificados e sacarose), lipídios (monoglicerídeos, diglicéridos e óleos hidrogenados) e proteínas (caseína, proteínas do soro do leite, gelatina e albumina) já foram estudados e mostram-se com potencial para serem utilizados no encapsulamento de probióticos, e, além destes outros podem ser usados desde que satisfaçam aos requisitos dos materiais geralmente reconhecidos como seguros (status GRAS) (Abd El-Salam & El-Shibiny, 2015; Antigo et al., 2020; Santos et al., 2014).

Segundo Dianawati; Mishra; Shah (2015) vários materiais de encapsulamento preparados a partir de carboidratos (álcoois de açúcar, açúcares, polissacarídeos, hidrocoloides) ou proteínas (à base de leite ou não) ou diversas combinações têm sido estudados, para verificar sua eficácia na proteção de bactérias probióticas. Leite desnatado e caseína e / ou açúcares como manitol, sorbitol, trealose e sacarose foram comprovadamente eficazes como materiais de encapsulamento de algumas cepas do gênero *Lactobacillus*. Por outro lado, o uso de polissacarídeos mostrou resultados variados, dependendo das cepas bacterianas e método de microencapsulamento, incluindo a etapa de liofilização ou *spray*. Cada um destes deve ser otimizado para aumentar a vida útil de bactérias probióticas sem alterações significativas nas suas características funcionais.

A pesquisa e desenvolvimento de novos biomateriais microencapsulante, tem sido uma área de crescente interesse contribuir para a eficácia na microencapsulamento de probióticos (Abd El-Salam & El-Shibiny, 2015).

a) Carboidratos

Os carboidratos são materiais muito utilizados para microencapsulamento, por sua capacidade de se ligar a compostos, além de sua diversidade e baixo custo. Entre os agentes protetores do tipo carboidrato, a trealose tem sido frequentemente investigada (Huang et al., 2017).

Outros compostos frequentemente estudados são o amido, a maltodextrina, a lactose e a goma xantana. O amido é um polissacarídeo de armazenamento constituído de unidades de α- D- glicose pelas ligações glicosídicas α 1 → 4 e α 1 → 6. Consiste em duas diferentes moléculas de polissacarídeos: uma é a amilose, com estrutura linear (20 a 30%); e a outra é a amilopectina, de alta ramificação (70-80%). A goma xantana mostra um comportamento bastante adequado à técnica de microencapsulamento devido à sua conformação semi-rígida e maior viscosidade, que leva a formar uma rede por ligação de hidrogênio e cadeia de polímero (Kavitake et al., 2018)

b) Proteínas

Proteínas são geralmente consideradas materiais adequados para microencapsulamento em aplicações alimentares, especialmente em alimentos líquidos e semissólidos. Possuem a vantagem de desenvolver matrizes de tamanhos controlados, sem qualquer efeito adverso no sabor dos alimentos (Abd El-Salam & El-Shibiny, 2015). Além disso, podem apresentar efeitos estabilizadores desejáveis na textura dos alimentos.

A gelatina é uma mistura complexa de polipeptídeos de cadeia simples ou múltipla utilizada no encapsulamento probiótico. Este gel termo reversível pode ser usado sozinho ou em cooperação com polissacarídeos (Huq et al., 2013).

As propriedades funcionais e nutricionais únicas das proteínas do leite são bem documentadas (Fox & McSweeney, 1998) e são universalmente valorizadas como ingrediente alimentar. Segundo Abd El-Salam; El- Shibiny (2015), as proteínas do leite têm várias vantagens específicas no microencapsulamento de compostos usados em alimentos:

1. Uma grande diversidade de produtos contendo proteína de leite está disponível comercialmente. Estes apresentam composição, teor de proteínas e propriedades funcionais diferentes. Podem apresentar caseínas, sais de caseinato, β-caseína, concentrados de proteína de soro de leite (WPC) e isolados (WPI) e β-lactoglobulina (β-LG) e frações ricas em α-lactalbumina (α-LA). Isso oferece uma ampla gama de opções para encapsulamento de probióticos em proteínas do leite;

2. As proteínas do leite são flexíveis para encapsular qualquer tipo de substância hidrofílica, hidrofóbica ou células probióticas viáveis;

3. As proteínas do leite podem formar géis por meio de diferentes mecanismos em condições amenas. Isso oferece diferentes opções para o sucesso quanto ao uso de proteínas do leite no microencapsulamento de bactérias viáveis

(Heidebach et al., 2012). A gelificação da caseína pode ser induzida com enzimas de coagulação do leite ou na presença de ácidos. A gelificação ácida da caseína ocorre próxima ao seu ponto isoelétrico, em que as interações hidrofóbicas atuam como as principais forças de agregação e gelificação. Além disso, a formação de novas ligações covalentes nas proteínas do leite pela ação da transglutaminase resulta na formação de géis;

4. As soluções concentradas de proteínas do leite têm viscosidades moderadas para facilitar a dispersão das células bacterianas e, quando usado no encapsulamento, fornece géis de alta densidade e melhor proteção para os probióticos aprisionados.

5. As proteínas do leite são fontes ricas de peptídeos bioativos de diferentes efeitos Tais peptídeos bioativos são gerados pela ação de enzimas do aparelho digestivo para exercer seus efeitos benéficos. Os peptídeos bioativos liberados podem oferecer um efeito sinérgico na ação probiótica.

c) Lipídios

Ao contrário da aplicação de carboidratos e proteínas, o uso de lipídios para o encapsulamento de probióticos por *spray dryer* ainda não foi bem explorado. O encapsulamento matricial pode ser alcançado misturando células probióticas com lipídios liquefeitos e, com subsequente resfriamento. No entanto, a dispersão de concentrados de células probióticas no óleo foi relatado como uma tarefa tecnológica de difícil emprego (Heidebach et al., 2012).

Microcápsulas à base de gordura atualmente parece menos adequado para a combinação de encapsulamento probiótico comparados com microcápsulas à base de polissacarídeos ou proteínas. Porém, mais estudos são necessários para aprimoramento deste composto no microencapsulamento microbiano (Liu et al., 2017).

4. Considerações Finais

O aumento da demanda por alimentos funcionais, buscando-se benefícios nutricionais e à saúde deve impulsionar o crescimento deste mercado nos próximos anos. Além disso, a crescente procura por tratamentos não farmacológicos devido ao menor custo pode estimular a busca por alimentos probióticos, por serem amplamente utilizados no tratamento de distúrbios neurológicos, problemas digestivos e doenças mentais (Huang et al., 2017). Neste contexto, vê-se que a tecnologia de microencapsulamento pode ser ampla e diversificada.

Células bacterianas microencapsuladas têm sido amplamente relatadas como sendo mais estáveis do que células livres. Os efeitos do encapsulamento na estabilidade das culturas produzidas estão intimamente ligados ao agente encapsulante e método de secagem. No entanto, um meio adequado para secagem pode não ser eficaz na conservação da viabilidade celular, e por isso, entender as interações entre o encapsulante e o material a ser encapsulado é relevante para a eficiência deste processo.

A literatura atual sinaliza a necessidade por informações, bem como por pesquisa e desenvolvimento para produção culturas de probióticos com alto rendimento, alta viabilidade celular e baixo custo. Estudos limitados foram realizados sobre a interação entre encapsulantes e micro-organismos, e sinalizam a importância do conhecimento deste importante tópico para o aprisionamento bem-sucedido de probióticos.

Portanto, para pesquisas futuras, sugere-se avaliar e elucidar os mecanismos de proteção que ocorrem entre diferentes agentes encapsulantes e células, variando-se métodos e condições de microencapsulamento, a fim de evitar que a eficiência do processo utilizado seja determinada por tentativa e erro. Desta forma, será possível guiar a produção e uso de culturas microbianas para atender, de forma mais adequada, as necessidades industriais na produção de alimentos.

Referências

Abd El-Salam, M. H., & El-Shibiny, S. (2015). Preparation and properties of milk proteins-based encapsulated probiotics: a review. *Dairy Science and Technology*, *95*(4), 393–412. https://doi.org/10.1007/s13594-015-0223-8

Ahuja, K., & Deb, S. (2018). Probiotics Market Size By Ingredients Bifidobacterium, Streptococcus, Bacillus), By End-Use (Human , Animal), By Application (Food and Beverages { Dairy Products , Non-Dairy Products , Cereals , Baked Food , Fermented Meat Products , Dry Food }, Die. *Food, Nutricion and Animal Feed*, 260. https://www.gminsights.com/industry-reports/food-nutrition-and-animal-feed

Alves, A. I., Rodrigues, M. Z., Ribeiro Pinto, M. R. M., Lago Vanzela, E. S., Stringheta, P. C., Perrone, Í. T., & Ramos, A. M. (2017). Morphological characterization of pequi extract microencapsulated through *spray* drying. *International Journal of Food Properties*, *20*(June), 1298–1305. https://doi.org/10.1080/10942912.2017.1343344

Antigo, J. L. D., Silva, J. ., Bergamasco, R. C., & Madrona, G. . (2020). Microencapsulation of beet dye (Beta vulgaris L.) using maltodextrin and xanthan gum as encapsulant agents and application in yogurt. *Research, Society and Development*, *9*(12), 1–25. https://doi.org/http://dx.doi.org/10.33448/rsd-v9i12.10896

Barroso, T. L. C. T., Alexandre, J. de B., Silva, L. C. da, Castelo, R. M., Ribeiro, L. B., Furtado, R. F., & Zambelli, R. A. (2021). Tecnologia de encapsulamento na área de alimentos: Uma revisão. *Research, Society and Development*, *10*(7), e6210716240. https://doi.org/10.33448/rsd-v10i7.16240

BRASIL. Ministério da Saúde/Agência Nacional de Vigilância Sanitária. (2018). *Resolução da Diretoria Colegiada - RDC n° 241, de 26 de julho de 2018*. Edição:144. Seção: 1. Pág.97.

Burgain, J., Gaiani, C., Linder, M., & Scher, J. (2011). Encapsulation of probiotic living cells: From laboratory scale to industrial applications. *Journal of Food Engineering*, *104*(4), 467–483. https://doi.org/10.1016/j.jfoodeng.2010.12.031

Chen, J., Wang, Q., Liu, C. M., & Gong, J. (2015). Issues deserve attention in encapsulating probiotics: Critical review of existing literature. *Critical Reviews in Food Science and Nutrition*, *57*(6), 1228–1238. https://doi.org/10.1080/10408398.2014.977991

Crovesy, L., Ostrowski, M., Ferreira, D. M. T. P., Rosado, E. L., & Soares-Mota, M. (2017). Effect of Lactobacillus on body weight and body fat in overweight subjects: A systematic review of randomized controlled clinical trials. *International Journal of Obesity*, *41*(11), 1607–1614. https://doi.org/10.1038/ijo.2017.161

Desai, K. G. H., & Park, H. J. (2005). Recent developments in microencapsulation of food ingredients. In *Drying Technology* (Vol. 23, Issue 7). https://doi.org/10.1081/DRT-200063478

Dianawati, D., Mishra, V., & Shah, N. P. (2015). Survival of Microencapsulated Probiotic Bacteria after Processing and during Storage: A Review. *Critical Reviews in Food Science and Nutrition*, *56*(10), 1685–1716. https://doi.org/10.1080/10408398.2013.798779

FAO / WHO. (2006). Probiotics in food - Health and Nutrition Properties of and guidelines for evaluation. In *FAO/ World Health Organization* (Issue 1). FAO and WHO.

Figueiredo, M. C. F., Araújo, D. S., Nascimento, J. M. F., Moura, F. V. P., Silva, T. R., Barros, F. D. D. B., Medeiros, S. R. A., Olivera, V. A., Souza, A. C. P., & Pereira-Freire, J. A. (2020). Efeitos dos probióticos sobre a microbiota intestinal e metabolismo de idosos. *Research, Society and Development*, *9*(4), 1–16. https://doi.org/http://dx.doi.org/10.33448/rsd-v9i4.2969

Food Ingredients Brasil. (2013). Microencapsulação a serviço da indústria alimentícia. *Food Ingredients Brasil*, *V. 25*, 30–36. www.revista-fi.com

Fox, P. F., & McSweeney, P. L. H. (1998). *Dairy Science and Technology Handbook*

(First Edit). Blackie Academic & Professional.

Freire, T. T., Silva, A. L. ., Ferreira, B. K. O., & Santos, T. M. (2021). Bactérias ácido lácticas suas características e importância: revisão. *Research, Society and Development*, *10*(11), 1–19. https://doi.org/http://dx.doi.org/10.33448/rsd-v10i11.19964

Goldin, B. R. (2011). Probiotics and health: From History to Future. In W. Kneifel & S. Salminen (Eds.), *Probiotics and Health Claims* (p. 13). Blackwell Publishing Ltd. Blackwell. https://doi.org/10.1097/mcg.0b013e31822c5718

Heidebach, T., Först, P., & Kulozik, U. (2012). Microencapsulation of Probiotic Cells for Food Applications. *Critical Reviews in Food Science and Nutrition*, *52*(4), 291–311. https://doi.org/10.1080/10408398.2010.499801

Huang, S., Vignolles, M. L., Chen, X. D., Le Loir, Y., Jan, G., Schuck, P., & Jeantet, R. (2017). *Spray* drying of probiotics and other food-grade bacteria: A review. *Trends in Food Science and Technology*, *63*, 1–17. https://doi.org/10.1016/j.tifs.2017.02.007

Huq, T., Khan, A., Khan, R. A., Riedl, B., & Lacroix, M. (2013). Encapsulation of Probiotic Bacteria in Biopolymeric System. *Critical Reviews in Food Science and Nutrition*, *53*(9), 909–916. https://doi.org/10.1080/10408398.2011.573152

Kailasapathy, K. (2002). Microencapsulation of Probiotic Bacteria: Technology and Potential Applications. *Curr. Issues Intest. Microbiol.*, 39–48.

Kavitake, D., Kandasamy, S., Devi, P. B., & Shetty, P. H. (2018). Recent developments on encapsulation of lactic acid bacteria as potential starter culture in fermented foods – A review. *Food Bioscience*, *21*(November 2017), 34–44. https://doi.org/10.1016/j.fbio.2017.11.003

Kuang, S. S., Oliveira, J. C., & Crean, A. M. (2010). Microencapsulation as a tool for incorporating bioactive ingredients into food. *Critical Reviews in Food Science and Nutrition*, *50*(10), 951–968. https://doi.org/10.1080/10408390903044222

Liu, H., Cui, S. W., Chen, M., Li, Y., Liang, R., Xu, F., & Zhong, F. (2017). Protective approaches and mechanisms of microencapsulation to the survival of probiotic bacteria during processing, storage and gastrointestinal digestion: A review. *Critical Reviews in Food Science and Nutrition*, *59*(17), 2863–2878. https://doi.org/10.1080/10408398.2017.1377684

Maciel, G. M., Chaves, K. S., Grosso, C. R. F., & Gigante, M. L. (2014). Microencapsulation of Lactobacillus acidophilus La-5 by *spray drying*using sweet whey and skim milk as encapsulating materials. *Journal of Dairy Science*, *97*(4), 1991–1998. https://doi.org/10.3168/jds.2013-7463

Madene, A., Jacquot, M., Scher, J., & Desobry, S. (2006). Flavour encapsulation and controlled release - A review. *International Journal of Food Science and Technology*, *41*(1), 1–21. https://doi.org/10.1111/j.1365-2621.2005.00980.x

Marques, C. G., Cruz, E. M. R. M. da, Bezerra, V. M., Costa, J. T. G., Lira, S. M., Holanda, M. O., Silva, J. Y., Canabrava, N. ., Silva, B. B., & Guedes, M. I. F. (2020). Prebióticos e probióticos na saúde e no tratamento de doenças intestinais: uma revisão integrativa. *Research, Society and Development*, *9*, 1–42. https://doi.org/http://dx.doi.org/10.33448/rsd-v9i10.9071

Martín, M. J., Lara-Villoslada, F., Ruiz, M. A., & Morales, M. E. (2015). Microencapsulation of bacteria: A review of different technologies and their impact on the probiotic effects. *Innovative Food Science and Emerging Technologies*, *27*, 15–25. https://doi.org/10.1016/j.ifset.2014.09.010

Martins, E., Cnossen, D. C., Silva, C. R. J., Cezarino, J. C., Nero, L. A., Perrone, I. T., & Carvalho, A. F. (2019). Determination of ideal water activity and powder temperature after *spray* drying to reduce Lactococcus lactis cell viability loss. *Journal of Dairy Science*, *102*(7), 6013–6022. https://doi.org/10.3168/jds.2019-16297

Martins, E., Poncelet, D., Rodrigues, R. C., & Renard, D. (2017). Oil encapsulation techniques using alginate as encapsulating agent : applications and drawbacks. *Journal of Microencapsulation*, *0*(0), 754–771. https://doi.org/10.1080/02652048.2017.1403495

Miranda, B. L., Lima, D. O., Barbosa, T., Barbosa, M. L. M. R., Silva, H. J. N., Nascimento, E. M. M., Meira, D. E. A. V., Rocha, R. E., Kananda, P. ., Soares, T. S., Passos, C. R. F., Oliveira, T. P., Nascimento, A. C. O., Machado, K. C., & Silva, A. M. (2021). Efeito dos probióticos na prevenção e tratamento de câncer e Diabetes mellitus. *Research, Society and Development,10*(5), 1–9. https://doi.org/http://dx.doi.org/10.33448/rsd-v10i5.14932

Panghal, A., Janghu, S., Virkar, K., Gat, Y., Kumar, V., & Chhikara, N. (2018). Potential non-dairy probiotic products – A healthy approach. *Food Bioscience*, *21*, 80–89. https://doi.org/10.1016/j.fbio.2017.12.003

Pereira, A., Shitsuka, D., Parreira, F., & Shitsuka, R. (2018). Metodologia da pesquisa científica. In UAB/NTE/UFSM (Ed.), *Núcleo de Tecnologia Educacional da Uni- versidade Federal de Santa Maria* (1st ed.). Universidade Federal de Santa Maria. https://repositorio.ufsm.br/bitstream/handle/1/15824/Lic_Computacao_Metodologia-Pesquisa-Cientifica.pdf?sequence=1. Acesso em: 03 de outubro de 2021.

