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MATHEUS HENRIQUE GOUVEIA GOMES

DEPOSITION OF RICE PROTEIN HYDROLYSATES ON THE MICROPARTICLES SURFACE OBTAINED BY SPRAY DRYING

DEPOSIÇÃO DE HIDROLISADOS PROTEICOS DE ARROZ NAS SUPERFÍCIES DAS MICROPARTÍCULAS OBTIDAS POR *SPRAY DRYING*

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Tese apresentada à Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do Título de Doutor em Engenharia de Alimentos.

Thesis presented to the School of Food Engineering of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Food Engineering

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"**E** tudo que pedirdes em
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RESUMO

Conhecer e compreender o mecanismo de formação da superfície das micropartículas obtidas via *spray dryer*, em termos de aspecto composicional, são de extrema importância na qualidade e desenvolvimento de novos produtos; portanto, a escolha do agente carreador é um passo decisivo na secagem. A modificação da estrutura da proteína, agente carreador bastante utilizado, através da hidrólise enzimática parcial pode aumentar a solubilidade da proteína e melhorar suas propriedades funcionais na formação do filme. Sendo assim, o objetivo principal deste trabalho foi avaliar a deposição de hidrolisados de proteína isolada de arroz (RPI) nas superfícies de micropartículas de óleo de linhaça e óleo essencial de laranja produzidas por *spray dryer* para verificar sua influência na permeação de oxigênio e liberação de compostos voláteis. A primeira parte do estudo consistiu em investigar os efeitos da hidrólise enzimática (DH) (2, 6 e 10%) pela protease Flavorzyme nas propriedades físico-químicas da RPI e da emulsão óleo/água utilizando diferentes concentrações de hidrolisados (0,5, 1,0 e 1,5 %). Os resultados mostraram que o DH10% aumentou a solubilidade em quase 20%. Além disso, obteve-se efeito positivo na atividade emulsificante (EAI) (31,5 m^2/g) com menor concentração de hidrolisado (0,5%) e estabilidade emulsificante (ESI) (39,49-74,22 min) nas diferentes concentrações. Em seguida, as emulsões foram secas por spray dryer e a microestrutura, composição superficial por espectroscopia de fotoelétrons de raios X (XPS) e estabilidade oxidativa das micropartículas foram investigadas. As propriedades físico-químicas dos pós também foram avaliadas e não foram afetadas pela DH e concentração de proteína. No entanto, foi encontrada uma correlação positiva entre DH e estabilidade oxidativa. O menor valor de peróxido (2,48 – 1,99 meq/kg de óleo) foi encontrado para o pó formulado com DH 10% e 1,5% de concentração de proteína durante o armazenamento. A microscopia confocal de varredura a laser e a microscopia de força atômica também permitiram identificar os efeitos dessas variáveis na estabilidade oxidativa. Por meio do XPS, observou-se que o DH10% favoreceu o acúmulo superficial de proteínas nas micropartículas. Esses resultados mostraram que o DH influenciou a distribuição dos componentes e a porcentagem de proteína na superfície, consequentemente afetando as propriedades de barreira das micropartículas. Essa influência também foi observada na superfície das micropartículas de óleo essencial de laranja produzidas por *spray drying.* As micropartículas formuladas com DH10% apresentaram maior presença de proteína (2,30%) na superfície do que as micropartículas RPI (1.07%) e consequentemente a retenção de óleo (49,97%) dobrou em relação ao RPI (23,03%). Em contraste, esta amostra apresentou alta higroscopicidade, atribuída ao percentual de proteína na superfície das micropartículas. As proteínas hidrolisadas de arroz (RPH) foram capazes de reduzir a tensão interfacial (3,78-4,04 mN/m) quando comparadas aos RPI (4,74 mN/m) e consequentemente o tamanho de gotas da emulsão (8,29-8,66 µm) e (9,06 µm), respectivamente. Assim, com base nos resultados da tensão interfacial e consequentemente no tamanho da gota, podemos supor que o RPH conseguiu permanecer na gota atomizada durante a secagem, conforme observado nos resultados de XPS. É importante determinar as correlações entre as propriedades da emulsão, a composição superficial do pó e as propriedades do produto final, uma vez que essas

correlações podem nos permitir determinar a formulação ideal de micropartículas e melhorar a proteção e a liberação controlada de compostos bioativos.

