

Development and characterisation of edible films and coatings with antimicrobial properties

Sofia Patrícia Meneses da Silva

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Tese de Doutoramento

UNIVERSIDADE DOS AÇORES
Faculdade de Ciências Agrárias e
do Ambiente

Rua Capitão João d'Ávila
9700-042 Angra do Heroísmo
Açores, Portugal

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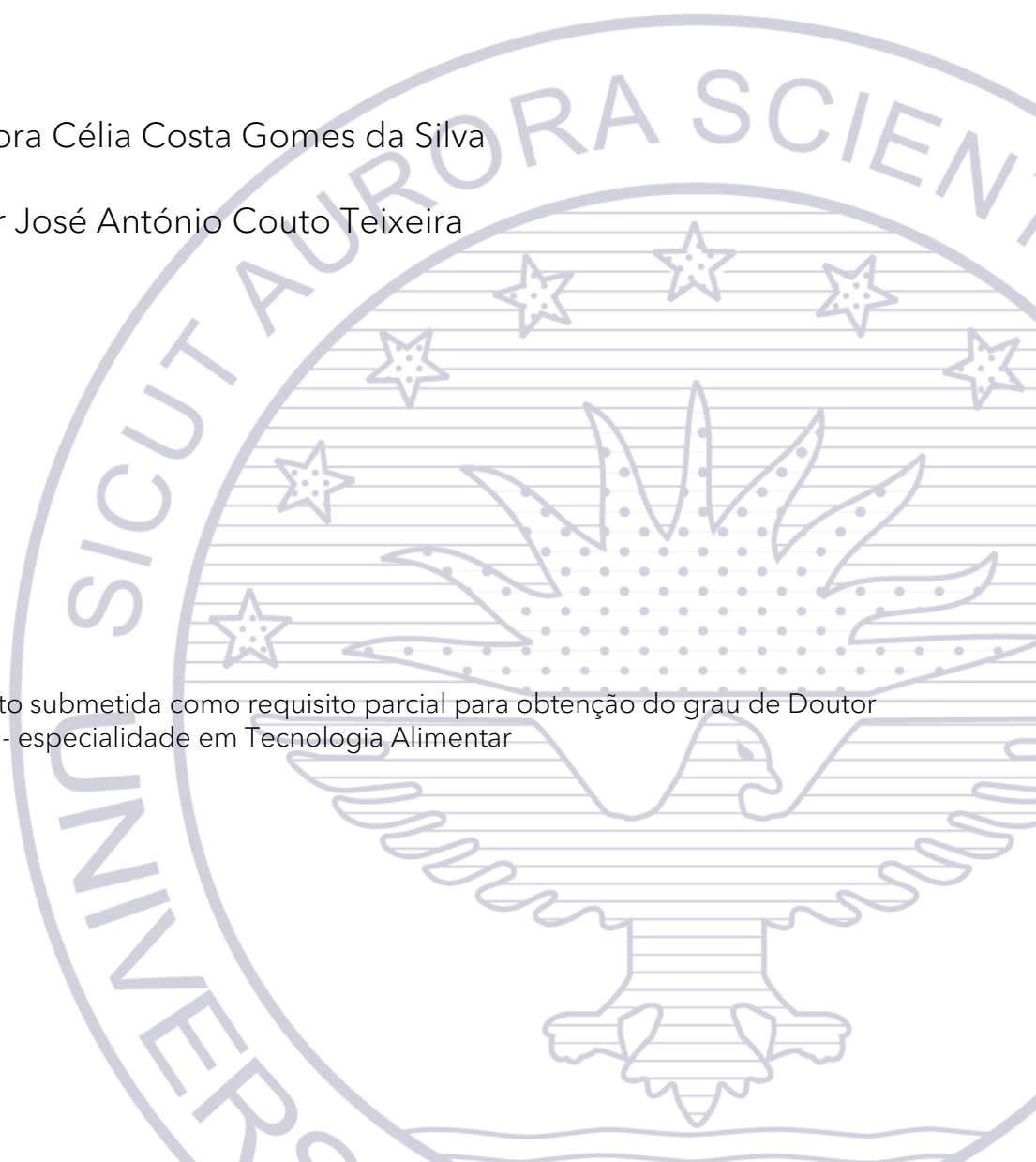
Sofia Patrícia Meneses da Silva