Rodrigues, F. J., Cedran, M. F., Bicas, J. L., & Sato, H. H. (2020). Encapsulated probiotic cells: Relevant techniques, natural sources as encapsulating materials and food applications – A narrative review. *Food Research International*, *137*, 109682. https://doi.org/10.1016/j.foodres.2020.109682

Rokka, S., & Rantamäki, P. (2010). Protecting probiotic bacteria by microencapsulation: Challenges for industrial applications. *European Food Research and Technology*, *231*(1), 1–12. https://doi.org/10.1007/s00217-010-1246-2

Rosolen, M. D., Bordini, F. W., de Oliveira, P. D., Conceição, F. R., Pohndorf, R. S., Fiorentini, Â. M., da Silva, W. P., & Pieniz, S. (2019). Symbiotic microencapsulation of Lactococcus lactis subsp. lactis R7 using whey and inulin by *spray* drying. *Lwt*, *115*, 108411. https://doi.org/10.1016/j.lwt.2019.108411

Saad, N., Delattre, C., Urdaci, M., Schmitter, J. M., & Bressollier, P. (2013). An overview of the last advances in probiotic and prebiotic field. *LWT - Food Science and Technology*, *50*(1), 1–16. https://doi.org/10.1016/j.lwt.2012.05.014

Santos, R. C. S. dos, Finkler, L., & Finkler, C. L. L. (2014). Microencapsulation of Lactobacillus casei by *spray* drying. *Journal of Microencapsulation*, *31*(8), 759–767. https://doi.org/10.3109/02652048.2014.932026

Sarao, L. K., & Arora, M. (2017). Probiotics, prebiotics, and microencapsulation: A review. *Critical Reviews in Food Science and Nutrition*, *57*(2), 344–371. https://doi.org/10.1080/10408398.2014.887055

Schuck, P. (2009). Understanding the factors affecting *spray*-dried dairy powder properties and behavior. In *Dairy-Derived Ingredients: Food and Nutraceutical Uses*. https://doi.org/10.1533/9781845697198.1.24

Schuck, P., Dolivet, A., Méjean, S., Hervé, C., & Jeantet, R. (2012). *Spray* drying of dairy bacteria: New opportunities to improve the viability of bacteria powders. *International Dairy Journal*, *31*(1), 12–17. https://doi.org/10.1016/j.idairyj.2012.01.006

Silva, P. T. da, Fries, L. L. M., Menezes, C. R. de, Holkem, A. T., Schwan, C. L., Wigmann, É. F., Bastos, J. de O., & Silva, C. de B. da. (2014). Microencapsulation: concepts, mechanisms, methods and some applications in food technology. *Ciência Rural*, *44*(7), 1304–1311. https://doi.org/10.1590/0103-8478cr20130971

Vaniski, R., Corti, D., & Drunkler, D. A. (2017). Técnicas e Materiais Empregados na Microencapsulação de Probióticos. *Brazilian Journal of Food Research*, *8*(1), 156. https://doi.org/10.3895/rebrapa.v8n1.3651

3. PAPER 2

MICROENCAPSULATION OF LACTIC ACID BACTERIA BY DRYING TECHNIQUES: THE PROTECTIVE ACTION OF THE ENCAPSULANT AGENT

Carolina Carvalho Ramos VIANA[a], Evandro MARTINS[a], Lucas Sales QUEIROZ[a], Isis Rodrigues Toledo RENHE[a], Rodrigo Stephani[a], Antônio Fernandes de CARVALHO[a], Ítalo Tuler PERRONE[a]

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Author affiliations

[a]INOVALEITE - Department of Food Technology, Universidade Federal de Viçosa (UFV), Avenida Peter Henry Rolfs, s/n - Campus Universitário, Viçosa - MG, 36570-900, Brazil.

ABSTRACT

Due to the increasing use of dehydrated ferments in the food industry, mainly containing lactic acid bacteria (LAB), we seek to optimize the production of these cultures. To achieve this objective, it is necessary to understand the negative effects of drying techniques related to cell culture. The present review lists the main data in the literature on LAB dehydration by lyophilization (freeze-drying) and spray drying, as well as on microencapsulation, since it is used as an important technique to prevent loss of viability during these processes. In addition, it is presented the main theories describing the potential of encapsulating agents as protective to cells. Analytical methods such as Isothermal Thermodynamic Calorimetry (ITC), Fourier Transform Infrared Spectroscopy (FTIR) and Raman Spectroscopy may be adequate, together, to elucidate the mentioned hypotheses.

Keywords: dehydrated ferment; lyophilization; spray drying; analytical methods

1. Introduction

Lactic acid bacteria (LAB) constitute a group of microorganisms of great industrial interest since they affect functional, technological, sensory and nutritional properties of foods, as illustrated in Figure 1 (Fusieger et al., 2019; Olvera-garcía et al., 2015; Stefanello et al., 2018). Although promising, the use of LAB by the food industry, whether as fermentative cultures or as probiotics, still has some challenges: the cultures need to be viable, in high population density and metabolically active before and after food processing (Corona-Hernandez et al., 2013). In the specific case of probiotics, the challenge is even greater since the microorganisms should be viable in the food during the entire shelf life of the product, endure the adverse conditions of the gastrointestinal tract and deliver health benefits to the consumer (Burgain et al., 2014; Gao et al., 2021; Gul, 2017).

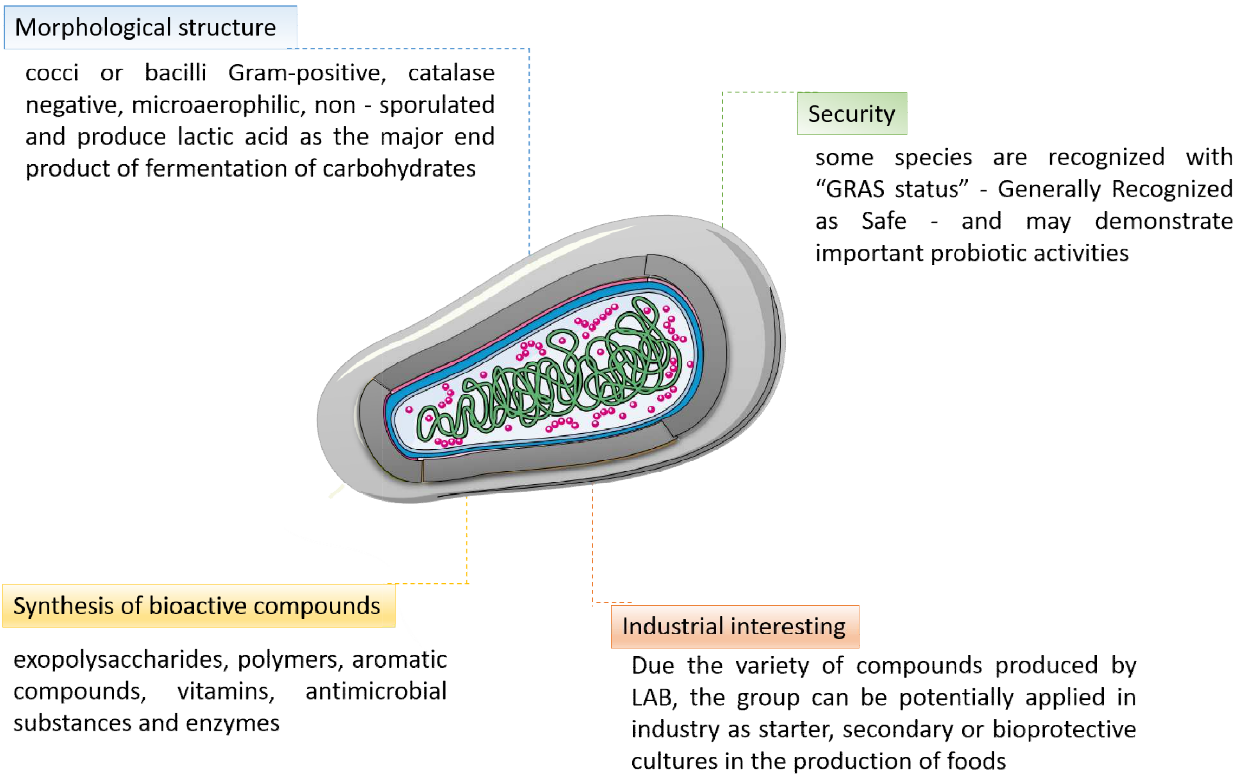

Figure 1 Characteristics and properties of lactic acid bacteria (LAB) and their industrial interest.
The various compounds produced by its primary or secondary metabolism allow application in foods, in order to affect nutritional, sensory and functional aspects.

As a strategy to protect the LAB against injury factors from the entire chain of food processing and consumption, several works have pointed to cell microencapsulation as the most successful alternative (Yao et al., 2020; Chen et al., 2015; Kavitake et al., 2018; Martín et al., 2015; Riaz & Masud, 2013). Microencapsulation consists of immobilization of the viable cells inside inert structures, whose size varies between 1 μm to 1000 μm, also known as microcapsules (Heidebach et al., 2012; Kavitake et al., 2018). The micrometric structures are produced by different encapsulation techniques especially when applying polysaccharides, proteins, lipids and waxes as the encapsulant material (Table 1).

Among the encapsulation techniques, those that utilize dehydration as their basis, such as the lyophilization and spray drying, stand out for providing cultures in powder form, which occupy smaller volumes, are easy to handle and more dispersible in the production vat (Chen et al., 2019; Martins et al., 2019; Mensink et al., 2017; Sarao & Arora, 2017). In both techniques, the operational parameters and any others are key factors to retain the LAB viability after dehydration (Figure 2).However, the cell survival is also linked to another essential factor: the encapsulating agent (Dianawati et al., 2015; Huang et al., 2017).

Several studies reported that substances such as carbohydrates and proteins improve the survival of lyophilized or spray-dried LAB (Table 1) and, in most of them, the best encapsulating agent, isolated or in combination, is determined arbitrarily due to not knowing the correct cellular protection mechanism. Despite everything that is known up to this moment about drying of LAB, the ideal encapsulating agent is still far from being identified.

In spite of not being well established, this review aims to describe and compile the main results, hypotheses and theories that could explain the exact mechanism of interaction between the encapsulating agent and the microbial cells in lyophilization and the spray drying process. In addition, it presents an overview of the most promising investigative techniques that can shed light on cell-encapsulating agent interaction.

Table 1 Examples of recently mentioned encapsulating agents for LAB encapsulation by freeze drying (FD) and spray drying (SD)

Morphology of capsules	Encapsuled Microorganism	Encapsulating agent	Size capsule	Encapsulation method	Survival rates	Reference
capsules with irregular shapes and surfaces, with the presence of bacteria on the surface in the medium with 5% total solid	*Lacticaseibacillus casei* BL23 *Propionibacterium freudenreichii* ITG P20	Different total solid content of sweet whey, with or without casein peptone supplementation	~10 µm	Spray drying	45% to 80%	Huang *et al.* (2016)
spherical microcapsules with no surface fissures or visible cracks, containing typical concavities of atomized powders	*Lacticaseibacillus casei Shirota*	different combinations of maltodextrin, reconstitute skim milk, and gum arabic	-	Spray drying	82.93 to 95.44%	Gul (2017)
-	*Lactiplantibacillus plantarum*	skim milk coated inulin-sodium alginate	1.5 ± 0.1mm	Freeze drying	~95%	Wang *et al.* (2016)
flake-like in shape with many vents present on the surface	*Lactobacillus kefiranofaciens* M1	Na-alginate, gellan gum and skim milk powder	20 µm to 50 µm	Freeze drying	92.4%	Wang *et al.* (2015)

did not have specific shape; irregularities such as dents/concavities (FD), and wrinkles (SD) on the surface	*Lacticaseibacillus rhamnosus*ATCC 7469	Whey protein isolate, whey protein isolate + inulin, and whey protein isolate + inulin + Persian gum	341nm to 364 nm (FD) 295.7nm to 353 nm (SD)	Freeze drying; spray drying	~60%	Moayyedi *et al.* (2018)
spherical shape and continuous walls, without cracking or fissures in the particle	*Lactococcus lactis* subsp. L*actis* R7	Inulin and whey	12.7 μm to 18.7 μm	Spray drying	94,61%	Rosolen *et al.* (2019)
Round shape, smooth surface and concavities	*Lactobacillus acidophilus*	Gum Arabic and Β-cyclodextrin	~24.06 μm	Spray drying	82-95%	Arslan-Tontul*et al.* (2017)
FOS: resembled gummy, cake like mass, with structural collapse; FOS+WPI: flake like structure with smooth surface; FOS+DWPI: porous, spongy flakes	*Lactiplantibacillus plantarum*MTCC 5422	Fructo-oligosaccharide (FOS) and FOS+whey proteins isolate (WPI) and FOS+denatured whey protein isolate (DWPI)	63.72 μm to 89.71 μm	Spray drying	~98%	Rajam et al., (2015)
Gum Arabic (GA) and maltodextrin (MD): an oval, irregular and	*Lactobacillus acidophilus*	Gum Arabic; maltodextrin; whey protein concentrate	5.65 μm to 11.15 μm	Spray drying	71.9% to 93.3%	Colín Cruz *et al.* (2019)

compact microstructure, large cavities and a coarse porous network; whey protein concentrate (WPC): more granular powder particles, with spherical shape						
small-sized particles	*Limosilactobacillus reuteri*	slurry fermentation with whey	~5 µm	Spray drying	~97%	Jantzen *et al.*, (2013)
spherical with slight surface roughness	*Lacticaseibacillus casei*	Maltodextrin	~15 µm	Spray drying	-	Santos et al., (2014)

2. Principle of dehydration by lyophilization and spray drying

Lyophilization is a drying method characterized by first freezing the material to be dried, and then subjecting the material to a pressure reduction which initiates water sublimation (Kavitake et al., 2018; Yudiastuti et al., 2019). In laboratory scale set-ups, the material to be dehydrated is normally placed in ampoules type flakes which are subjected to low temperatures (-40°C to -80°C) at normal pressure to freeze the water (Gaidhani et al., 2015). In the case of LAB cultures, the material to be dehydrated is composed of a concentrated cell suspension dispersed in a matrix which will recover the cells after water removal (Martín et al., 2015; Mensink et al., 2017; Stefanello et al., 2018). Next, the pressure of the system is reduced between 5 Pa to 20 Pa, depending on the material´s properties, for approximately 24 hours to promote water removal by sublimation (Bjelošević et al., 2020; Gaidhani et al., 2015; Nowak & Jakubczyk, 2020). The freezing process can last up to 72 hours depending on the amount of material to be dried and the equipment performance (Yudiastuti et al., 2019).

Lyophilization is considered the reference technology for dehydration of microorganism since low temperatures are used throughout the process which significantly reduces the thermal damage inflicted on the cells (Chen et al., 2019; Stefanello et al., 2018). Furthermore, the drying is not conducted by an air flow which also minimizes the possibility of oxidative stresses on the microorganisms (Chavarri et al., 2012). Although to be considered the less stressful drying method for biological systems, some loss of viability is expected due to ice crystal formation and the osmotic stress that can damage cell structures (Broeckx et al., 2016; Liu et al., 2017; Wang et al., 2020).

From a commercial point of view, lyophilization is carried out in long batch processes which reduce the productivity and results in high energy costs (Pretoro & Manenti, 2020; Wang et al., 2020). On the other hand, LAB spray drying has been preferred and extensively studied by different research groups, since this technology can be utilized in a continuous operation and can use up to 10 times less heat energy than lyophilization (Schuck et al., 2012).

The spray drying technique consists basically of atomization of the material to be dried to form small droplets (10 μm to 150 μm) which are dispersed into a hot and dry air flow (Dolinsky, 2001; Huang et al., 2017; Thybo et al., 2008). Due to the small size of the droplets and the high difference between the temperature and water content of the droplet and air, the water is removed by evaporation almost instantly without overheating of the material (around 45°C) (Haque & Adhikari, 2015; Perrone et al., 2016; Moreira et al., 2021). This makes it possible to produce dried LAB cultures even using air drying with high temperatures (between 140°C and 200°C) (Schuck et al., 2012).

When compared to lyophilization, this process has some advantages such as: flexibility in controlling drying properties; the possibility of large-scale production and continuous operation, even with simple equipment; the production of relatively uniform and spherical particles; good process efficiency and low cost; better storage stability compared to cultures kept frozen or fresh cultures and the possibility of storage at room temperature (Huang et al., 2017; Maciel et al., 2014; Martins et al., 2017; Schuck et al., 2012).According to Moreira et al., (2021) the spray dryer technique can be potentially used for the production of dehydrated LAB cultures, and has been researched for applications in new products in several areas, including food.

In contrast, if the operational parameters have not been well defined, the microbial cultures can be exposed to high temperatures and oxygen concentrations, which reduce the survival rate of cells (Chen et al., 2019; Dianawati et al., 2015). Several studies have demonstrated that the accumulation of multiple stresses that the microorganisms can undergo during spray drying, can cause a significant reduction in cell viability after drying (Gong et al., 2014).

Perdana et al., (2013) evaluated the kinetic inactivation at different drying temperatures for *Lactiplantibacillus plantarum* WCFS1 and suggested that the loss of viability is strongly correlated with the moisture content. That research demonstrated that, the marked loss of viability can be explained by two phenomena or a combination of these: (i) during drying, water is removed from the hydration layer of the lipid bilayer of cells causing destabilization, especially related to low humidity in the medium. In this case, water is removed not only from outside the cell but intracellular water is depleted as well. and (ii) the removal of water leads to an accumulation of solutes in the medium outside the cell, which generates an increase in the difference in osmotic pressure across the cell membrane. This phenomenon becomes a driving force for water migration through the cell membrane, which can lead to irreversible damage and lost viability (Crowe et al., 1989; Perdana et al., 2013).