Palavras-chave: proteína de arroz, hidrólise enzimática, propriedade funcional, composição de superfície, microencapsulação, *spray drying*,

ABSTRACT

Knowing and understanding the mechanism of surface formation of spray dried microparticles, in terms of compositional aspect, are extremely important in the quality and development of new products; therefore, the choice of carrier agent is a decisive step in drying. Modification of protein structure through partial enzymatic hydrolysis can increase protein solubility and improve its functional properties in film formation. Thus, the main objective of this work was to evaluate the deposition of rice protein hydrolysates (RPI) on the surfaces of microparticles of progressive linseed oil by spray drying to verify its influence on oxygen permeation. The first part of the study consisted of investigating the effects of enzymatic hydrolysis (DH) (2, 6 and 10%) by protease Flavorzyme on the physicochemical properties of RPI and oil/water emulsion using different concentrations of hydrolysates (0.5, 1 .0 and 1.5 %). The results appreciated that DH10% increased solubility by almost 20%. In addition, there was a positive effect on emulsifying activity (EAI) (31.5 m2/g) with lower concentration (0.5%) and emulsifying stability (ESI) (39.49-74.22 min) with different concentrations. Then, the emulsions were spray dried and the microstructure, surface composition by X-ray photoelectron spectroscopy (XPS), and oxidative stability of the microparticles were investigated. The physicochemical properties of the powders were not affected by DH and protein concentration. However, a positive relationship was found between DH and oxidative stability. The lowest peroxide value (2.48 – 1.99 meq/kg of oil) was found for the powder formulated with 10% DH and 1.5% protein concentration during treatment. Confocal laser scanning microscopy and atomic force microscopy also allowed identifying the effects of these variables on oxidative stability. Through XPS, DH10% favored the superficial accumulation of proteins on the microparticles. These results showed that DH influenced the distribution of components and the percentage of protein on the surface, consequently affecting the barrier properties of microparticles. This influence was also observed on the surface of orange essential oil spray dried microparticles. The microparticles formulated with DH10% showed a higher presence of protein (2.30%) on the surface than the RPI microparticles (1.07%) and consequently doubled the oil retention (49.97%) and in relation to the RPI (23 .03%). In contrast, this sample showed high hygroscopicity, attributed to the percentage of protein on the surface of the microparticles. The hydrolyzed rice proteins (RPH) were able to reduce the interfacial tension (3.78-4.04 mN/m) when compared to the RPI (4.74 mN/m) and consequently the droplet size (8.29- 8.66 μm) and (9.06 μm), respectively. Thus, based on the interfacial tension results and consequently on the droplet size, we can assume that the RPH was able to remain in the atomized droplet during drying, as observed in the XPS results. It is important to determine correlations between emulsion properties, surface powder composition and final product properties, as these correlations may allow us to determine the optimal microparticle formulation and improve protection and controlled release of bioactive compounds.

Keywords: rice protein, enzymatic hydrolysis, functional property, surface composition, microencapsulation, spray drying.