Orientadores

Professora Doutora Célia Costa Gomes da Silva

Professor Doutor José António Couto Teixeira

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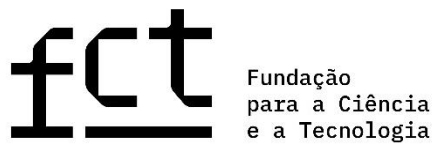
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THESIS OUTLINE

The thesis is divided into five chapters, one of which refers to the bibliographical review of the literature on the subject of this thesis (CHAPTER I), three chapters describe the experimental work (CHAPTER II, CHAPTER III and CHAPTER IV), the last chapter contains the main conclusions and future perspectives of this thesis (CHAPTER V).

This thesis is based on the following articles, which will be referred in the text by chapters:

II. Application of an alginate-based edible coating with bacteriocin-producing *Lactococcus* strains in fresh cheese preservation

Silva, S. P., Ribeiro, S. C., Teixeira, J. A., & Silva, C. C.
LWT (2022) 153, 112486

III. Application of enterocin-whey films to reduce *Listeria monocytogenes* contamination on ripened cheese

Silva, S. P., Teixeira, J. A., & Silva, C. C.
Food Microbiology (2023) 109, 104134

IV. Prevention of Fungal Contamination in Semi-Hard Cheeses by Whey-Gelatin Film Incorporated with *Levilactobacillus brevis* SJC120

Silva, S. P., Teixeira, J. A., & Silva, C. C.
Foods (2023) 12(7), 1396

OTHER PUBLICATIONS BY THE AUTHOR

i. Production of low-cholesterol butter with *Lactocaseibacillus paracasei* immobilized in calcium-alginate beads

Teixeira, M. F. B., Silva, S. P. M., Domingos-Lopes, M. F. P., Bessa, R. J. B., Prates, J. A. M., Rosa, H. J. D., & Silva, C. C. G.
Food Chemistry (2022) 393, 133419

ABSTRACT

Conventional food packaging made of synthetic materials has caused numerous environmental concerns in recent years, and consumer awareness of this problem is forcing the food industry to look for alternatives. Edible films and coatings are sustainable packaging made from biomaterials that are biodegradable. As they are an integral part of the food, they can be consumed together with the food, reducing waste. In addition, the incorporation of lactic acid bacteria (LAB) and their metabolites as a biopreservation approach against microbial contamination has gained attention in recent years. Therefore, the main objective of this thesis was to develop new edible coatings and films containing bacteriocins and/or lactic acid bacteria (LAB) with antimicrobial properties that can be used for cheese preservation.

In the first study, the bacteriocin-producing strains *Lactococcus lactis* L3A21M1 and *Lactococcus garvieae* SJM17 isolated from artisanal Azorean cheeses were incorporated into an edible fresh-cheese coating composed of alginate, maltodextrin and glycerol. The immobilization of *Lactococcus* cells into the coating had no negative effect on their viability and antibacterial activity throughout 10 days storage at 4 °C and 10 °C. The application of coating with immobilized *Lactococcus* cells on fresh cheese reduced significantly ($p < 0.05$) the contamination by *Listeria monocytogenes* on surface and prevented the growth of mesophilic bacteria by the 6th and 8th day of storage at 4 °C. Moreover, the coating reduced moisture and weight losses of fresh cheeses during storage, without affecting the pH and titratable acidity. The coating also prevented the migration of *L. monocytogenes* from the surface into the cheeses. These findings indicate that alginate-maltodextrin-glycerol formulation provided an excellent matrix to support *Lactococcus* cells viability and bacteriocin production. This bioactive coating can act as protective antimicrobial barrier in fresh cheeses by reducing bacterial contamination after processing.