However, the hydration not only affects the external structures. Cytoplasmic proteins, ribosomes, enzymes, RNA and DNA, can also be degraded or damaged during water removal (Abee & Wouters, 1999). Considering only the damage caused by heat stress, researchers also assess the occurrence of simultaneous damage to multiple cellular structures including ribosome, nucleus, etc. Experimental evidence is still needed to determine, for example, whether ribosome damage is the critical factor responsible for the thermal death of microorganisms (Fu & Chen, 2011). Santivarangka and Kulozik, et al. (2008) suggested that ribosomes can be considered a critical component if large amounts of these structures are damaged, which compromises protein synthesis. In addition, critical components can also be cell envelopes, DNA and RNA polymerase.

Lee and Kaletunc (2002) used differential scanning calorimetry (DSC) to assess the thermal stability and reversibility of various cellular components of *Escherichia coli* (gram-negative) and *Lactiplantibacillus plantarum* (gram-positive) after heat application. Their results indicated changes in ribosomes, cell wall components and DNA of both microorganisms. It was also reported a higher heat inactivation temperature for *Escherichia coli* than for *Lactiplantibacillus plantarum*, depending on growing conditions as well as cell structure.

Membrane-phase transitions were detected by Kilimann et al. (2006). The heat inactivation of *Lactococcus lactis* was studied by determination of cell counts, and by FT-IR spectroscopy recording the average structure of cell proteins. FT-IR spectra results showed information about structural changes in the membrane and in proteins induced by increasing the temperature.

On the other hand, high survival rates (~100 %) are reported by other studies that applied microbiological and technological strategies to reach this goal (Schuck et al., 2012). These studies have been the starting point to scale up the production of spray dried LAB.

3. LAB microencapsulation by lyophilization and spray drying

The microencapsulation process involves entrapment of the active substance in another material, promoting capsules, immobilization or compartmentalization, of size between 1 µm to 1000 µm in diameter (Heidebach et al., 2012; Kavitake et al., 2018). The encapsulated material is generally called the core or internal phase, while the material used for encapsulation is called either the encapsulating agent, coating membrane, external phase or matrix (Burgain et al., 2014b; Heidebach et al., 2012; Martins et al., 2017; Sarao & Arora, 2017).

For decades methods of dehydration have been used in the production of bacterial cultures, but its combination with microencapsulation is relatively recent (Heidebach et al., 2012). Encapsulating agents, together with the control of processing parameters, can be crucial to prevent cell damage and protect microorganisms during drying, either by lyophilization or spray drying (Chen et al., 2015; Martín et al., 2015; Sohail et al., 2013).

In lyophilization or spray drying process (Figure 2), the material containing living bacteria is dried and, as the water is removed, the solids components are deposited on the surface of cells. When drying is complete, capsules are obtained in powder form in which the cells are in the interior or surface of capsules (Sarao & Arora, 2017). In the specific case of spray drying technology, the material is first converted in droplets before drying (Boel et al., 2020; Kavitake et al., 2018). Thus, the capsules produced can be less aggregated and more spherical than the capsules produced by lyophilization. In addition, due to the air expansion during drying, the capsules can also show occluded air structures inside (Behboudi-Jobbehdar et al., 2013).

Moayyedi et al. (2018) studied the mechanism and characterization of coating formation by different wall materials used in microencapsulation. The capsules produced did not have a specific shape. In the encapsulation by lyophilization, irregularities in the surfaces, such as dents/concavities, were noticed, unlike the capsules produced by spraying, which showed wrinkles. This can be attributed to low drying temperature, as well as the mixed effect of atomization, respectively.

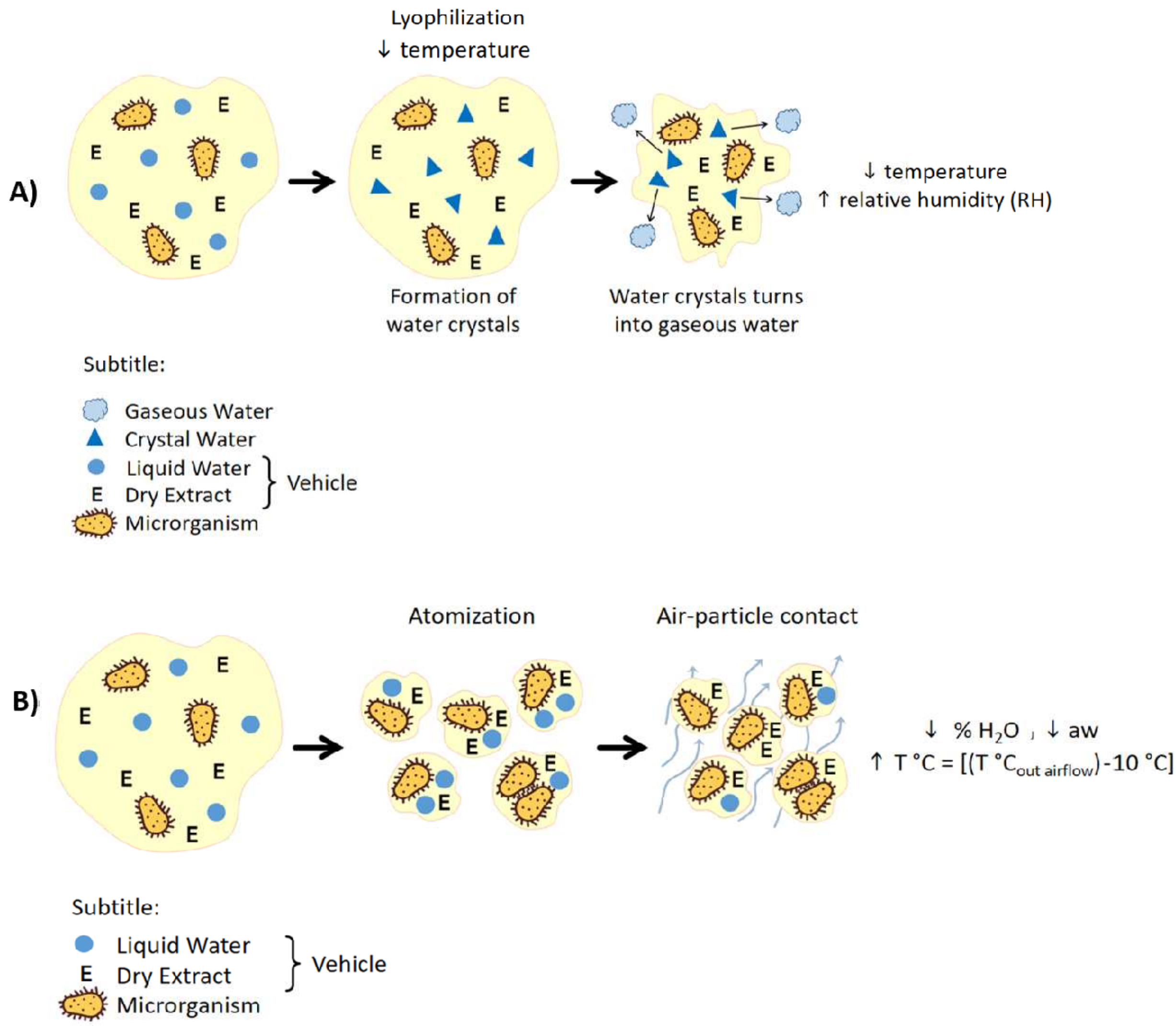

Figure 2 Schematic representation of microencapsulation.
A) Microencapsulation by lyophilization: with the lowering of the temperature, the water present in the cell-encapsulating agent mixture is frozen and then, by pressure reduction, it is sublimated, leaving only solid and dry components;
B) Microencapsulation by spray drying: the mixer of microorganisms and the encapsulating agents it is atomized inside the dryer, the liquid from the solution of the encapsulating agent evaporates with trapping of the active material

If one considers that LAB is the core material, the capsules produced by lyophilization and spray drying can be classified as multinuclear where the cores (or cells) are distributed on the interior and surface of the capsules (Dianawati et al., 2015) (Figure 3). In term of shape, the capsules can be classified as spherical or have an irregular or amorphous form (Boel et al., 2020).

The chemical composition of the encapsulating agent affects the final particle morphologies after spray drying, and based on that a general classification can be made (Boel et al., 2020). Three distinct categories have been identified, namely skin-like, crystalline, and agglomerate materials (Walton, 2000; Walton & Mumford, 1999). A particle can be considered skin-like when the solid phase is continuous and is composed of sub-microcrystalline materials, and usually occurs when the encapsulating agent is an organic compound. In crystalline materials, the final particle is presented in the form of macrocrystals delimited by a continuous microcrystalline phase, while the agglomerated material is composed of grains connected by particles of the same material. Both cases can be associated with inorganic compounds. Exceptions to this categorization may occur (Boel et al., 2020; Walton & Mumford, 1999).

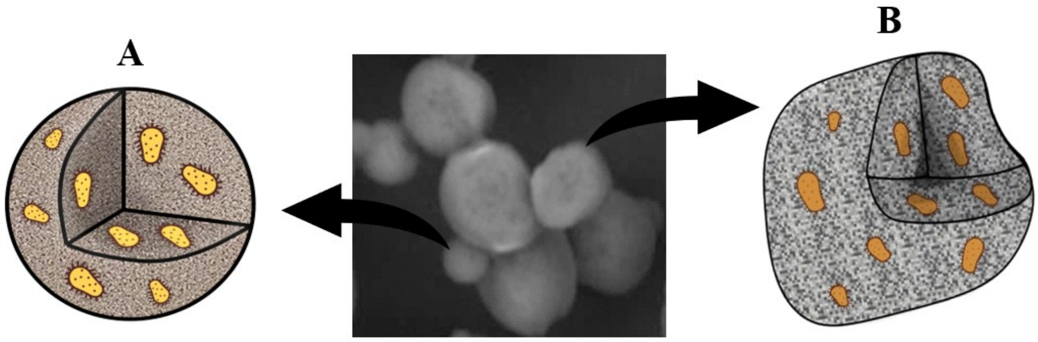

Figure 3 Schematic representation of microcapsule structures produced by spray drying: A) spherical form; B) Irregular form.

Choosing the optimal encapsulating agent for a specific application takes into consideration a series of factors that include: price, toxicity, solubility, viscosity in solution, glass transition temperature, covering property, among others (Rodrigues et al., 2020; Tee et al., 2014). In term of the core, the success of dehydration is linked to features of the LAB strain itself such as thermal, osmotic and oxidative resistance, growth stage, physiological state and type of interaction with the cell- encapsulating agent (Bekhit et al., 2016).

Numerous materials have already been studied and show potential for use in the encapsulation of microorganisms (Table 1). The efficacy of these materials must be accompanied by a conformance to requirements of GRAS status (generally recognized as safe) (Abd El-Salam & El-Shibiny, 2015; Silva et al., 2014). In addition, the capsules must be large enough to protect the encapsulated core material but, they must be small enough to avoid negative impacts on the sensory attributes (Würth et al., 2017).

As shown in the literature, there are a variety of candidates for encapsulating-agents for LAB, however, there is no consensus among the authors about which is the best. This can be explained by the fact that LAB strains from different species behaves differently when dehydrated and each drying process is peculiar to each microorganism (De Prisco & Mauriello, 2016). Furthermore, the final application of the capsules differs between the studies and each research follows a specific protocol for preparing and drying the cells (Huang et al., 2017). Thus, determining the best encapsulating agent is not an easy task, but understanding how these materials interact with the cells is the starting point to choosing the most appropriate one.

4. Mechanism of cell-encapsulating agent interactions

To understand the protective action of the encapsulating agent, it is necessary to have a global view of the most important cell structures that can interact with them.

LAB are Gram-positive and are externally recovered by a heterogeneous and macromolecular glycopolymeric wall (Figure 4) (Filloux & Whitfield, 2016). It consists mainly of peptidoglycan, formed by a network of carbohydrates, peptides, protein and teichoic and lipoteichoic acids (Vollmer & Seligman, 2010). This results in a hydrophobic structure due to the existence of anionic phosphate groups in the repetitive units of glycerol phosphate of teichoic acids (Oh et al., 2018).

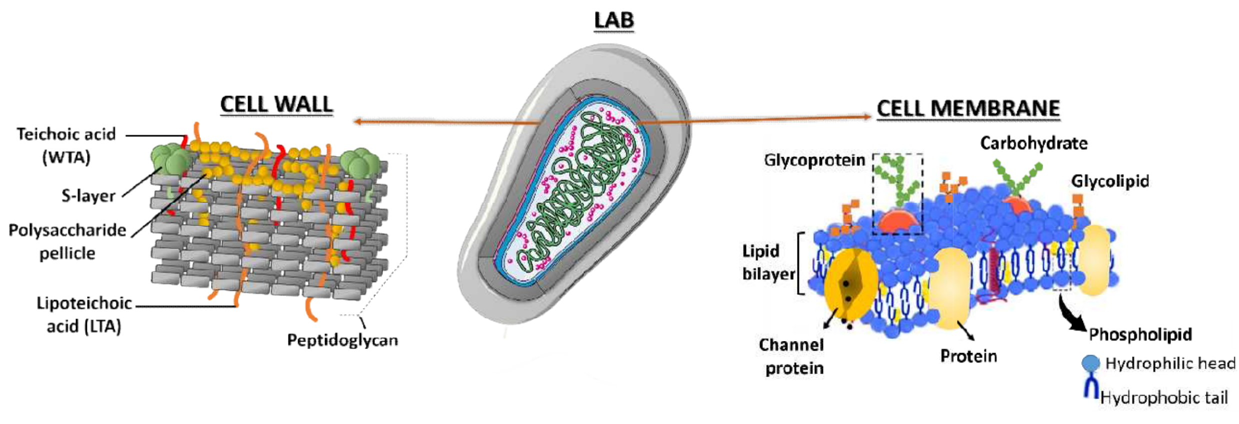

Figure 4 Schematic representation of basic compounds of the cell wall and cell membrane of a LAB.
Cell wall: the peptidoglycan is the main component of the wall cell LAB; polysaccharides, proteins (S-layer) and wall teichoic acid (WTAs) are covalently linked to the peptidoglycan; lipoteichoic acid (LTAs) anchored in the cytoplasmic membrane. Cell membrane: a lipid bilayer, where the phospholipid-protein ratio can reach 70:20, with a thickness of around 5nm to 10nm. It is also composed of carbohydrates, both on the intracellular and extracellular sides.

Source: Adapted from Delcour (1999), Alimohamadi and Rangamani (2018) and Matinez et al. (2020)

Right below the wall, there is a cytoplasmic membrane, illustrated in Figure 4, formed by lipid bilayer of phospholipids with amphipathic character (Watson, 2015). The phospholipids that compose the bilayer have the negative charged phosphate groups oriented outward which cause the membranes to assume a negative liquid charge (Mallikarjunaiah et al., 2019). As well as the wall, the cytoplasmic membrane is embedded with multiple proteins and glycoproteins (Li et al., 2016; Mallikarjunaiah et al., 2019).

Although the composition of the wall and the cytoplasmic membranes has been quite well defined, it should be kept in mind that these structures as well as their components can change in function with environmental conditions (Carvalho et al., 2004).

In its physiological state, the lipid bilayer of the membrane is in the fluid phase and its components are distributed and organized according to their hydrophilicity, interacting or not with water molecules (Liu et al., 2017). With dehydration, this bilayer can undergo a transition to the gel phase, and with that, hydrophobic and hydrophilic groups near each other resulting in leakage of intracellular components, and consequently cell death (Broeckx et al., 2016; Wolfe, 1987). Using flow cytometry, bacterial membranes were identified as the main site of injury during spray drying (Ananta et al., 2005).

Considering that the wall and the membrane are the outermost structures of the cell, it is expected that most of the encapsulating agent interactions occur preferentially on these structures during the drying, in order to protect the cell from the negative effects of drying and avoiding the loss of viability (Crowe et al., 1987). This hypothesis is supported by many authors, like Leslie et al. (1995), Fu and Chen (2011), Dianawati et al. (2013a).

Ananta et al. (2005) described in their studies that disaccharides were found to be effective in protecting bacterial membranes and cell proteins during drying, and suggested that lactose may play an important protective role through direct interaction with sensitive biomolecules.

Bustos and Bórquez (2013) demonstrated that the use of protective materials is absolutely needed, principally for stabilization of cell membranes and the outer coatings of the encapsulant, especially when this is made up of proteins and/or sugars. In their research, samples that were not protected, have their viability diminished by more than 98%, but with the use of protective materials loss of viability between 44% to 83%, using whey or a mixture of whey, maltodextrin and pectin, respectively.

Studies showed that other cell structures can also interact in the microencapsulation and protection process of the microbial cell (Fu & Chen, 2011). For example, research has shown that trehalose can act as an effective thermoprotector against protein denaturation. It was demonstrated by whole cell DSC that trehalose was able to stabilize cytoplasmic structures such as nucleus, mitochondria and ribosomes under thermal stresses (Obuchi et al., 2000).

Thus, in addition to creating a protective shell over the cell during drying, the optimal encapsulating agent should interact with cellular components to minimize the adverse structural modifications caused by dehydration (Liu et al., 2017).

Several theories are suggested to explain the mechanisms of cellular protection during drying and these are directly related to the chemical nature of the encapsulating material (Figure 5).