LISTA DE FIGURAS

Capítulo 3

Capítulo 4

Capítulo 5

Capítulo 6

LISTA DE TABELAS

Capítulo 3

Capítulo 4

Capítulo 5

Capítulo 6

SUMÁRIO

CAPÍTULO 1 - INTRODUÇÃO GERAL

INTRODUÇÃO E JUSTIFICATIVA

O aumento da demanda por produtos de alto valor agregado tem afetado substancialmente as tendências do mercado global. No entanto, a incorporação de algumas substâncias a um determinado produto se torna inviável devido às limitações tecnológicas. A microencapsulação é uma técnica ou um conjunto de técnicas que permite aprisionar ingredientes ativos usando um material circundante, comumente chamado material de parede. Tal técnica é tida como uma das soluções para estabilização de substâncias, viabilização tecnológica e financeira na obtenção dos produtos (DIAS; FERREIRA; BARREIRO, 2015; FAVARO-TRINDADE *et al.*, 2010). O tamanho do mercado global de encapsulamento de alimentos movimentou, em 2022, U$ 11.896,2 milhões, e espera-se que as vendas em valor de alimentos encapsulados apresentem uma taxa de crescimento anual composta de 10,3% de 2023 a 2030 (MARKET ANALYSIS REPORT, 2023).

A microencapsulação permite o manuseio e incorporação do material ativo em uma formulação e fornece proteção contra reações adversas desencadeadas pela interação com o meio ambiente, evitando a perda de sabor e *flavors*. Além disso, pode ser usada para controlar a liberação de compostos durante o processamento, ingestão e armazenamento de alimentos (JAFARI *et al.*, 2008). Dentre tantas técnicas viáveis para a microencapsulação, o método via *spray drying* merece uma atenção especial.

A secagem por *spray drying*, método que mantém o composto ativo como fase dispersa, e é uma das ferramentas mais utilizadas para transformar uma ampla variedade de produtos alimentícios líquidos para a forma de pó. Compreender o mecanismo da formação da superfície das partículas do pó e a capacidade de controlar sua composição superficial é necessário na melhoria da qualidade e no desenvolvimento de novos produtos. Diversos estudos mostraram que a composição da superfície das partículas

dos pós influenciou significativamente as interações partícula-líquido (por exemplo, higroscopicidade e propriedades de reconstituição dos pós, tais como dispersibilidade e molhabilidade); as interações partícula-partícula (fluidez do material particulado, aglomeração); as propriedades de barreira e retenção de compostos voláteis; e desempenho de secagem (ADHIKARI *et al.*, 2009; JAYASUNDERA *et al.*, 2011a; MCCARTHY *et al.*, 2013; ROSENBERG; SHEU, 1996; SADEK *et al.*, 2016).

Além da composição superficial da partícula, outro importante fenômeno a ser considerado é a formação de poros na parede das partículas durante o processo de secagem e que tem sido amplamente relatada (DRUSCH; BERG, 2008; EDRIS *et al.*, 2016; WANG *et al.*, 2014). Embora tenha apresentado resultados relevantes em muitas aplicações, como boa eficiência de encapsulação e recuperação, a formação de poros necessita de uma abordagem mais detalhada, visto que estes poros servem como caminho para o oxigênio se difundir através da micropartícula para reagir com o material ativo, bem como permite a saída do composto volátil do interior da partícula.

Para minimizar boa parte desses problemas, existem algumas abordagens fundamentadas no processo e na ciência por trás da formação da parede das partículas. Diversas modificações no processo de secagem por *spray drying* podem ser feitas, visando aumentar a recuperação de sólidos, melhorar as propriedades de barreiras e tecnológicas dos pós obtidos. Em muitos casos, uma modificação na temperatura de saída do ar no *spray dryer* é tida como solução, mantendo a temperatura do ar abaixo de 50°C ou mesmo à temperatura ambiente (JAYASUNDERA *et al.*, 2011b). No entanto, os pós obtidos a baixas temperaturas costumam ter altos teores de umidade residual e valores de atividade da água que afetam negativamente seu posterior armazenamento. Além disso, outras soluções como o resfriamento da parede da câmara de secagem ou a

introdução de ar frio na parte inferior do secador e a varredura mecânica, são muitas vezes financeiramente inviáveis.