In the second study, an enterocin whey solution, obtained by growing *Enterococcus faecalis* L2B21K3 and *E. faecalis* L3A21K6 in sweet whey (enterocin whey solution-EWS), was incorporated into gelatin-glycerol films that were tested for the control of *L. monocytogenes*. The films containing enterocins produced by either strain (EWS L2 and EWS K6 films) were shown to serve as a suitable matrix for bacteriocin release and preserve the anti-listerial activity for up to 90 days. When applied in cheese (cheddar), EWS L2 and EWS K6 films were able to reduce *L. monocytogenes* contamination to undetected levels after 20 or 30 days, respectively, and prevented the migration

of this pathogen from the films to cheese. The incorporation of EWS into films did not affect ($p < 0.05$) moisture content, solubility, permeability (water vapor and limonene), and elongation at break compared to control films (without EWS). However, thickness, swelling index and tensile strength were higher ($p < 0.05$) in EWS films. These results suggest that active EWS gelatin-glycerol films could be an effective, and safe application to control *L. monocytogenes* in cheese. In addition, the use of cheese whey as a culture medium for the production of the bacteriocins complemented with the incorporation in films formulation as a packaging material represents an alternative approach to reuse this by-product of cheese production.

In the third study, cheese whey fermented by LAB was used to develop an edible film with antifungal properties. Five LAB strains isolated from artisanal cheeses were screened for antifungal activity and incorporated into a whey-gelatin film. Of the strains tested, *Levilactobacillus brevis* SJC120 showed the strongest activity against five filamentous fungi isolated from cheese and cheese-making environment, at both 10 °C and 20 °C. The cell-free supernatant from *L. brevis* inhibited fungal growth by more than 80%. Incorporation of bacterial cells into the film did not alter the moisture content, water vapor permeability, or mechanical and optical properties. The whey-gelatin film was also able to maintain the viability of *L. brevis* cells at 10^7 CFU/g after 30 days at 10 °C. In cheeses wrapped with *L. brevis* film, the size of fungal colonies decreased by 55% to 76%. Furthermore, no significant differences ($p > 0.05$) were observed in cheese proteolysis or in the moisture, fat, and protein content of the cheese wrapped with films. The results showed that whey-gelatin film with *L. brevis* SJC120 can reduce the contamination of cheese with filamentous fungi and could be used as an alternative to conventional cheese preservation and packaging.

Overall, the present work proposes three new formulations for edible coatings and films with the incorporation of LAB or LAB fermented whey with antimicrobial activity, which showed promising results in protecting cheese from spoilage and pathogenic microorganisms.

Keywords: edible coatings; edible films; lactic acid bacteria; antilisteria; antifungal; biopreservation; cheese; whey.

RESUMO

Nos últimos anos, as embalagens convencionais de alimentos produzidas com materiais sintéticos têm despertado inúmeras preocupações ambientais. A conscientização dos consumidores sobre esse problema tem forçando a indústria alimentar a procurar alternativas para estas embalagens. Os revestimentos e filmes comestíveis são embalagens sustentáveis feitas de biomateriais biodegradáveis e que podem ser consumidos juntamente com o alimento, reduzindo o desperdício. Nos últimos anos tem também ganhado notoriedade a incorporação de bactérias do ácido láctico (BAL), bem como os seus metabolitos, como forma de biopreservação dos alimentos contra a contaminação microbiana. Desta forma, o presente trabalho tem como objetivo o desenvolvimento de novos revestimentos e filmes edíveis contendo bacteriocinas e/ou BAL com propriedades antimicrobianas que possam ser utilizados na conservação de queijos.

No primeiro estudo, as estirpes produtoras de bacteriocinas *Lactococcus lactis* L3A21M1 e *Lactococcus garvieae* SJC17, previamente isoladas de queijos artesanais dos Açores, foram incorporadas num revestimento edível de queijo fresco composto por alginato, maltodextrina e glicerol. A imobilização de células de *Lactococcus* no revestimento não teve efeito negativo sobre sua viabilidade e atividade antibacteriana ao longo de 10 dias de armazenamento a 4 °C e 10 °C. No entanto, a aplicação no queijo fresco, do revestimento contendo células imobilizadas de *Lactococcus*, reduziu significativamente ($p < 0,05$) a contaminação por *Listeria monocytogenes* na superfície e impediu o crescimento de bactérias mesófilas no 6º e 8º dia de armazenamento a 4 °C. Além disso, o revestimento reduziu as perdas de humidade e o peso dos queijos frescos durante o armazenamento, sem afetar o pH e a acidez titulável. O revestimento também impediu a migração de *L. monocytogenes* da superfície para os queijos. Estes resultados indicam que a formulação de alginato-maltodextrina-glicerol forneceu uma excelente matriz para sustentar a viabilidade das células de *Lactococcus* e a produção da respectiva bacteriocina. Este revestimento bioativo pode assim atuar como barreira antimicrobiana protetora em queijos frescos, reduzindo a contaminação bacteriana após o processamento.