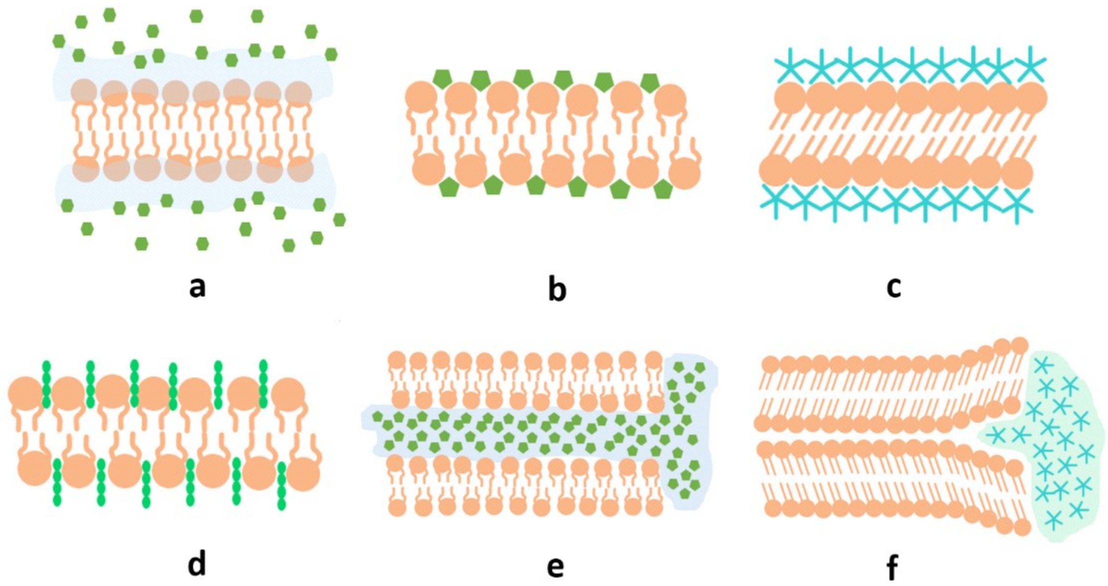

Figure 5 Membrane representation of suggested theories to explain the mechanisms of cellular protection during drying:
5a) Representation of the preferential exclusion of sugars (green pentagons) of a cell membrane encapsulated by agents in the form of compatible solutes; 5b, 5c, 5d) Representation of water substitution hypothesis, with different carbohydrates as encapsulation agent: this interaction is facilitated by sugar from smaller chains (Fig 5b); polymeric sugars with a rigid structure may not enter between groups (Fig 5c); flexible sugars

(Fig 5d) may fit into the spaces; 5e, 5f,) Representation of high relative molecular mass protectors: sugar molecules can concentrate in the space between membranes and provide protection as a "physical barrier" to the effects of dehydration (5e); sugars with a more rigid structure, may not have this effect (5f).
Source: Adapted from Santivarangkna; Higl; Foerst (2008)

In the next section, the main results and hypotheses found in the literature will be discussed in detail.

4.1 Encapsulating agents in the form of compatible solutes

Compatible solutes are small organic molecules such as sugars, such as trehalose and sucrose (Broeckx et al., 2016), and amino acid, or even electrolytes, such as Na^+ and K^+ which have high solubility and the ability to accumulate in the cytoplasm of cells (Carvalho et al., 2004). NaCl, and different concentrations of undefined components that are sources of compatible solutes (e. g. peptones, tryptone, and meat and yeast extracts) have been used with high frequency in the drying process of LAB (Table 1). The compatible solutes are substances whose specific transport systems available in the cytoplasmic membrane allow their passage and accumulation inside the cell (Brown, 1976).

The most accepted hypotheses that explains the molecular interaction between compatible solutes and the cell components are based on the concept that was introduced by Santivarangkna, Higl and Foerst (2008). According to them, the solute takes up more space and has a worse geometric adjustment than water in the membrane. For this reason, the compatible solutes did not interact directly with the membrane but they form a physical barrier on the solvation layer that cover it (Figure 5a) Thus, during drying, the movement of the water molecules is limited by this barrier which reduces the structural damage resulting from the sudden loss of water (Santivarangkna, Higl, et al., 2008; Yancey, 2001).

This hypothesis is corroborated by studies of Kilimann et al. (2006) that demonstrated that high intracellular sucrose concentrations prevent the denaturation or misfolding of proteins and increase the heat resistance of *Lactococcus lactis*. These results reaffirm the role of compatible solutes in bacterial freezing, drying, and heat resistance.

This mechanism has been also studied by Carvalho et al. (2003) who found that the addition of both sucrose and NaCl or of NaCl alone to the MRS growth medium significantly improved the viability of *Lactobacillus delbrueckii ssp.*

Bulgaricus dried cells during storage. In contrast, no improvement was found when cells were grown in sucrose alone.

Carvalho et. al. (2004) and Broeckx et. al. (2016) suggested that in addition to protecting cells from the effects of lyophilization and spray drying, the compatible solutes do not alter enzymatic activity and may even protect enzymes from denaturation during such processes.

In the case of macromolecules such as proteins, enzymes and nucleic acids, the protecting mechanism involves the phenomenon of preferential exclusion (Fig 5a). Carpenter et al. (1991) suggested that a wide variety of compounds, including sugar, polyols and certain salts are effective at minimizing protein denaturation during drying. The accumulation of high concentrations of solutes can provide protection to cells and their biomolecules, as they create a thermodynamically unfavorable situation. Solutes tend to induce polymerization, since the formation of contact sites between the constituent monomers will reduce the surface area of the protein exposed to the solvent.

It should be noted that the intracellular accumulation of compatible solutes and their protective effects are probably different in each LAB strain, and depend on the dedicated transport systems of the solutes and permeability of salts and sugars (Le Marrec et al., 2007).

4.2 Carbohydrates as encapsulating agents

The most remarkable carbohydrates used in LAB encapsulation are classified as monosaccharides (fructose), disaccharides (lactose, trehalose), oligosaccharides (fructooligosaccharides (FOS) and galactooligosaccharides (GOS)) and polysaccharides (inulin, maltodextrin) (Table 1).The polyalcohol-aldehyde or polyalcohol-ketone molecules are carbohydrates whose composition contains carbon, hydrogen and oxygen, and may contain nitrogen, phosphorus and sulfur (Fennema, 2008).

These substances are widely used for microencapsulation when they exhibit high solubility, low viscosity in high concentration, low cost, high glass transition temperature and quick drying property (Abd El-Salam & El-Shibiny, 2015).

Studies of Leslie et al. (1995) indicated that cells dried with a sugar solution had higher survival rates than cells dried in water alone. These studies supported the theory that sucrose can protect liposomes, isolated biological membranes, and some intact cells from the adverse effects of freezing and drying.

Carvalho et al. (2003) suggested that addition of sucrose to the drying medium significantly increases survival of *Lactobacillus bulgaricus* during storage as good survival was attained under all drying conditions tested.

Perdana et al. (2014) investigated the influence of formulation parameters on the survival of *Lactiplantibacillus plantarum* WCFS1 after drying. According to them the highest survival rates, and therefore the best protection against cellular damage in spray drying, were obtained by using low relative molecular mass carbohydrates (less than 2 kDa) with high glass transition temperatures, at low drying temperatures (25 °C) and high (50 °C or higher). However, some high relative molecular mass carbohydrates with high glass transition temperatures, such as sorbitol and mannitol, also offered acceptable rates (above 80%) under the conditions tested.

Lin et al. (2019) evaluated the physicochemical and physiological properties of microcapsules with different compositions of maltodextrin (MD) and isomaltooligosaccharide (IMOS), and their potential application in the encapsulation of *Bifidobacterium adolescentis* and *Lactobacillus acidophilus*. The results suggested that the composition of 95% MD + 5% IMOS had the highest microcapsule survival rate, and therefore, it was considered efficient for microencapsulation of the probiotics they evaluated.

Several hypotheses are found in the literature to explain the protective effect of carbohydrates in bacterial cells, including: (4.2.1) the water substitution/ replacement hypothesis and (4.2.2) the vitrification theory (Ananta et al., 2005; Broeckx et al., 2016; Rokka & Rantamäki, 2010).

4.2.1 Water substitution/ replacement hypothesis

The theory of water substitution/replacement contributes to cell stabilization during dehydration from a thermodynamic point of view has been corroborated by Mensink et al., (2017). This theory was first proposed by Timasheff (1982), and it suggested that carbohydrates act as water substitutes around proteins and polar residues of membrane phospholipids during drying (Carpenter et al., 1991; Crowe et al., 1987)(Figure 5b, 5c, 5d).

To support this theory, Crowe et al. (1987), showed that certain sugars, like trehalose and maltose, are capable of preventing damage from dehydration not only by inhibiting fusion between adjacent vesicles during drying, but also by maintaining the lipids in a fluid phase in the absence of water.

Carpenter and Crowe (1989), based on the experiments with Fourier-transform infrared spectroscopy, characterized the interaction of stabilizing carbohydrates with dried proteins. This research demonstrated that the hydrogen bonding of carbohydrate to the protein's polar groups, appears to be responsible for preventing dehydration-induced inactivation.

The nature of this interaction was reported by Carpenter et al. (1991). These researchers support the theory that the increase in cell survival can be attributed to protection of protein functionality, owing to formation of a glassy matrix during freeze-drying that possesses high viscosity and low mobility, and/or to solute binding to the dried protein, thus serving as a water substitute when the hydration shell of the proteins is removed).

Based on the studies, it is believed that the water substitution mechanism is based on the formation of hydrogen bonds between the hydroxyl groups of a stabilizing sugar and the polar groups present in cell membrane proteins (Ananta et al., 2005; Bjelošević et al., 2020; Mensink et al., 2017).

It is generally accepted that low relative molecular mass sugars (less than 2 kDa) such as trehalose and lactose may provide stabilization by closely interacting with the lipid bilayer of the cell membranes (Perdana et al., 2014) and depressing the cell membrane phase transition during drying (Broeckx et al., 2016; Santivarangkna, Higl, et al., 2008). Thus, as water is removed, sugars can interact with phospholipids by hydrogen bonding between their hydroxyl groups and phosphate groups on the bilayer surface. To provide such protection, specific interactions between phospholipids and sugars must occur properly (Liu et al., 2017).

There is a strong likelihood that sugars, such as sucrose, lactose and maltose, are capable of forming hydrogen bonds with the protein as water is removed and prevent denaturation (Dianawati et al., 2015; Liu et al., 2017; Suryabhan et al., 2019). This interaction of hydrogen has been demonstrated by Fourier Transform Infrared Spectroscopy (FTIR) (Dianawati et al., 2015; Santivarangkna et al., 2010).

Smaller and molecularly more flexible oligosaccharides are better able to stabilize after drying, as compared to larger and molecularly more rigid structures as demonstrated by studies of Mensink et al. (2015). According to these authors, this can be attributed to the greater ability to form hydrogen bonds with proteins, that are less sterically hindered in interacting with proteins (Mensink et al., 2017). In this way, they reduce destabilizing the membrane, avoiding the negative effects of dehydration (Broeckx et al., 2016; Mensink et al., 2015; Santivarangkna, Higl, et al., 2008).

On the other hand, some authors argue that sugars can depress the membrane phase transition without interacting with the polar groups of the lipid bilayers, but due to osmotic and bulky effects and by vitrification between membranes (Mensink et al., 2017; Santivarangkna, Kulozik, et al., 2008; Zhao et al., 2017).

This hypothesis is corroborated by studies of Wang et al. (2015) that demonstrated that a mixture of sodium alginate, gellan gum and skim milk powder was successfully used to encapsulated *Lactobacillus kefiranofaciens*. According to them, skim milk powder, a non-permeable cryoprotectant, adsorbs on the cell surface forming a viscous layer. It may cause a partial efflux of water from the cell, thus inhibiting the growth of ice crystals and maintain ice in an amorphous form close to the cell (Wang et al., 2015).

Polymeric sugars with high relative molecular mass such as mannitol or sorbitol are not able to depress the phase transition because they are excluded from the inter membrane space during water withdrawal. High relative molecular mass carbohydrates can provide a certain degree of protection by fixing cells in a vitreous matrix, which hinders the mobility of the lipid bilayer (Perdana et al., 2014) (Figure 5e, 5f). In this case, the vitrification hypothesis seems to be more appropriate and it is shared by different studies (Dianawati et al., 2013; Santivarangkna, Higl, et al., 2008; Santivarangkna, Kulozik, et al., 2008).

4.2.2 Hypothesis of vitrification

This hypothesis is based on the concept of immobilization in a rigid matrix of amorphous sugar, which drastically reduces the possibility of modifying the freeze frame, and therefore guards against changes in its structure (Bjelošević et al., 2020; Mensink et al., 2017). High relative molecular mass protectors such as polysaccharides have a more rigid structure, so they may not interact easily with the cell wall or membrane (Perdana et al., 2014). However, they can prevent harmful consequences of dehydration by adsorbing on the surface of microorganisms, forming a viscous and vitrified layer (Broeckx et al., 2016; Liu et al., 2017; Stefanello et al., 2018; Vaessen et al., 2019).

This concept was established by Wang et al. (2016) in a study which the results indicated that skim milk coated inulin-sodium alginate encapsulation beads improved the survival of *Lactiplantibacillus plantarum* during freeze-drying. According to this study, it was assumed that the inulin with a low degree of polymerization penetrated between the cell wall and the membrane and acted as a buffer layer against ice formation when the bead encapsulated cells were frozen. The inulin with a high degree of polymerization, which adsorbed on the surface of cells to form a viscous layer, further reduced the rate of ice growth by increasing the solution viscosity.

Perdana et al. (2013) studied the inactivation of *Lactiplantibacillus plantarum* WCFS1 due to dehydration, comparing single drop drying, lyophilization and spray drying, on a laboratory scale, using different carbohydrates as encapsulating agents. The main result points out that rapid spray drying can induce instantaneous and stable fixation of cell suspensions in a vitrification matrix and, thus, avoid inactivation by dehydration.

Perdana et al. (2014) demonstrated that encapsulating agents with higher glass transition temperatures (for example, with mannitol or sorbitol) offered high viability rates for *Lactiplantibacillus plantarum* WCFS1 (above 80%). This can be related to the occurrence of the phenomenon of vitrification at high outlet temperatures in spray drying. The crystallization of solid carriers during drying was considered beneficial for cell survival, and the rates are likely to be dependent on the shape of the crystal.

Polysaccharides, in general, are characterized by a glass transition temperature (Tg), above which there is kinetic immobilization (Mensink et al., 2017). In the glassy state, cell processes controlled by diffusion and mobility become slower, due to the high viscosity (above 1014 Pa.s) generated by the concentration of sugars (Aschenbrenner et al., 2014; Broeckx et al., 2016) By incorporating cells in this matrix, there is a gain in chemical and physical stability, which helps to maintain cell integrity (Liu et al., 2017).

Therefore, for good stabilization of the cell to undergo dehydration, it is desirable that the carbohydrate used as an encapsulating agent, forms the maximum possible interactions with cellular compounds, in order to reduce local mobility (Mensink et al., 2015). In addition, it must have a high glass transition temperature to maintain the glazing under the prescribed conditions. Larger oligosaccharides (i.e.

those with more flexible backbones) or a combination of smaller disaccharides and large polysaccharides can be used to increase the glass transition temperatures of around 10ºC to 20 ºC above storage temperature, and thus resulting in a protective effect (Mensink et al., 2017).

Although different underlying molecular mechanisms explain how carbohydrates as encapsulating agents protect cells from dehydration, they are probably not exclusive of the other theories. According to Broeckx et al. (2016) in low concentrations of sugar, it is likely that interaction with the main groups is predominant (water replacement hypothesis). However, at higher sugar concentrations (above 0.2 M), the theory of preferential exclusion seems to be a more likely explanation.

According to Mensink et al. (2017) the hypotheses of vitrification and makeup water can together result in cell preservation, molecular mobility and the prevention of changes to protein structure, respectively. Therefore, it is possible that the theories and hypotheses described in some way contribute to the maintenance of cell viability.

4.3. Proteins as encapsulating agents

In contrast to commonly used carbohydrates, the use of proteins as an encapsulating agent stands out due to their good emulsifying properties and relatively low viscosity. This facilitates the formation of microcapsules that provide a protective barrier between the microorganism and the surrounding environment (Heidebach et al., 2012; Vaniski et al., 2017). In recent studies, milk and whey proteins have been frequently considered for use as encapsulating agents in the drying of LAB by lyophilization or spray drying (Table 1).

Ferreira et al. (2017) studied the effects of freeze drying and spray drying of the strain *Lactiplantibacillus plantarum* (UFV-Lb26) isolated from Marajó cheese. They used a carrier medium with 20% concentration (wt/wt), of reconstituted skim milk powder (RSM), reconstituted sweet whey powder (RSW), and reconstituted sweet whey permeate powder (RSWP). The results showed that the encapsulating agents were effective in maintaining the viability of spray-dried samples. Furthermore, high viability was reported after 60 days of storage in all *Lactiplantibacillus plantarum* samples, and the cell concentration values found for spray- dried samples are higher than those for freeze-dried samples.

A hypothesis found in the literature about the interactions of proteins used as encapsulating agents for microorganisms is related to the hydrophobic interactions between proteins and cell surface components (Alimohamadi & Rangamani, 2018). Considering that cell wall has hydrophobic properties, it is likely that most proteins interact with this structure (Haque & Adhikari, 2015). This kind of interaction was demonstrated by Burgain et al. (2014). Their studies demonstrated that in the microencapsulation of *Lacticaseibacillus rhamnosus* with whey proteins, due to their surface properties, probiotic bacteria tend to form aggregates through hydrophobic interactions. The high adhesion that exists between pili and whey proteins explains the better retention of probiotic bacteria tested in the protein matrix during microencapsulation.

Khem et al. (2016) verified that, in drying *Lactiplantibacillus plantarum* by spray dryer, using whey protein as an encapsulating agent, the encapsulating proteins unfolded, thus exposing their hydrophobic portions to interact with the cell surface, which presented this same characteristic. Thus, the inactivation of bacteria cells by drying was prevented.

Studies carried out by Duongthingoc et al. (2013) investigated the effect of whey protein agglomeration on the survival of a spray-dried probiotic yeast strain *Saccharomyces boulardii* and established a relationship between the physicochemical characteristics of the polymer matrix and its effect on probiotic resistance after spray drying. It was reported that during the spray drying process, clumps of whey protein formed, which induced the formation of a scab, like a cohesive network, which encapsulated the yeast cells. Before spray drying the cell count was about log 8.5 cfu/ml, and after drying, the best treatment achieved a cell count of log 8.07 cfu/ml. Thus, the protective effect of proteins was demonstrated, through the use of whey protein isolate as the wall ingredient, at relatively low pH values.