A abordagem baseada nos materiais de parede também tem suas próprias limitações. Os carboidratos, base principal dos produtos encapsulados, geralmente têm propriedades interfaciais ruins como, por exemplo, a capacidade emulsificante. Entretanto, ao microencapsular um material lipofílico, uma das características que o material de parede deve apresentar é a capacidade de formação de uma emulsão estável. Assim, os carboidratos devem ser modificados quimicamente para melhorar sua atividade interfacial, uma vez que são moléculas polares e não conseguem romper ou minimizar a tensão interfacial entre dois líquidos com polaridades distintas. A incorporação de carboidratos na composição de parede permite a formação de um filme e altera significativamente as características de secagem da matriz, uma vez que aumenta a temperatura de transição vítrea do material da partícula e acelera a formação de uma crosta seca ao redor das gotículas de secagem. Entretanto, a adição de quantidades elevadas desse material na composição da parede das partículas, geralmente acima de 35%, aumenta o custo e pode alterar o sabor original do produto, arriscando a desaprovação do consumidor (FANG; BHANDARI, 2011; TONON *et al.*, 2009).

Uma alternativa para melhorar as propriedades de barreira das micropartículas e a retenção de compostos voláteis é modificar as propriedades da superfície das gotículas/partículas com adição de proteínas na formulação da parede da partícula. As proteínas de origem animal têm sido extensivamente pesquisadas e utilizadas em diversos processos devido às suas excelentes propriedades funcionais. Porém, a produção e obtenção dessas proteínas tem um impacto ambiental significativo, como mostram os dados da Food and Agriculture Organization (FAO) de (2018). Sendo assim, a utilização de proteínas vegetais em diversos processos recebeu atenção

considerável nos últimos anos devido ao seu valor nutricional, disponibilidade e baixo custo, além de possuírem atividade antimicrobiana e antioxidante (BOYE *et al.*, 2010; KARACA; LOW; NICKERSON, 2011; NESTERENKO *et al.*, 2014).

Apesar disso, é interessante reforçar que no processo de microencapsulação as proteínas de origem animal são mais efetivas como material de parede quando comparadas com as proteínas de origem vegetal. As proteínas de origem animal apresentam menor tamanho molecular, o que permite uma rápida difusão e adsorção à interface de uma emulsão e posterior estabilização das gotas. Elas ainda tendem a ser mais solúveis que as proteínas de origem vegetal em uma maior faixa de pH (KARACA; LOW; NICKERSON, 2015). As proteínas de origem vegetal apresentam estrutura globular que resultam em propriedades funcionais limitadas devido a sua estrutura terciária compacta, grupos hidrofóbicos no interior da estrutura e baixa flexibilidade, tendo assim maior dificuldade em estabilizar a interface do que as proteínas menores e mais flexíveis de origem animal (NESTERENKO *et al.*, 2014; TAMM *et al.*, 2016).

Alguns trabalhos na literatura mostram que as propriedades funcionais das proteínas vegetais podem ser melhoradas através da modificação da sua estrutura. A utilização da hidrólise enzimática parcial, geralmente entre 1% e 10% de hidrólise das ligações peptídicas, pode aumentar a solubilidade da proteína e melhorar suas propriedades emulsificantes ao expor grupos hidrofóbicos, aumentando assim sua hidrofobicidade superficial. Além disso, hidrolisados proteicos têm uma vantagem cinética de migrar mais facilmente para superfície da gotícula da emulsão pulverizada durante secagem no *spray dryer* através do mecanismo de difusão e possivelmente formar um filme superficial nas partículas formadas, conferindo uma barreira de proteção, como reportado por Tamm et al. (2015). Ao comparar o efeito de proteínas e

surfactantes de baixo peso molecular na secagem por *spray drying* de soluções ricas em açúcar, Jayasunderera et al. (2011a) observaram que a proteína caseinato de sódio, que apresenta menor peso molecular e maior solubilidade, migrou mais rapidamente para a interface ar-água quando comparada com a proteína isolada de ervilha. A razão para uma maior quantidade de caseinato de sódio na interface ar-água pode ser atribuída às diferenças estruturais e de composição entre essas duas proteínas, como a solubilidade e tamanho (GOMES; KUROZAWA, 2020; TAMM *et al.*, 2016; ZANG *et al.*, 2019). A influência de proteínas vegetais nas propriedades de barreiras de filmes e partículas é ainda pouco explorada.