No segundo estudo, o soro de queijo fresco foi utilizado como substrato para o crescimento de *Enterococcus faecalis* L2B21K3 e *E. faecalis* L3A21K6, e produção de bacteriocinas (enterocinas). O soro fermentado contendo estas enterocinas (EWS) foi incorporado em filmes de gelatina-glicerol para avaliação no controle do crescimento de *L. monocytogenes*. Os filmes contendo enterocinas produzidos por ambas as estirpes (filmes EWS L2 e EWS K6) demonstraram

uma boa capacidade para a liberação de enterocinas, além de preservarem a atividade anti-listéria durante 90 dias. No revestimento de queijos modelo (tipo cheddar), os filmes EWS L2 e EWS K6 foram capazes de reduzir a contaminação por *L. monocytogenes* a níveis abaixo do limite de detecção, após 20 ou 30 dias, respectivamente, além de funcionarem como barreira à migração deste patógeno para o queijo. A incorporação de EWS em filmes não afetou ($p < 0.05$) o teor de humidade, solubilidade, permeabilidade (vapor de água e limoneno) e alongamento na rotura, em comparação com os filmes controlo (sem EWS). No entanto, a espessura, inchamento e resistência à tração foram mais elevados ($p < 0.05$) nos filmes EWS. Estes resultados sugerem que os filmes ativos de gelatina-glicerol e EWS podem constituir uma aplicação eficaz e segura para controlar *L. monocytogenes* no queijo. Acresce ainda, que a utilização do soro de queijo como meio de cultura para a produção de bacteriocinas, bem como a sua incorporação na formulação dos filmes, apresenta-se como uma alternativa sustentável para o reaproveitamento deste subproduto.

No terceiro estudo, o soro de queijo fermentado por BAL foi usado para desenvolver um filme comestível com propriedades antifúngicas. Cinco estirpes de BAL isoladas de queijos artesanais foram avaliadas quanto à atividade antifúngica e incorporadas num filme de gelatina e soro de leite. Das estirpes testadas, a *Levilactobacillus brevis* SJC120 mostrou uma atividade mais elevada contra cinco fungos filamentosos isolados de queijos ou ambiente de fabricação de queijo, tanto a 10°C quanto a 20 °C. O sobrenadante livre de células de *L. brevis* inibiu o crescimento fúngico em mais de 80%. A incorporação de células bacterianas não alterou o teor de humidade, a permeabilidade ao vapor de água ou as propriedades mecânicas e óticas do filme. O filme de soro-gelatina também foi capaz de manter a viabilidade das células de *L. brevis* (10^7 UFC/g) após 30 dias de armazenamento a 10 °C. Nos queijos envolvidos com o filme contendo *L. brevis*, o crescimento dos fungos diminuiu em 55% a 76%. Não foram também observadas diferenças significativas ($p > 0.05$) na proteólise ou no teor de humidade, gordura e proteína do queijo embalado com os filmes. Os resultados mostraram que o filme de gelatina-soro, com a incorporação de *L. brevis* SJC120, pode reduzir a contaminação do queijo com fungos filamentosos, podendo ser usado como uma alternativa à embalagem e preservação convencional dos queijos.

No geral, o presente trabalho propõe três novas formulações para revestimentos e filmes comestíveis com a incorporação de BAL ou soro de leite fermentado com BAL com atividade antimicrobiana, com resultados promissores na proteção do queijo contra microrganismos deteriorantes e patogénicos.

Palavras-chave: revestimentos edíveis; filmes edíveis; bactérias do ácido láctico; anti-listéria; antifúngico; biopreservação; queijo; soro de leite.

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