According to Huang et al. (2017) proteins in skimmed milk can prevent cell damage by stabilizing the constituents of the cell membrane. In addition, they can form a protective coating on the bacterial cell wall when interacting with the calcium in milk. Therefore, milk proteins have been considered good choices for nano and microencapsulation of nutrients and bacteria (Abd El-Salam & El-Shibiny, 2015; Heidebach et al., 2012).

4.4 Lipids as encapsulating agents

Unlike the application of carbohydrates and proteins, the use of lipids for microbial encapsulation has not been well explored, especially if used as a sole encapsulating agent for drying technologies such as lyophilization or spray drying. However, these substances can be combined with carbohydrates and/or proteins in LAB drying processes.

Recently, it was found that lipids with low melting points, used as encapsulating agents have the potential to protect probiotic bacteria from heat damage during spray drying (Huang et al., 2017). In temperatures above their melting point, the encapsulating lipid transforms quickly from solid to liquid, during which it absorbs substantial thermal energy, as reported by Liu et al. (2017).

Low melting point fat (LMF) has also been reported to protect *Lactobacillus* sp. cells from damage when added to sodium caseinate during spray drying. This protection can be attributed to the ability of this encapsulant to absorb part of the thermal energy present during spray drying (Liu et al., 2015).

Therefore, fat-based microcapsules currently seem less suitable for bacterial encapsulation compared to polysaccharide or protein-based microcapsules. However, the addition of lipids to encapsulating agent materials seems to be a viable strategy to reduce thermal injury undergone by the cells.

Studies involving the interaction of lipids with cells structures are not available in the literature. Thus, the thermal protection by phase change is the only mechanism suggested at this time.

5. Methodologies for assessing the efficiency of cell- encapsulation and bacterial viability

It is important to investigate the efficiency of the encapsulation process and several parameters can be evaluated and modified to produce the highest yield of viable bacterial cells (Figure 6). The literature has shown that several methods have been suggested to measure the efficiency of bacterial microencapsulation, however the criteria typically addressed in these methods aremicrobial viability and stability, capsule density and micro geometric properties of the spheres, cell concentration and encapsulation / entrapment yield (Heidebach; Först; Kulozik, 2012; Kavitake et al., 2018).

It is noted that the interaction between cells and encapsulating agents is complex, and for that several hypotheses are considered. A deeper study can be performed when the interactions of the membrane/cell wall of the microorganism of interest with the matrix are analyzed (Chen et al., 2015).

Therefore, it is necessary to use optimized physical-chemical and analytical techniques, which reflect the structural, thermodynamic and viability characteristics of the bacterial cell and the applied matrix (Abraham et al., 2005; Santos et al., 2015).

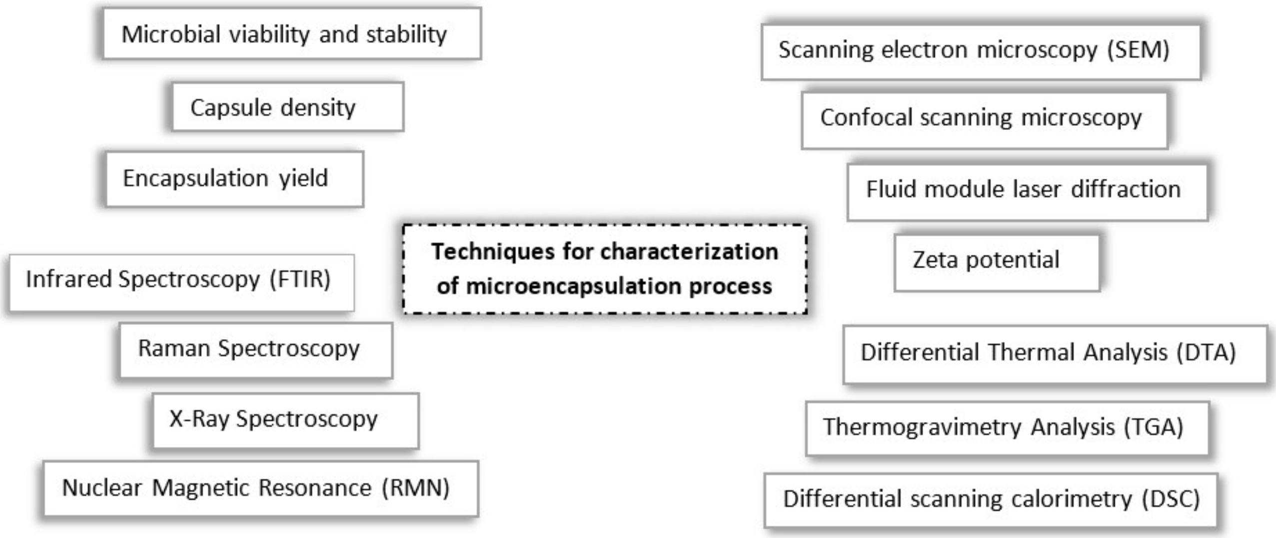

Figure 6 Examples of techniques for characterization and evaluation of microencapsulation of microorganisms.
Several techniques can be used to assess the morphology, size and properties of microcapsules, in addition to the interactions between the encapsulated microorganisms and the encapsulating agent, in a way that complements traditionally methods used to assess encapsulation efficiency.

As illustrated in the figure 6, several techniques have been studied for the characterization of encapsulated and particulate materials, in addition to the methodologies traditionally used in the evaluation of microencapsulation efficiency. As an example, it is possible to cite spectroscopic techniques, such as Nuclear Magnetic Resonance (Aschenbrenner et al., 2011, 2014), X-ray spectroscopy (Wei, Yang, Geng, et al., 2021; Wei, Yang, van der Mei, et al., 2021) Infrared Spectroscopy (FTIR) (Kilimann et al., 2006; Mensink et al., 2015; Santivarangkna et al., 2010) and Raman (Rodrigues Júnior et al., 2016; Vaishanavi & Preetha, 2021), combined with chemometric tools; thermodynamics techniques such as Differential Thermal Analysis (DTA), Differential scanning calorimetry (DSC) and Thermogravimetry Analysis (TGA) (Colín-Cruz et al., 2019; Lopes et al., 2017; Santos et al., 2014; Schebor et al., 2000); morphological characterization and properties of microcapsules, by zeta potentials, optical and scanning electron microscopy (SEM), beside evaluation of particle size by laser diffraction (Arslan-Tontul & Erbas, 2017; Silva et al., 2019; Silva et al., 2018).

Among the main methods that can contribute to a better understanding of cell-encapsulating agent interactions as well as the effectiveness of microencapsulation, in this review, Fluid module laser diffraction, Isothermal Titration Calorimetry (ITC), spectroscopic techniques such as FTIR and Raman spectroscopy (Al- Kaddah et al., 2010; San Keskin et al., 2018; M. Santos et al., 2015). (Al-Kaddah et al., 2010; San Keskin et al., 2018; Santos et al., 2015) were highlighted.

5.1 Fluid module laser diffraction for particle-size distribution analysis

The stability and efficiency of microencapsulation of microorganisms is influenced by, among other factors, the average particle size of the microcapsule (Zhao et al., 2007). The structure and morphology of the particles, as well as the microbial survival rate after drying, are affected by the process parameters as well as by the encapsulating agent used and the viscosity of the mixture to be dehydrated (Würth et al., 2018).

Regarding the structure of the microcapsule, the literature suggests the need for a high volume-to-surface ratio to increase the protective effect, and at the same time, that the microcapsule needs to be small enough to avoid a negative sensory impact (Anal & Singh, 2007).

Microcapsules with larger particles generally offer greater protection in relation to the material contained in its nucleus, when compared to smaller particles. However, in contrast to, their dispersion in the final product maybe impaired. Very thin powders can present hollow interiors and little migration into the particle core, and consequently low encapsulation efficiency (Zhao et al., 2007).

Particle size distribution is determined by total solids and drying conditions used for the production of dehydrated crops (Kim et al., 2009; Walton, 2000). It was observed that there is an increase in the negative effects of drying on microbial viability with the highest content of solids and larger particles and, due to the longer residence time in the spray dryer. In addition, the higher the solids content will cause a proportionally lower water content available for cooling after evaporation. Thus, lower survival rates are achieved due to thermal stress resulting from the combination of these factors (Zhao et al., 2007).

Determination of particle size distribution is already used in several industrial branches, because it has as advantages ease of operation, speed and reading amplitude. It can be done by fluid module laser diffraction method, whose technique is based on the phenomenon of light diffraction combined with mathematical tools to indicate particle diameter. In this method, the particles are dispersed in a moving fluid, and are detected by an incident light and correlated with the size of the microcapsule. Thus, the smaller the particle size, the greater the diffraction angle of a light beam that crosses a population of particles (Campos et al., 2020).

This technique can also be used to understand the culture rehydration process obtained after dehydration, as proposed in the work of Francisquini et al., (2020), which demonstrated that this can be a simple and efficient method to determine the properties of dispersibility and solubility.

Zhao et al. (2007) studied b-cyclodextrin and acacia gum as encapsulating agents of *Lactobacillus acidophilus* by spray dryer, at different concentrations (15, 20, 25 and 30% [w/v]) with a core material concentration of 6% (v/v). The results showed that the ideal concentration of wall material was 20% (p/ v), which generated microcapsules with a relatively uniform size and high survival rate 1.50 x 10^9 c.f.u./ml. In addition, after eight weeks of storage at 4ºC, the number of viable bacteria remained above 10^7 c.f.u./ml, compared to *Lactobacillus acidophilus*not encapsulated, which presented 10^4 - 10^5 c.f.u./ml.

Burgain et al. (2013) studied the microencapsulation of *Lacticaseibacillus rhamnosus GG* within a dense protein matrix in order to evaluate its resistance to gastric conditions. The results showed that the protein nature seems to influence particle size in addition to other factors such as porosity, surface and internal microstructure, since proteins have variable physical properties (density, refraction index), size, load and stability (Laclair & Etzel, 2010). It was also discussed that the presence of denatured whey proteins was beneficial for the production of microparticles with a small dispersion and high elasticity. By modifying these properties, a more or less porous structure can be obtained. And, consequently, a porous structure should allow easier access and release of an encapsulated bioactive, while a dense structure would limit access and release (Burgain et al., 2013).

Wurth et al. (2018) sought to correlate the survival rate of *Lacticaseibacillus paracasei ssp. paracasei* F19 with the particle size of different powders. As a result, survival rates ranged from 64% to 0.2%, according to experimental conditions. There was a high correlation between the survival rate of bacteria and the size of the powder particle, since the drying behavior is influenced by particle volume and residence times. In addition, the drying behavior is also directly influenced by the content of total solids in the material to be dried.

It is important to monitor the particle diameter, not only to have a good protection of the encapsulated microorganisms and achieve maximum shelf stability, but also to have a good distribution of microcapsule in the products (Zhao et al., 2007).

From the literature data, it can be seen that evaluating the particle size of cultures produced by dehydration has already been widely investigated (De Castro-Cislaghi et al., 2012; Gul, 2017; Huang et al., 2016; Silva et al., 2018; Nowak & Jakubczyk, 2020) and is an important tool for characterizing and measuring process efficiency.

5.2 Isothermal Titration Calorimetry (ITC)

The heat involved during the encapsulation of LAB is important for understanding the formation of complex structures and the specific interaction between these macromolecules. These interactions can occur between the main external structure of a bacterial cell and an encapsulating agent (Abraham et al., 2005). Isothermal Titration Calorimetry (ITC), permits the determination of a full set of thermodynamic parameters involved in such interactions, such as the equilibrium association constant (Ka), the enthalpy changes (ΔH), the entropy changes (ΔS), the change in Gibbs free energy (ΔG), and the dissociation constant (Kd) (Filloux & Ramos, 2014). Even though in this context, the technique has been primarily applied to understand the binding between the main components present in the bacterial cell/membrane and antibacterial peptides, it is possible to apply ITC to comprehending the interaction between encapsulating agents such as proteins and carbohydrates and bacterial structures.

An Isothermal titration calorimeter measures the temperature differences between a reaction medium and a reference cell. The titrations are automatic injections of any ligand solution into a stirred solution containing macromolecules of interest (importance). The heat absorbed or released after each injection is correlated to the reaction cell (µcal/s) and compared to its baseline value. This technique has been extensively used to study the binding of Pseudomonas proteins to DNA fragments (Filloux & Ramos, 2014).

An interesting real time-monitoring analysis has been carried out in live bacterial cells. The authors Zhang (2018) reported an exothermic cleavage reaction after cefazolin was added into different solutions containing New Delhi Metallo-β-lactamase (NDM-1) and E. coli cells expressing NDM-1. All heat involved in the reaction was monitored during the experiment and it shows the versatility of ITC. The method can be further applied to screen and analyze small molecule inhibitors of Metallo-β-lactamase in whole cells (Zhang et al., 2018).

The interaction between the lantibiotic gallidermin and the glycopeptide antibiotic vancomycin with DOPC/lipid II membranes was simulated using ITC (Al-Kaddah et al., 2010). ITC was reported to be a promising thermoanalytical technique and has been largely applied in the study of peptides/membrane interaction studies. Rekharsky (2006) investigated the thermodynamic interaction between a synthetic peptide glycan from the cell wall and vancomycin. The study was able to confirm the 1:1 binding stochiometry between the antimicrobial peptide and the D-Ala-D-Ala moiety. The binding of Magainin 2 amide to lipid membranes was also studied for calorimetric detection with ITC (Wenk & Seelig, 1998) and the calorimetry technique was used to detect the binding of decapeptide gramicidin S (GS) to some lipid bilayer model membrane systems (Abraham et al., 2005) all thermodynamic measurements were taken and indicated that the GS binding to phospholipid bilayer is mainly entropy driven.

Besides demonstrating the efficacy of ITC in characterizing the thermal flux and interactions between bacterial cellular structures and any ligand, through simulated studies the binding affinity between encapsulating agents and the main molecules in the bacterial cell/membrane can be thoroughly understood.

5.3 Fourier Transform Infrared Spectroscopy (FTIR)

Bacterial typing and characterization are vital to different venues of research such as hospital infections, epidemiological studies, monitoring the presence of resistant bacteria, environmental waste treatment and food technology applications (Garon et al., 2010; Jin et al., 2017; Sabat et al., 2013). Due to the unique cell composition and specificity for each bacterial species, spectro-techniques can be used to identify specific fingerprint-like patterns. Among those techniques, Fourier Transform Infrared Spectroscopy (FTIR) has been able to produce rapid bacterial identification through low-cost processing (Burgula et al., 2007; Naumann, 2006). In general, bacterial typing and identification can be performed in all three IR-spectroscopy measurements, which includes the normal absorbance and transmission (A/T), attenuated total reflection (ATR) and diffuse reflectance modes (Quintelas et al., 2018).

FT-IR is an efficient tool for bacterial typing (fingerprint) and identification. The bacterial signals are specific and reproductible for finger-print analyses (Ji et al., 2019; Muhamadali et al., 2016). Each bacterial species shows a complex cell composition that presents a specific fingerprint-like pattern and can be well identified by FT-IR spectra (Yang et al., 2020). To characterize and identify the main absorbance peaks, bacterial FTIR can be divided into five main sub-ranges, the membrane amphiphile region (3000-2800 cm^{-1}), the biological infrared-silent region (2800-1800 cm^{-1}), the protein and peptide region (1500-800 cm^{-1}), the protein and fatty acid region (1500-1200 cm^{-1}), and the nucleic acid and polysaccharide region (1200-~900 cm^{-1}) (Naumann, 2006; Yang et al., 2020).

The association of materials to design a protective shell and encapsulate the bacteria, may also be characterized by FTIR. The FTIR fingerprint can detect the presence of bacteria in the external encapsulation material by identifying specific absorption peaks for the bacteria alone and comparing them with the absorption peaks for encapsulated bacteria.

Vodnar et al. (2010), investigated the encapsulation of a mix of two lactic acid bacteria (*Streptococcus thermophilus*and *Lactobacillus delbrueckii* subsp. *bulgaricus*) in sodium alginate and chitosan matrices to enhance and protect their survivability in the digestive tract. Through FTIR technique the free bacteria fingerprints were compared to alginate and chitosan spectrums and the encapsulated bacteria revealed specific absorption peaks in 1750, 2852 and 2926 cm^{-1}.

FTIR spectroscopy was assessed to determine the probiotic stability after microencapsulation, by spray drying, of *Lacticaseibacillus casei* 01 with soy protein isolate and alginate microparticles (Hadzieva et al., 2017). The study focused on the stability of bacteria after microencapsulation by comparing the FTIR fingerprint before and after spray-drying.

Due to the ability to form micro, submicro and nanocapsules, protein and carbohydrates are widely used as encapsulation materials. A study reported the encapsulation of *Bifidobacteriumsp.* for food applications, using whey protein concentrate and pullulan, a carbohydrate. The main changes in the molecular structure upon incorporation of bifidobacterial cells by the protein and the carbohydrate were measured by infrared spectroscopy (Lopez-Rubio et al., 2006). The study reveals how the technique can help with the fingerprint of the lactic acid bacteria cell wall (carbohydrates, peptides, teichoic acid, and anchored proteins) or even the whole cells, and compare it to the encapsulated bacteria after the drying process. The aim is to identify alterations in the spectrum and thereby determine which molecular interactions are occurring between organic functional groups in order to form a new and stable structure.

Even though FTIR has been mentioned as one of the main techniques for bacteria identification and characterization of encapsulated probiotics, there is still a lack of standardization. Differences in instruments, operators, sample preparation and environmental conditions can provide no-analogue results which make it difficult to compare all the identified peaks in a fingerprint approach between different works.

5.4 Raman Spectroscopy

Several techniques have been used for bacterial typing and characterization over the years, including plate counts, molecular and immunological methods (He et al., 2014). Although these are considered reliable methods, they can be time-consuming and not the best option for the high demand in real time analysis. In this context, Raman can represent a good alternative as a non-invasive technique, minimal sample preparation, quick registration and data generation and no need for chemicals. In addition, basically any sample can be analyzed by Raman spectroscopy (Santos et al., 2015).