Na microencapsulação de óleos, a composição da superfície das partículas formadas é governada principalmente pelo emulsificante usado (DRAPALA *et al.*, 2017; MUNOZ-IBANEZ *et al.*, 2016; SADEK *et al.*, 2016; TAMM *et al.*, 2015) e a maltodextrina, material encapsulante bastante utilizado, é responsável pela estrutura da partícula. As proteínas são moléculas anfifílicas naturais com boas propriedades emulsificantes e formadoras de filmes com excelentes barreiras ao oxigênio e a incorporação delas na interface de uma emulsão pode se dar de duas maneiras: por adsorção direta na interface óleo/água ou ar/água durante a formação e estabilização de gotículas, e/ou através da interação associativa com uma camada de biopolímero existente já localizada na interface (ENCINA *et al.*, 2016; LE PRIOL *et al.*, 2019; REINECCIUS; YAN, 2016). De fato, as proteínas podem adsorver na interface óleo/água e formar um filme viscoelástico que fornece estabilidade física à emulsão durante o processo e armazenamento subsequentes. Durante a secagem, uma nova interface ar/líquido é criada e os componentes de superfície (proteínas, peptídeos, surfactantes de baixo peso molecular) presentes na emulsão migram e adsorvem na nova

interface, reduzindo efetivamente a energia livre de superfície e alcançando a estabilidade termodinâmica do sistema (MUNOZ-IBANEZ *et al.*, 2016).

O farelo de arroz é um subproduto da produção de arroz que contém 12 a 20% de proteína, rico em aminoácidos essenciais. Geralmente, este produto é descartado ou vendido com baixo valor de mercado como ração, combustível e fertilizante. No entanto, a aplicação de proteína hidrolisada de arroz pode ser viável tecnologicamente no processo de microencapsulação, como apresentado por nosso grupo de pesquisa em trabalhos recentes (GOMES; KUROZAWA, 2020).

Diante disso, o principal objetivo deste trabalho foi avaliar a deposição da proteína de arroz não-hidrolisada e hidrolisada, nas superfícies das micropartículas obtidas por *spray drying* e correlacionar a influência dos componentes da formulação sobre a retenção do ativo e as propriedades de barreira das micropartículas. Portanto, foram investigados os efeitos da hidrólise enzimática controlada (2, 6 e 10%) pela protease Flavourzyme nas propriedades físico-químicas da proteína do arroz e na emulsão óleo/água utilizando diferentes concentrações de hidrolisados (0,5, 1,0 e 1,5%) e maltodextrina (34,5, 34,0 e 33,5%) e consequentemente seus efeitos na formação e proteção das micropartículas. Para essa proposta, utilizaram-se duas matrizes diferentes que se deseja microencapsular: um óleo insaturado suscetível à oxidação (óleo de linhaça) e um óleo volátil (óleo essencial de laranja).

CAPÍTULO 2 – OBJETIVOS E ESTRUTURA DA TESE

OBJETIVOS

Entender os mecanismos associados à estabilização de emulsões e a microestrutura final de partículas secas por *spray drying* utilizando proteínas hidrolisadas para reduzir os problemas tecnológicos associados ao processo de microencapsulação e as propriedades de barreira da micropartícula.