Raman spectroscopy and Infrared are considered complementary methods and can be useful and reveal important details for bacteria typing and characterization of encapsulated probiotics. In sum, the Infrared method requires changes in the intrinsic dipole moment with molecular vibration that makes polar groups appear as strong infrared stretching bands, whereas in Raman spectroscopy non-polar groups appear as intense bands as the method depends on changes in the polarizability of functional groups when atoms vibrate. Therefore, the presence of water has a weak interference in Raman spectroscopy which makes this method more appropriate for in vivo and in situ studies of biological systems which include food applications (Santos et al., 2015).

Vaishanavi & Preetha (2021) incorporated soy protein into nanoemulsion achieving increased stability of probiotics (*Lactobacillus delbrueckii* subsp. *bulgaricus*). The study used Raman spectroscopy to analyze the interaction between components and provide more detailed information on chemical structure of the encapsulated product.

Keskin (2018) reported the use of Raman spectroscopy to characterize the encapsulation of *Lysinibacillus sp.* NOSK bacteria with cyclodextrin fiber (CD-F) through an electrospinning process. CD-F protects the bacterial cell from the external environment and can also serve as a carbon source for the bacteria. The Raman spectra of CD-F, bacteria cell and CD-F/bacteria were assigned and compared. The main peaks for skeletal CCH structure of CD-F could be observed for the amide and amide I vibrations from the bacterial cell and two evident peaks (1048 and 995cm^{-1}) observed at the polysaccharide region (1200-900 cm^{-1}) possibly arising from vibrations in the peptidoglycan of bacterial cell wall and then made it possible to confirm the presence of the bacterial cells in the fibrous matrix (San Keskin et al., 2018).

Vibrational spectroscopy methods have been used to investigate the optimization of storage techniques such as freezing, freeze-drying and spray-drying on lactic acid bacteria and other probiotics. Due to different materials having different amounts and affinities for water, these methods might cause a significant loss of structural water and consequently induce damage of membranes and proteins that are responsible for bacterial cell viability. Akanny et al., (2019) developed an encapsulation system for *Lacticaseibacillus rhamnosus* GG delivery. The system used spray-dried Eudragit® S100 microparticles as encapsulating agent and in order to assess the survival ratio of bacteria after drying and encapsulation Raman spectroscopy was employed and the method showed that the presence of carbohydrates increased the survival ratio to more than 50%. In this study Surface Enhanced Raman Scattering (SERS) was applied in order to increase the sensitivity of the bacterial quantification analysis compared to the conventional Raman. In sum, this increase can be explained by greater enhancement of Raman scattering due to molecules adsorbed on a nanostructured noble metal surface where the electromagnetic effect and chemical effect are the mechanism responsible for SERS effect. Due to its simplicity and high sensitivity SERS has been used in many studies for probiotic bacterial quantification, identification and typing (Akanny et al., 2019; Ankamwar et al., 2016; Mircescu et al., 2014; Premasiri et al., 2005; Zhou et al., 2014).

6. CONCLUSION

In reviewing the available literature, one sees a variety of hypotheses regarding the effect of microbial microencapsulation in relation to encapsulating agents and their benefits. However, there is still a lack of consensus in the published results on the protective effectiveness of various encapsulation materials. In this sense, to develop and refine this technique and apply it to LAB, it is important to investigate further the interaction between bacterial surface molecules and the encapsulating matrices, which will enable an increase in yield, stability and cell viability among other beneficial things.

Several studies indicate the importance of knowledge about the bacterial surface to better understand the mechanisms and factors that affect the interaction between encapsulating agents and microorganisms. A major challenge is the identification of the molecular processes responsible for the injury or bacterial adaptation. Maximum residual viability can be achieved by mapping the ideal drying parameters or designing better means of protection. More knowledge and research is needed to elucidate this important topic.

Even today, the main obstacle in the industrial process is the maintenance and viability of the initial culture in industrial ferment. Microencapsulation is able to provide protection for cells and, therefore, increase the viability of the ferments produced. However, the production of microcapsules capable of withstanding several months or years of continuous use, without deteriorating cellular activity and economical viability is a challenge. For it to be successful, it is important not only to use suitable encapsulating agents and methods, but also to know how they interact with the microorganism of interest.

The application of analytical techniques at the molecular and structural levels in foods, may enable the clarification of existing hypotheses and fill related gaps in the literature regarding microencapsulation of microorganisms. Research advances in this context are essential for the optimized production of dehydrated ferments.

Author Contributions

I. Perrone, I. Renhe, R. Stephani and A. Carvalho gave a guide to professional knowledge and interpreted the literature sources. E. Martins designed the study as well as planned and reviewed the article. C. Viana prepared, interpreted, edited and drafted the manuscript. L. Queiroz wrote part of the article.

Conflicts of Interest

The authors declare no conflicts of interest.

6. References

Abd El-Salam, M. H., & El-Shibiny, S. (2015). Preparation and properties of milk proteins-based encapsulated probiotics: a review. *Dairy Science and Technology*, 95(4), 393–412. https://doi.org/10.1007/s13594-015-0223-8

Abee, T., & Wouters, J. A. (1999). Microbial stress response in minimal processing. *International Journal of Food Microbiology*, 50(1–2), 65–91. https://doi.org/10.1016/S0168-1605(99)00078-1

Abraham, T., Lewis, R. N. A. H., Hodges, R. S., & McElhaney, R. N. (2005). Isothermal Titration Calorimetry Studies of the Binding of a Rationally Designed Analogue of the Antimicrobial Peptide Gramicidin S to Phospholipid Bilayer Membranes. *Biochemistry*, 44(6), 2103–2112. https://doi.org/10.1021/bi048077d

Ahuja, K., & Deb, S. (2018). Probiotics Market Size By Ingredients Bifidobacterium, Streptococcus, Bacillus), By End-Use (Human, Animal), By Application (Food and Beverages {Dairy Products, Non-Dairy Products, Cereals, Baked Food, Fermented Meat Products, Dry Food}, Die. *Food, Nutricion and Animal Feed*, 260. https://www.gminsights.com/industry-reports/food-nutrition-and-animal-feed

Akanny, E., Bonhommé, A., Commun, C., Doleans-Jordheim, A., Bessueille, F., Bourgeois, S., & Bordes, C. (2019). Development of uncoated near-spherical gold nanoparticles for the label-free quantification of Lactobacillus rhamnosus GG by surface-enhanced Raman spectroscopy*. Analytical and Bioanalytical Chemistry*, 411(21), 5563–5576. https://doi.org/10.1007/s00216-019-01938-4

Al-Kaddah, S., Reder-Christ, K., Klocek, G., Wiedemann, I., Brunschweiger, M., & Bendas, G. (2010). Analysis of membrane interactions of antibiotic peptides using ITC and biosensor measurements. *Biophysical Chemistry*, 152(1–3), 145–152. https://doi.org/10.1016/j.bpc.2010.09.002

Alimohamadi, H., & Rangamani, P. (2018). Modeling membrane curvature generation due to membrane–protein interactions. *Biomolecules,* 8(4), 35–38. https://doi.org/10.3390/biom8040120

Anal, A. K., & Singh, H. (2007). Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. *Trends in Food Science and Technology,* 18(5), 240–251. https://doi.org/10.1016/j.tifs.2007.01.004

Ananta, E., Volkert, M., & Knorr, D. (2005). Cellular injuries and storage stability of spray-dried Lactobacillus rhamnosus GG. *International Dairy Journal*, 15(4), 399–409. https://doi.org/10.1016/j.idairyj.2004.08.004

Ankamwar, B., Sur, U. K., & Das, P. (2016). SERS study of bacteria using biosynthesized silver nanoparticles as the SERS substrate. *Analytical Methods*, 8(11), 2335–2340. https://doi.org/10.1039/C5AY03014E

Arslan-Tontul, S., & Erbas, M. (2017). Single and double layered microencapsulation of probiotics by spray drying and spray chilling. *LWT*, 81, 160–169. https://doi.org/10.1016/j.lwt.2017.03.060

Aschenbrenner, M., Grammueller, E., Kulozik, U., & Foerst, P. (2014). The Contribution of the Inherent Restricted Mobility of Glassy Sugar Matrices to the Overall Stability of Freeze-Dried Bacteria Determined by Low-Resolution Solid-State H-NMR. *Food Bioprocess Technol*, 1012–1014. https://doi.org/10.1007/s11947-013-1095-7

Aschenbrenner, M., Kulozik, U., & Först, P. (2011). The role of the glassy state in production and storage of freeze-dried starter cultures. *Procedia Food Science*, 1, 347–354. https://doi.org/10.1016/j.profoo.2011.09.054

Behboudi-Jobbehdar, S., Soukoulis, C., Yonekura, L., & Fisk, I. (2013). Optimization of Spray-Drying Process Conditions for the Production of Maximally Viable Microencapsulated L. acidophilus NCIMB 701748. *Drying Technology*, 31(11), 1274–1283. https://doi.org/10.1080/07373937.2013.788509

Bekhit, M., Sánchez-González, L., Ben Messaoud, G., & Desobry, S. (2016). Design of microcapsules containing Lactococcus lactis subsp. lactis in alginate shell and xanthan gum with nutrients core. *LWT - Food Science and Technology*, 68, 446–453. https://doi.org/10.1016/j.lwt.2015.12.037

Bjelošević, M., Zvonar Pobirk, A., Planinšek, O., & Ahlin Grabnar, P. (2020). Excipients in freeze-dried biopharmaceuticals: Contributions toward formulation stability and lyophilisation cycle optimisation. *International Journal of Pharmaceutics*, 576, 119029. https://doi.org/10.1016/j.ijpharm.2020.119029

Boel, E., Koekoekx, R., Dedroog, S., Babkin, I., Vetrano, M. R., Clasen, C., & Van den Mooter, G. (2020). Unraveling particle formation: From single droplet drying to spray drying and electrospraying. *Pharmaceutics,* 12(7), 1–58. https://doi.org/10.3390/pharmaceutics12070625

Broeckx, G., Vandenheuvel, D., Claes, I. J. J., Lebeer, S., & Kiekens, F. (2016). Drying techniques of probiotic bacteria as an important step towards the development of novel pharmabiotics. *International Journal of Pharmaceutics*, 505(1–2), 303–318. https://doi.org/10.1016/j.ijpharm.2016.04.002

Brown, A. D. (1976). Microbial water stress. *Bacteriological Reviews*, 40(4), 803–846. https://doi.org/10.1128/mmbr.40.4.803-846.1976

Burgain, J., Gaiani, C., Cailliez-Grimal, C., Jeandel, C., & Scher, J. (2013). Encapsulation of Lactobacillus rhamnosus GG in microparticles: Influence of casein to whey protein ratio on bacterial survival during digestion. *Innovative Food Science and Emerging Technologies*, 19, 233–242. https://doi.org/10.1016/j.ifset.2013.04.012

Burgain, Jennifer, Scher, J., Lebeer, S., Vanderleyden, J., Cailliez-Grimal, C., Corgneau, M., Francius, G., & Gaiani, C. (2014a). Significance of bacterial surface molecules interactions with milk proteins to enhance microencapsulation of

Lactobacillus rhamnosus GG. *Food Hydrocolloids*, 41(2014), 60–70. https://doi.org/10.1016/j.foodhyd.2014.03.029

Burgain, Jennifer, Scher, J., Lebeer, S., Vanderleyden, J., Cailliez-Grimal, C., Corgneau, M., Francius, G., & Gaiani, C. (2014b). Significance of bacterial surface molecules interactions with milk proteins to enhance microencapsulation of Lactobacillus rhamnosus GG. *Food Hydrocolloids*, 41(2014), 60–70. https://doi.org/10.1016/j.foodhyd.2014.03.029

Burgula, Y., Khali, D., Kim, S., Krishnan, S. S., Cousin, M. A., Gore, J. P., Reuhs, B. L., & Mauer, L. J. (2007). Review of mid-infrared Fourier Transform-Infrared Spectroscopy applications for bacterial detection. *Journal of Rapid Methods and Automation in Microbiology,* 15(2), 146–175. https://doi.org/10.1111/j.1745-4581.2007.00078.x

Bustos, P., & Bórquez, R. (2013). Influence of Osmotic Stress and Encapsulating Materials on the Stability of Autochthonous Lactobacillus plantarum after Spray Drying. *Drying Technology*, 31(1), 57–66. https://doi.org/10.1080/07373937.2012.717325

Campos, N. da S., Almeida, M. R. de, Viana, C. C. R., Maurício, E. F., Stephani, R., & Oliveira, L. F. C. de. (2020). CAPÍTULO 8 - Técnicas analíticas para o controle de qualidade e desenvolvimento de produtos na indústria de secagem de soro. *Química e tecnologia do soro de leite: by the whey*. (p. 24).

Carpenter, J. F., Arakawa, T., & Crowe, J. H. (1991). *Interactions of stabilizing additives with proteins during freeze-thawing and freeze-drying*. Developments in Biological Standardization, 74.

Carpenter, John F., & Crowe, J. H. (1989). An infrared spectroscopic study of the interactions of carbohydrates with dried proteins. *Biochemistry*, 28(9), 3916–3922. https://doi.org/10.1021/bi00435a044

Carvalho, A., Teixeira, P., Gibbs, P., Carvalho, A. S., Silva, J., Ho, P., Teixeira, P., Malcata, F. X., & Gibbs, P. (2004). Relevant factors for the preparation of freeze-dried lactic acid bacteria. Int Dairy J Relevant factors for the preparation of freeze-dried lactic acid bacteria. *International Dairy Journal*, 14(October), 835–847. https://doi.org/10.1016/j.idairyj.2004.02.001

Chavarri, M., Maranon, I., & Carmen, M. (2012). Encapsulation Technology to Protect Probiotic Bacteria. *Probiotics*. https://doi.org/10.5772/50046

Chen, C.-C., Chen, S.-T., & Hsieh, J.-F. (2015). Proteomic Analysis of Polysaccharide-Milk Protein Interactions Induced by Chitosan. *Molecules,* 20(5), 7737–7749. https://doi.org/10.3390/molecules20057737

Chen, H., Tian, M., Chen, L., Cui, X., Meng, J., & Shu, G. (2019). Optimization of composite cryoprotectant for freeze-drying Bifidobacterium bifidum BB01 by response surface methodology. *Artificial Cells, Nanomedicine and Biotechnology*, 47(1), 1559–1569. https://doi.org/10.1080/21691401.2019.1603157

Chen, J., Wang, Q., Liu, C. M., & Gong, J. (2015). Issues deserve attention in encapsulating probiotics: Critical review of existing literature. *Critical Reviews in Food*

Science and Nutrition, 57(6), 1228–1238. https://doi.org/10.1080/10408398.2014.977991

Colín-Cruz, M. A., Pimentel-González, D. J., Carrillo-Navas, H., Alvarez-Ramírez, J., & Guadarrama-Lezama, A. Y. (2019). Co-encapsulation of bioactive compounds from blackberry juice and probiotic bacteria in biopolymeric matrices. *LWT,* 110(April), 94–101. https://doi.org/10.1016/j.lwt.2019.04.064

Corona-Hernandez, R. I., Álvarez-Parrilla, E., Lizardi-Mendoza, J., Islas-Rubio, A. R., de la Rosa, L. A., & Wall-Medrano, A. (2013). Structural stability and viability of microencapsulated probiotic bacteria: A review. *Comprehensive Reviews in Food Science and Food Safety*, 12(6), 614–628. https://doi.org/10.1111/1541-4337.12030

Colombo, M., Castilho, N. P. A., Todorov, S. D., & Nero, L. A. (2018). Beneficial properties of lactic acid bacteria naturally present in dairy production. *BMC Microbiology*, 1–12. https://doi.org/10.1186/s12866-018-1356-8

Cosentino, S., Viale, S., Deplano, M., Fadda, M. E., & Pisano, M. B. (2018). Application of Autochthonous Lactobacillus Strains as Biopreservatives to Control Fungal Spoilage in Caciotta Cheese. *Biomed Research*. https://doi.org/https://doi.org/10.1155/2018/3915615

Crowe, J. H., Crowe, L. M., Carpenter, J. F., & Aurell Wistrom, C. (1987). Stabilization of dry phospholipid bilayers and proteins by sugars. *The Biochemical Journal,* 242(1), 1–10. https://doi.org/10.1042/bj2420001

Crowe, John H., Crowe, L. M., & Hoekstra, F. A. (1989). Phase transitions and permeability changes in dry membranes during rehydration. *Journal of Bioenergetics and Biomembranes*, 21(1), 77–91. https://doi.org/10.1007/BF00762213

De Castro-Cislaghi, F. P., Silva, C. D. R. E., Fritzen-Freire, C. B., Lorenz, J. G., & Sant'Anna, E. S. (2012). Bifidobacterium Bb-12 microencapsulated by spray drying with whey: Survival under simulated gastrointestinal conditions, tolerance to NaCl, and viability during storage. *Journal of Food Engineering*, 113(2), 186–193. https://doi.org/10.1016/j.jfoodeng.2012.06.006

De Prisco, A., & Mauriello, G. (2016). Probiotication of foods: A focus on microencapsulation tool. Trends in Food Science and Technology, 48, 27–39. https://doi.org/10.1016/j.tifs.2015.11.009

Dianawati, D., Mishra, V., & Shah, N. P. (2015). Survival of Microencapsulated Probiotic Bacteria after Processing and during Storage: A Review. *Critical Reviews in Food Science and Nutrition*, 56(10), 1685–1716. https://doi.org/10.1080/10408398.2013.798779

Dolinsky, A. A. (2001). High-temperature spray drying. *Drying Technology*, 19(5), 785–806. https://doi.org/10.1081/DRT-100103770

Domingos-lopes, M. F. P., Stanton, C., Ross, P. R., Dapkevicius, M. L. E., & Silva, C. C. G. (2017). Genetic diversity, safety and technological characterization of lactic acid bacteria isolated from artisanal Pico cheese. *Food Microbiology*, 63, 178–190. https://doi.org/10.1016/j.fm.2016.11.014

Duongthingoc, D., George, P., Katopo, L., Gorczyca, E., & Kasapis, S. (2013). Effect of whey protein agglomeration on spray dried microcapsules containing Saccharomyces boulardii. *Food Chemistry*, 141(3), 1782–1788. https://doi.org/10.1016/j.foodchem.2013.04.093

Fennema, O. R. (2008). *Food Chemistry* (S. Damodaran, K. L. Parkin, & O. R. Fennema (eds.); Fourth). CRC Press. https://doi.org/10.1080/01932691.2011.584482

Filloux, A., & Ramos, J.-L. (2014). *Pseudomonas - Methods and Protocols*. Humana Press - Springer.

Filloux, A., & Whitfield, C. (2016). Editorial: The many wonders of the bacterial cell surface. FEMS Microbiology Reviews, 40(2), 161–163. https://doi.org/10.1093/femsre/fuv047

Francisquini, J. d'Almeida, Martins, E., Renhe, I. R. T., Oliveira, L. F. C. de, Stephani, R., Perrone, Í. T., & Carvalho, A. F. de. (2020). Particle size distribution applied to milk powder rehydration. *Quimica Nova*, 43(2), 226–230.

Fu, N., & Chen, X. D. (2011). Towards a maximal cell survival in convective thermal drying processes. *Food Research International*, 44(5), 1127–1149. https://doi.org/10.1016/j.foodres.2011.03.053

Fusieger, A., Martins, M. C. F., Freitas, R. De, Nero, L. A., & Carvalho, A. F. de. (2019). Technological properties of Lactococcus lactis subsp . lactis bv . diacetylactis obtained from dairy and non-dairy niches. *Brazilian Journal of Microbiology*, 51.

Gaidhani, K. A., Harwarlkar, M., Bhambere, D., & Nirgude, P. S. (2015). Lyophilization / freeze drying – a review. *World Journal of Pharmaceutical Research*, 4(8), 516–543.

Gao, J., Li, X., Zhang, G., Sadiq, F. A., Simal-Gandara, J., Xiao, J., & Sang, Y. (2021). Probiotics in the dairy industry—Advances and opportunities. *Comprehensive Reviews in Food Science and Food Safety*, 20(4), 3937–3982. https://doi.org/10.1111/1541-4337.12755

Garon, D., El Kaddoumi, A., Carayon, A., & Amiel, C. (2010). FT-IR Spectroscopy for Rapid Differentiation of Aspergillus flavus, Aspergillus fumigatus, Aspergillus parasiticus and Characterization of Aflatoxigenic Isolates Collected from Agricultural Environments. *Mycopathology,* 170(2), 131–142. https://doi.org/10.1007/s11046-010-9304-7

Golowczyc, M. A., Silva, J., Abraham, A. G., De Antoni, G. L., & Teixeira, P. (2010). Preservation of probiotic strains isolated from kefir by spray drying. *Letters in Applied Microbiology*, 50(1), 7–12. https://doi.org/10.1111/j.1472-765X.2009.02759.x

Gong, P., Zhang, L., Han, X., Shigwedha, N., Song, W., Yi, H., Du, M., & Cao, C. (2014). Injury Mechanisms of Lactic Acid Bacteria Starter Cultures During Spray Drying: A Review. *Drying Technology*, 32(7), 793–800. https://doi.org/10.1080/07373937.2013.860458

Gul, O. (2017). Microencapsulation of Lactobacillus casei Shirota by spray drying using different combinations of wall materials and application for probiotic dairy dessert. *Journal of Food Processing and Preservation*, 41(5). https://doi.org/10.1111/jfpp.13198

Hadzieva, J., Mladenovska, K., Simonoska Crcarevska, M., Glavaš Dodov, M., Dimchevska, S., Geškovski, N., Grozdanov, A., Popovski, E., Petruševski, G., Chachorovska, M., Petreska Ivanovska, T., Petruševska-Tozi, L., Ugarkovic, S., & Goracinova, K. (2017). Lactobacillus casei loaded Soy Protein-Alginate Microparticles prepared by Spray-Drying. *Food Technology and Biotechnology*, 55(2), 173–186. https://doi.org/10.17113/ftb.55.02.17.4991

Haque, M. A., & Adhikari, B. (2015). *Proteins in Spray Drying Process.* Handbook of Industrial Drying, April 2015, 971–983.

He, H.-J., Sun, D.-W., & Wu, D. (2014). Rapid and real-time prediction of lactic acid bacteria (LAB) in farmed salmon flesh using near-infrared (NIR) hyperspectral imaging combined with chemometric analysis*. Food Research International*, 62, 476–483. https://doi.org/10.1016/j.foodres.2014.03.064

Heidebach, T., Först, P., & Kulozik, U. (2012). Microencapsulation of Probiotic Cells for Food Applications. *Critical Reviews in Food Science and Nutrition*, 52(4), 291–311. https://doi.org/10.1080/10408398.2010.499801

Huang, S., Cauty, C., Dolivet, A., Le Loir, Y., Chen, X. D., Schuck, P., Jan, G., & Jeantet, R. (2016). Double use of highly concentrated sweet whey to improve the biomass production and viability of spray-dried probiotic bacteria. *Journal of Functional Foods*, 23, 453–463. https://doi.org/10.1016/j.jff.2016.02.050

Huang, S., Vignolles, M. L., Chen, X. D., Le Loir, Y., Jan, G., Schuck, P., & Jeantet, R. (2017). Spray drying of probiotics and other food-grade bacteria: A review. *Trends in Food Science and Technology*, 63, 1–17. https://doi.org/10.1016/j.tifs.2017.02.007

Jantzen, M., Göpel, A., & Beermann, C. (2013). Direct spray drying and microencapsulation of probiotic Lactobacillus reuteri from slurry fermentation with whey. *Journal of Applied Microbiology*, 115(4), 1029–1036. https://doi.org/10.1111/jam.12293

Ji, S. Y., Jeong, D. H., Hassan, M., & Ilev, I. K. (2019). Signature Infrared Bacteria Spectra Analyzed by an Advanced Integrative Computational Approach Developed for Identifying Bacteria Similarity. IEEE *Journal of Selected Topics in Quantum Electronics,* 25(1). https://doi.org/10.1109/JSTQE.2018.2846034

Jin, N., Zhang, D., & Martin, F. L. (2017). Fingerprinting microbiomes towards screening for microbial antibiotic resistance. *Integrative Biology*, 9(5), 406–417. https://doi.org/10.1039/c7ib00009j

Kavitake, D., Kandasamy, S., Devi, P. B., & Shetty, P. H. (2018). Recent developments on encapsulation of lactic acid bacteria as potential starter culture in fermented foods – A review. *Food Bioscience*, 21(November 2017), 34–44. https://doi.org/10.1016/j.fbio.2017.11.003

Kilimann, K. V., Doster, W., Vogel, R. F., Hartmann, C., & Gänzle, M. G. (2006). Protection by sucrose against heat-induced lethal and sublethal injury of Lactococcus lactis: An FT-IR study. *Biochimica et Biophysica Acta - Proteins and Proteomics*, 1764(7), 1188–1197. https://doi.org/10.1016/j.bbapap.2006.04.016

Kim, E. H. J., Chen, X. D., & Pearce, D. (2009). Surface composition of industrial spray-dried milk powders. 2. Effects of spray drying conditions on the surface composition. *Journal of Food Engineering*, 94(2), 169–181. https://doi.org/10.1016/j.jfoodeng.2008.10.020

Laclair, C. E., & Etzel, M. R. (2010). Ingredients and pH are key to clear beverages that contain whey protein. *Journal of Food Science*, 75(1), 21–27. https://doi.org/10.1111/j.1750-3841.2009.01400.x

Le Marrec, C., Bon, E., & Lonvaud-Funel, A. (2007). Tolerance to high osmolality of the lactic acid bacterium Oenococcus oeni and identification of potential osmoprotectants. *International Journal of Food Microbiology*, 115(3), 335–342. https://doi.org/10.1016/j.ijfoodmicro.2006.12.039

Lee, J., & Kaletunç, G. (2002). Evaluation of the heat inactivation of Escherichia coli and Lactobacillus plantarum by differential scanning calorimetry. *Applied and Environmental Microbiology*, 68(11), 5379–5386. https://doi.org/10.1128/AEM.68.11.5379-5386.2002

Leroy, F., & Vuyst, L. De. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science and Technology*, 15, 67–78. https://doi.org/10.1016/j.tifs.2003.09.004

Leslie, S. B., Israeli, E., Lighthart, B., Crowe, J. H., & Crowe, L. M. (1995). Trehalose and sucrose protect both membranes and proteins in intact bacteria during drying. *Applied and Environmental Microbiology*, 61(10), 3592–3597. https://doi.org/10.1128/aem.61.10.3592-3597.1995

Li, Z. L., Ding, H. M., & Ma, Y. Q. (2016). Interaction of peptides with cell membranes: Insights from molecular modeling. *Journal of Physics Condensed Matter*, 28(8), 83001. https://doi.org/10.1088/0953-8984/28/8/083001

Lin, T. C., Chen, B. Y., Chen, C. Y., Chen, Y. S., & Wu, H. (2019). Comparative analysis of spray-drying microencapsulation of Bifidobacterium adolescentis and Lactobacillus acidophilus cultivated in different growth media. *Journal of Food Process Engineering*, 42(7). https://doi.org/10.1111/jfpe.13258

Linares, D. M., Gómez, C., Renes, E., Fresno, J. M., Tornadijo, M. E., Ross, R. P., & Stanton, C. (2017). Lactic Acid Bacteria and Bifidobacteria with Potential to Design Natural Biofunctional Health-Promoting Dairy Foods. In *Frontiers in Microbiology* (Vol. 8, Issue MAY). Frontiers Media S.A. https://doi.org/10.3389/fmicb.2017.00846

Liu, H., Cui, S. W., Chen, M., Li, Y., Liang, R., Xu, F., & Zhong, F. (2017). Protective approaches and mechanisms of microencapsulation to the survival of probiotic bacteria during processing, storage and gastrointestinal digestion: A review. *Critical Reviews in Food Science and Nutrition,* 59(17), 2863–2878. https://doi.org/10.1080/10408398.2017.1377684

Liu, H., Gong, J., Chabot, D., Miller, S. S., Cui, S. W., Ma, J., Zhong, F., & Wang, Q. (2015). Protection of heat-sensitive probiotic bacteria during spray-drying bysodium caseinate stabilized fat particles. *Food Hydrocolloids*, 51, 459–467. https://doi.org/10.1016/j.foodhyd.2015.05.015

Lopes, S., Bueno, L., De Aguiar Júnior, F., & Finkler, C. L. L. (2017). Preparation and characterization of alginate and gelatin microcapsules containing Lactobacillus Rhamnosus. Anais Da Academia Brasileira de Ciencias, 89(3), 1601–1613. https://doi.org/10.1590/0001-3765201720170071

Lopez-Rubio, A., Gavara, R., & Lagaron, J. M. (2006). Bioactive packaging: turning foods into healthier foods through biomaterials. *Trends in Food Science & Technology*, 17(10), 567–575. https://doi.org/10.1016/j.tifs.2006.04.012

Maciel, G. M., Chaves, K. S., Grosso, C. R. F., & Gigante, M. L. (2014). Microencapsulation of Lactobacillus acidophilus La-5 by spray-drying using sweet whey and skim milk as encapsulating materials. *Journal of Dairy Science*, 97(4), 1991–1998. https://doi.org/10.3168/jds.2013-7463

Mallikarjunaiah, K. J., Kinnun, J. J., Petrache, H. I., & Brown, M. F. (2019). Flexible lipid nanomaterials studied by NMR spectroscopy. *Physical Chemistry Chemical Physics*, 21(34), 18422–18457. https://doi.org/10.1039/c8cp06179c

Martín, M. J., Lara-Villoslada, F., Ruiz, M. A., & Morales, M. E. (2015). Microencapsulation of bacteria: A review of different technologies and their impact on the probiotic effects. *Innovative Food Science and Emerging Technologies*, 27, 15–25. https://doi.org/10.1016/j.ifset.2014.09.010

Martins, E., Cnossen, D. C., Silva, C. R. J., Cezarino, J. C., Nero, L. A., Perrone, I. T., & Carvalho, A. F. (2019). Determination of ideal water activity and powder temperature after spray drying to reduce Lactococcus lactis cell viability loss. *Journal of Dairy Science*, 102(7), 6013–6022. https://doi.org/10.3168/jds.2019-16297

Martins, E., Poncelet, D., Rodrigues, R. C., & Renard, D. (2017). Oil encapsulation techniques using alginate as encapsulating agent : applications and drawbacks. *Journal of Microencapsulation*, 0(0), 754–771. https://doi.org/10.1080/02652048.2017.1403495

Mensink, M. A., Frijlink, H. W., van der Voort Maarschalk, K., & Hinrichs, W. L. J. (2017). How sugars protect proteins in the solid state and during drying (review): Mechanisms of stabilization in relation to stress conditions. *European Journal of Pharmaceutics and Biopharmaceutics,* 114, 288–295. https://doi.org/10.1016/j.ejpb.2017.01.024

Mensink, M. A., Van Bockstal, P. J., Pieters, S., De Meyer, L., Frijlink, H. W., Van Der Voort Maarschalk, K., Hinrichs, W. L. J., & De Beer, T. (2015). In-line near infrared spectroscopy during freeze-drying as a tool to measure efficiency of hydrogen bond formation between protein and sugar, predictive of protein storage stability. *International Journal of Pharmaceutics*, 496(2), 792–800. https://doi.org/10.1016/j.ijpharm.2015.11.030

Mircescu, N. E., Zhou, H., Leopold, N., Chiş, V., Ivleva, N. P., Niessner, R., Wieser, A., & Haisch, C. (2014). Towards a receptor-free immobilization and SERS detection of urinary tract infections causative pathogens. *Analytical and Bioanalytical Chemistry*, 406(13), 3051–3058. https://doi.org/10.1007/s00216-014-7761-4

Moayyedi, M., Eskandari, M. H., Rad, A. H. E., Ziaee, E., Khodaparast, M. H. H., & Golmakani, M. T. (2018). Effect of drying methods (electrospraying, freeze drying and spray drying) on survival and viability of microencapsulated Lactobacillus rhamnosus ATCC 7469. *Journal of Functional Foods*, 40(November 2017), 391–399. https://doi.org/10.1016/j.jff.2017.11.016

Moreira, M. T. C., Martins, E., Perrone, Í. T., de Freitas, R., Queiroz, L. S., & de Carvalho, A. F. (2021). Challenges associated with spray drying of lactic acid bacteria: Understanding cell viability loss. In *Comprehensive Reviews in Food Science and Food Safety* (Vol. 20, Issue 4, pp. 3267–3283). Blackwell Publishing Inc. https://doi.org/10.1111/1541-4337.12774

Motta, A. D. S., & Gomes, M. da S. M. (2015). Technological and functional properties of lactic acid bacteria: the importance of these microorganisms for food. *Rev. Inst. Latic. Cândido Tostes*, 172–184. https://doi.org/10.14295/2238-6416.v70i3.403

Muhamadali, H., Subaihi, A., Mohammadtaheri, M., Xu, Y., Ellis, D. I., Ramanathan, R., Bansal, V., & Goodacre, R. (2016). Rapid, accurate, and comparative differentiation of clinically and industrially relevant microorganisms via multiple vibrational spectroscopic fingerprinting. *The Analyst*, 141(17), 5127–5136. https://doi.org/10.1039/C6AN00883F

Naumann, D. (2006). *Infrared Spectroscopy in Microbiology*. Encyclopedia of Analytical Chemistry. John Wiley & Sons, Ltd. https://doi.org/10.1002/9780470027318.a0117

Nowak, D., & Jakubczyk, E. (2020). The freeze-drying of foods - the characteristic of the process course and the effect of its parameters on the physical properties of food materials. *Foods,* 9(10). https://doi.org/10.3390/foods9101488

Obuchi, K., Iwahashi, H., Lepock, J. R., & Komatsu, Y. (2000). Calorimetric characterization of critical targets for killing and acquired thermotolerance in yeast. *Yeast,* 16(2), 111–119. https://doi.org/10.1002/(SICI)1097-0061(20000130)16:2<111::AID-YEA507>3.0.CO;2-V

Oh, J. K., Yegin, Y., Yang, F., Zhang, M., Li, J., Huang, S., Verkhoturov, S. V., Schweikert, E. A., Perez-Lewis, K., Scholar, E. A., Taylor, T. M., Castillo, A., Cisneros-Zevallos, L., Min, Y., & Akbulut, M. (2018). The influence of surface chemistry on the kinetics and thermodynamics of bacterial adhesion. *Scientific Reports*, 8(1), 1–13. https://doi.org/10.1038/s41598-018-35343-1

Olvera-garcía, M., Serrano-maldonado, C. E., & Quirasco, M. (2015). Detección de Proteínas con Actividad Antibacteriana Producidas por Bacterias Ácido Lácticas. *BioTecnologia*, 19(July).

Perdana, J., Bereschenko, L., Fox, M. B., Kuperus, J. H., Kleerebezem, M., Boom, R. M., & Schutyser, M. A. I. (2013). Dehydration and thermal inactivation of Lactobacillus plantarum WCFS1: Comparing single droplet drying to spray and freeze drying. *Food Research International,* 54(2), 1351–1359. https://doi.org/10.1016/j.foodres.2013.09.043

Perdana, J., Fox, M. B., Siwei, C., Boom, R. M., & Schutyser, M. A. I. (2014). Interactions between formulation and spray drying conditions related to survival of lactobacillus plantarum WCFS1. *Food Research International*, 56, 9–17. https://doi.org/10.1016/j.foodres.2013.12.007

Perrone, Í. T., Silveira, A. C. P., Martins, E., Carvalho, A. F., & Schuck, P. (2016). Soro em pó: estado vítreo e condições de operação do spray dryer. *Revista do Instituto de Laticínios Cândido Tostes*, 71(2), 106. https://doi.org/10.14295/2238-6416.v70i2.527

Premasiri, W. R., Moir, D. T., Klempner, M. S., Krieger, N., Jones, G., & Ziegler, L. D. (2005). Characterization of the Surface Enhanced Raman Scattering (SERS) of Bacteria. *The Journal of Physical Chemistry B*, 109(1), 312–320. https://doi.org/10.1021/jp040442n

Pretoro, A. Di, & Manenti, F. (2020). *Non-conventional Unit Operations - Solving Practical Issues*. Applied Sciences and Technology. Springer Briefs

Quintelas, C., Ferreira, E. C., Lopes, J. A., & Sousa, C. (2018). An Overview of the Evolution of Infrared Spectroscopy Applied to Bacterial Typing. *Biotechnology Journal*, 13(1), 1700449. https://doi.org/10.1002/biot.201700449

Rajam, R., Kumar, S. B., Prabhasankar, P., & Anandharamakrishnan, C. (2015). Microencapsulation of Lactobacillus plantarum MTCC 5422 in fructooligosaccharide and whey protein wall systems and its impact on noodle quality. *Journal of Food Science and Technology,* 52(7), 4029–4041. https://doi.org/10.1007/s13197-014-1506-4

Riaz, Q. U. A., & Masud, T. (2013). Recent Trends and Applications of Encapsulating Materials for Probiotic Stability. *Critical Reviews in Food Science and Nutrition*, 53(3), 231–244. https://doi.org/10.1080/10408398.2010.524953

Rodrigues, F. J., Cedran, M. F., Bicas, J. L., & Sato, H. H. (2020). Encapsulated probiotic cells: Relevant techniques, natural sources as encapsulating materials and food applications – A narrative review. *Food Research International*, 137, 109682. https://doi.org/10.1016/j.foodres.2020.109682

Rodrigues Júnior, P. H., De Sá Oliveira, K., Almeida, C. E. R. De, De Oliveira, L. F. C., Stephani, R., Pinto, M. D. S., Carvalho, A. F. De, & Perrone, Í. T. (2016). FT-Raman and chemometric tools for rapid determination of quality parameters in milk powder: Classification of samples for the presence of lactose and fraud detection by addition of maltodextrin. *Food Chemistry*, 196, 584–588. https://doi.org/10.1016/j.foodchem.2015.09.055

Rokka, S., & Rantamäki, P. (2010). Protecting probiotic bacteria by microencapsulation: Challenges for industrial applications. *European Food Research and Technology*, 231(1), 1–12. https://doi.org/10.1007/s00217-010-1246-2

Sabat, A. J., Budimir, A., Nashev, D., Sá-Leão, R., van Dijl, J. M., Laurent, F., Grundmann, H., Friedrich, A. W., & on behalf of the ESCMID Study Group. (2013). Overview of molecular typing methods for outbreak detection and epidemiological surveillance. *Eurosurveillance*, 18(4). https://doi.org/10.2807/ese.18.04.20380-en

San Keskin, N. O., Celebioglu, A., Sarioglu, O. F., Uyar, T., & Tekinay, T. (2018). Encapsulation of living bacteria in electrospun cyclodextrin ultrathin fibers for bioremediation of heavy metals and reactive dye from wastewater. Colloids and

Surfaces B. *Biointerfaces,* 161, 169–176. https://doi.org/10.1016/j.colsurfb.2017.10.047

Santivarangkna, C., Higl, B., & Foerst, P. (2008). Protection mechanisms of sugars during different stages of preparation process of dried lactic acid starter cultures. *Food Microbiology*, 25(3), 429–441. https://doi.org/10.1016/j.fm.2007.12.004

Santivarangkna, Chalat, Naummann, D., Kulosik, U., & Foerst, P. (2010). Protective effects of sorbitol during the vacuum drying of Lactobacillus helveticus : An FT-IR study. *Annals of Microbiology*, 60(June), 235–242. https://doi.org/10.1007/s13213-010-0032-5

Santos, R. C. S. dos, Finkler, L., & Finkler, C. L. L. (2014). Microencapsulation of Lactobacillus casei by spray drying. *Journal of Microencapsulation*, 31(8), 759–767. https://doi.org/10.3109/02652048.2014.932026

Santos, M., Gerbino, E., Tymczyszyn, E., & Gomez-Zavaglia, A. (2015). Applications of Infrared and Raman Spectroscopies to Probiotic Investigation. *Foods*, 4(4), 283–305. https://doi.org/10.3390/foods4030283

Sarao, L. K., & Arora, M. (2017). Probiotics, prebiotics, and microencapsulation: A review. *Critical Reviews in Food Science and Nutrition*, 57(2), 344–371. https://doi.org/10.1080/10408398.2014.887055

Schebor, C., Galvagno, M., Del Pilar Buera, M., & Chirife, J. (2000). Glass transition temperatures and fermentative activity of heat-treated commercial active dry yeasts. *Biotechnology Progress*, 16(2), 163–168. https://doi.org/10.1021/bp990147y

Schuck, P., Dolivet, A., Méjean, S., Hervé, C., & Jeantet, R. (2012). Spray drying of dairy bacteria: New opportunities to improve the viability of bacteria powders. *International Dairy Journal*, 31(1), 12–17. https://doi.org/10.1016/j.idairyj.2012.01.006

Silva, P. T. da, Fries, L. L. M., Menezes, C. R. de, Holkem, A. T., Schwan, C. L., Wigmann, É. F., Bastos, J. de O., & Silva, C. de B. da. (2014). Microencapsulation: concepts, mechanisms, methods and some applications in food technology. *Ciência Rural*, 44(7), 1304–1311. https://doi.org/10.1590/0103-8478cr20130971

Silva, T. M., Barin, J. S., Lopes, E. J., Cichoski, A. J., De Moraes Flores, E. M., De Bona Da Silva, C., & De Menezes, C. R. (2019). Development, characterization and viability study of probiotic microcapsules produced by complex coacervation followed by freeze-drying. *Ciencia Rural*, 49(7). https://doi.org/10.1590/0103-8478cr20180775

Silva, T. M., Jacob Lopes, E., Codevilla, C. F., Cichoski, A. J., Flores, É. M. de M., Motta, M. H., da Silva, C. de B., Grosso, C. R. F., & de Menezes, C. R. (2018). Development and characterization of microcapsules containing Bifidobacterium Bb-12 produced by complex coacervation followed by freeze drying. *LWT - Food Science and Technology*, 90. https://doi.org/10.1016/j.lwt.2017.12.057

Sohail, A., Turner, M. S., Coombes, A., & Bhandari, B. (2013). The Viability of Lactobacillus rhamnosus GG and Lactobacillus acidophilus NCFM Following Double Encapsulation in Alginate and Maltodextrin. *Food and Bioprocess Technology*, 6(10), 2763–2769. https://doi.org/10.1007/s11947-012-0938-y

Stefanello, R. F., Nabeshima, E. H., Iamanaka, B. T., Ludwig, A., Fries, L. L. M., Bernardi, A. O., & Copetti, M. V. (2018). Survival and stability of Lactobacillus fermentum and Wickerhamomyces anomalus strains upon lyophilisation with different cryoprotectant agents. *Food Research International*, 115(April), 90–94. https://doi.org/10.1016/j.foodres.2018.07.044

Suryabhan, P., Lohith, K., & Anu-Appaiah, K. A. (2019). Sucrose and sorbitol supplementation on maltodextrin encapsulation enhance the potential probiotic yeast survival by spray drying. *LWT*, 107, 243–248. https://doi.org/10.1016/j.lwt.2019.03.002

Tee, W. F., Nazaruddin, R., Tan, Y. N., & Ayob, M. K. (2014). Effects of encapsulation on the viability of potential probiotic Lactobacillus plantarum exposed to high acidity condition and presence of bile salts. *Food Science and Technology International*, 20(6), 399–404. https://doi.org/10.1177/1082013213488775

Timasheff, S.N. (1982). *Preferential interactions in protein-water-cosolvent systems*. In Biophysics of Water, ed. F. Franks, pp. 70-2. London: John Wiley.

Thybo, P., Hovgaard, L., Andersen, S. K., & Lindeløv, J. S. (2008). Droplet size measurements for spray dryer scale-up. *Pharmaceutical Development and Technology*, 13(2), 93–104. https://doi.org/10.1080/10837450701830957

Vaessen, E. M. J., den Besten, H. M. W., Esveld, E. D. C., & Schutyser, M. A. I. (2019). Accumulation of intracellular trehalose and lactose in Lactobacillus plantarum WCFS1 during pulsed electric field treatment and subsequent freeze and spray drying. *LWT,* 115(July), 108478. https://doi.org/10.1016/j.lwt.2019.108478

Vaishanavi, S., & Preetha, R. (2021). Soy protein incorporated nanoemulsion for enhanced stability of probiotic (Lactobacillus delbrueckii subsp. bulgaricus) and its characterization. Materials Today: *Proceedings*, 40, S148–S153. https://doi.org/10.1016/j.matpr.2020.05.008

Vaniski, R., Corti, D., & Drunkler, D. A. (2017). Técnicas e Materiais Empregados na Microencapsulação de Probióticos. *Brazilian Journal of Food Research*, 8(1), 156. https://doi.org/10.3895/rebrapa.v8n1.3651

Vodnar, D. C., Socaciu, C., Rotar, A. M., & Stãnilã, A. (2010). Morphology, FTIR fingerprint and survivability of encapsulated lactic bacteria (Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus) in simulated gastric juice and intestinal juice. *International Journal of Food Science and Technology*, 45(11), 2345–2351. https://doi.org/10.1111/j.1365-2621.2010.02406.x

Vollmer, W., & Seligman, S. J. (2010). Architecture of peptidoglycan: more data and more models. *Trends in Microbiology*, 18(2), 59–66. https://doi.org/10.1016/j.tim.2009.12.004

Walton, D. E. (2000). The morphology of spray-dried particles a qualitative view. *Drying Technology*, 18(9), 1943–1986. https://doi.org/10.1080/07373930008917822

Walton, D. E., & Mumford, C. J. (1999). The morphology of spray-dried particles. The effect of process variables upon the morphology of spray-dried particles. *Chemical Engineering Research and Design*, 77(5), 442–460. https://doi.org/10.1205/026387699526296

Wang, G. Q., Pu, J., Yu, X. Q., Xia, Y. J., & Ai, L. Z. (2020). Influence of freezing temperature before freeze-drying on the viability of various Lactobacillus plantarum strains. *Journal of Dairy Science*, 103(4), 3066–3075. https://doi.org/10.3168/jds.2019-17685

Wang, L., Yu, X., Xu, H., Aguilar, Z. P., & Wei, H. (2016). Effect of skim milk coated inulin-alginate encapsulation beads on viability and gene expression of Lactobacillus plantarum during freeze-drying. *LWT - Food Science and Technology*, 68, 8–13. https://doi.org/10.1016/j.lwt.2015.12.001

Watson, H. (2015). Biological membranes. *Essays in Biochemistry*, 59, 43–70. https://doi.org/10.1042/BSE0590043

Wei, H., Yang, X. Y., Geng, W., Van Der Mei, H. C., & Busscher, H. J. (2021). Interfacial interactions between protective, surface-engineered shells and encapsulated bacteria with different cell surface composition. *Nanoscale,* 13(15), 7220–7233. https://doi.org/10.1039/d0nr09204e

Wei, H., Yang, X. Y., van der Mei, H. C., & Busscher, H. J. (2021). X-Ray Photoelectron Spectroscopy on Microbial Cell Surfaces: A Forgotten Method for the Characterization of Microorganisms Encapsulated With Surface-Engineered Shells. *Frontiers in Chemistry*, 9, 1–15. https://doi.org/10.3389/fchem.2021.666159

Wenk, M. R., & Seelig, J. (1998). Magainin 2 amide interaction with lipid membranes: Calorimetric detection of peptide binding and pore formation. *Biochemistry*, 37(11), 3909–3916. https://doi.org/10.1021/bi972615n

Wolfe, J. (1987). Lateral Stresses in Membranes at Low Water Potential. *Functional Plant Biology*, 14(3), 311. https://doi.org/10.1071/pp9870311

Würth, R., Wiesner, S., Foerst, P., & Kulozik, U. (2017). Impact of the CaCl2 content in the rehydration media on the microcapsule formation out of spray dried capsule precursors for the immobilization of probiotic bacteria. *International Dairy Journal*, 68, 75–79. https://doi.org/10.1016/j.idairyj.2017.01.008

Würth, Rebecca, Foerst, P., & Kulozik, U. (2018). Effects of skim milk concentrate dry matter and spray drying air temperature on formation of capsules with varying particle size and the survival microbial cultures in a microcapsule matrix. *Drying Technology*, 36(1), 93–99. https://doi.org/10.1080/07373937.2017.1301952

Yancey, P. H. (2001). Water stress, osmolytes and proteins. *American Zoologist*, 41(4), 699–709. https://doi.org/10.1093/icb/41.4.699

Yang, H., Wu, F., Xu, F., Tang, K., Ding, C., Shi, H., & Yu, S. (2020). Bacterial Typing and Identification Based on Fourier Transform Infrared Spectroscopy. *Research Square*, 1–58. https://doi.org/10.21203/rs.2.23337/v1

Yao, M., Xie, J., Du, H., McClements, D. J., Xiao, H., & Li, L. (2020). Progress in microencapsulation of probiotics: A review. In *Comprehensive Reviews in Food Science and Food Safety* (Vol. 19, Issue 2, pp. 857–874). Blackwell Publishing Inc. https://doi.org/10.1111/1541-4337.12532

Yudiastuti, S., Sukarminah, E., Mardawati, E., & Kastaman, R. (2019). Evaluation study of Lactobacillus acidophilus drying. IOP Conference Series: Earth and Environmental Science, 250(1). https://doi.org/10.1088/1755-1315/250/1/012016

Zhang, Y.-J., Wang, W.-M., Oelschlaeger, P., Chen, C., Lei, J.-E., Lv, M., & Yang, K.-W. (2018). Real-Time Monitoring of NDM-1 Activity in Live Bacterial Cells by Isothermal Titration Calorimetry: A New Approach To Measure Inhibition of Antibiotic-Resistant Bacteria. *ACS Infectious Diseases*, 4(12), 1671–1678. https://doi.org/10.1021/acsinfecdis.8b00147

Zhao, R., Sun, J., Torley, P., Wang, D., & Niu, S. (2007). Measurement of particle diameter of Lactobacillus acidophilus microcapsule by spray drying and analysis on its microstructure. *World Journal of Microbiology and Biotechnology*, 24(8), 1349–1354. https://doi.org/10.1007/s11274-007-9615-0

Zhou, H., Yang, D., Ivleva, N. P., Mircescu, N. E., Niessner, R., & Haisch, C. (2014). SERS Detection of Bacteria in Water by in Situ Coating with Ag Nanoparticles. *Analytical Chemistry*, 86(3), 1525–1533. https://doi.org/10.1021/ac402935p

4. OTHER PUBLICATIONS DEVELOPED DURING THE DOCTORATE

4.1 - Published papers

Viana, CCR. Finamore, ALB. Renhe, IRT. Lima, SP. Perrone, IT (2020). Fórmulas infantis para lactentes: Uma breve revisão. *Revista Indústria de Laticínios*. ISSN 1678-7250. Ano XXV - nº 145 - julho/agosto.

Pinto CB dos A, Renhe IRT, Viana CCR, Perrone ÍT, Oliveira LFC de and Stephani R (2020). Lactose hydrolysis and milk homogenization effects on the pasty dulce de leche characteristics. *Principia – Caminhos da Iniciação Científica*, Juiz de Fora, v. 20, n. 1.

Pinto C.B. dos A, Renhe IRT, Viana CCR, Perrone ÍT, Oliveira LFC de and Stephani R (2021). Lactose-free Dulce de leche: compositional characterization, browning and texture profile. *Journal of Dairy Research.* https://doi.org/10.1017/S0022029921000820

4.2 - Published book chapter

Campos, N. da S., Almeida, M. R. de, Viana, C. C. R., Maurício, E. F., Stephani, R., & Oliveira, L. F. C. de. (2020). CAPÍTULO 8 - Técnicas analíticas para o controle de qualidade e desenvolvimento de produtos na indústria de secagem de soro. In *Química e tecnologia do soro de leite: by the whey*.

5. GENERAL CONCLUSION AND PERSPECTIVES

As already presented, the microencapsulation market is broad, diversified and can impact financial flows around the world, related to the application of microorganisms in food, especially probiotics. These data indicate the need for research and development of methodologies for the production of microbial powder cultures with high yield, high cell viability and low cost, in order to meet industrial needs in food production.

According to **Paper 1**, it can be seen that there are different techniques available to produce dehydrated probiotic cultures, and that, to microencapsulate them, there are also a variety of encapsulating agents, especially carbohydrates and proteins. The stability and viability of dehydrated microorganisms is closely related to the conditions chosen. This makes the need for research in this area increasing, to guide in a more assertive and profitable way the definitions of the conditions of the microencapsulation process.

Paper 2 brings a review of issues raised in Paper 1, in order to complement it. Spray drying and lyophilization stand out in the production of dehydrated cultures. However, the process can lead to irreversible damage, mainly to the cell membrane, causing loss of viability.

There are many hypotheses in the literature that discuss the probable effect of microbial microencapsulation in relation to encapsulating agents, which mainly depends on their properties and nature. Cell protection can occur by physical factors, such as vitrification, or chemical factors, for example, by forming hydrogen bonds between the encapsulant and membrane components.

The maximum viability of the cultures produced can be achieved by mapping the ideal drying parameters or designing protection means, in relation to the encapsulating agents. Physicochemical analytical techniques, such as spectroscopy, microscopy and thermodynamic methods, together with the microbiological methodologies already used, can help to elucidate and prove theories present in the scientific literature.

More knowledge and research are needed to elucidate this important topic. Therefore, as future perspectives, it is proposed to carry out applied research, with the suggested analytical methods, in order to seek an understanding of the protective effect of different encapsulating agents in the microencapsulation of bacteria, especially probiotics. The understanding of the specificities of the interaction of bacterial surface molecules with the encapsulating matrices will allow, among other advantages, to increase the yield, stability and cell viability in the production of yeasts for the food industry.