Objetivos específicos

- Verificar a influência do grau de hidrólise da proteína de arroz sobre o comportamento interfacial de filmes adsorvidos nas interfaces óleo/água;
- Avaliar os efeitos da hidrólise enzimática do RPI nas propriedades físico-químicas e verificar o efeito nas propriedades funcionais das proteínas na emulsão óleo/água;
- Microencapsular por *spray drying* um óleo insaturado suscetível à oxidação (óleo de linhaça) e um óleo volátil (óleo essencial de laranja) usando como materiais de parede maltodextrina e proteína de arroz ou hidrolisado proteico de arroz;
- Investigar a topografia e a possível mobilidade molecular das proteínas não-hidrolisadas e hidrolisada para a superfície das partículas obtidas por *spray drying* através da microscopia de força atômica (AFM);
- Verificar a influência do grau de hidrólise da proteína de arroz na composição elementar de cada superfície das micropartículas;
- Estudar o efeito da concentração e do grau de hidrólise das proteínas hidrolisadas sobre as propriedades de barreiras das micropartículas produzidas por *spray drying* e verificar possíveis falhas na deposição dos hidrolisados na superfície das partículas, não formando um filme proteico contínuo.

ESTRUTURA DA TESE

No processo de microencapsulação por *spray drying* existem algumas abordagens fundamentadas na ciência por trás da formação da parede e superfície das micropartículas. Porém existe uma lacuna de como esses materiais podem ser manipulados para aumentar a vida de prateleira dos pós. Assim, uma alternativa para melhorar as propriedades emulsificantes do material encapsulante, de barreira das micropartículas e a retenção de compostos voláteis é modificar as propriedades da superfície das gotículas/partículas com adição de proteínas modificadas na formulação da parede da micropartícula.

Considerando que o processo de microencapsulação por *spray drying* compreende duas etapas importantes e distintas: emulsão (“fase líquida”) e secagem por *spray dryer* (“fase sólida”), as quais interferem significativamente nas propriedades das micropartículas obtidas, o trabalho foi estruturado da seguinte forma:

Capítulo 1 e 2: Apresentam uma introdução geral, com a justificativa do trabalho, objetivos e a estrutura da tese.

Capítulo 3 - Surface composition and microstructure of spray-dried microparticles: their effect on functional properties: Neste capítulo é apresentada uma revisão detalhada, que será a primeira sobre o tema, sobre a modificação da superfície das micropartículas obtidas por *spray drying*. Esse levantamento visa trazer uma melhor compreensão sobre o tema e como essas modificações podem melhorar a eficiência da microencapsulação, estabilidade do composto bioativo, qualidade do pó e design do processo de microencapsulação nas indústrias farmacêutica e alimentícia. Além disso, são destacados os fundamentos teóricos, mecanismos e métodos usados para entender a modificação da superfície de alimentos e emulsões em pó e sua influência nas propriedades funcionais.

Capítulo 4 - Performance of rice protein hydrolysates as a stabilizing agent on oil-in-water emulsions: Este capítulo apresenta os resultados obtidos na "fase líquida" com enfoque para a avaliação dos efeitos de diferentes graus de hidrólise da proteína de arroz e diferentes concentrações de proteína na formulação das emulsões utilizando um óleo insaturado como fase dispersa (óleo de linhaça). Os hidrolisados obtidos foram caracterizados e correlacionados com as características de estabilidade das emulsões.

Capítulo 5 - Compositional aspect and mechanism of surface formation of spray-dried microparticles with surface-active rice protein hydrolysates: O desempenho melhorado dos RPH nas emulsões com as mesmas concentrações (**Capítulo 4**) foram também correlacionadas com as propriedades de barreira das micropartículas de óleo de linhaça obtidas por *spray drying.* Esse estudo mostrou que o conhecimento do efeito dos hidrolisados de proteína de arroz e da concentração de proteína nas emulsões e, consequentemente, a microencapsulação por *spray drying* melhora a proteção e as propriedades funcionais das micropartículas. As análises XPS e AFM possibilitaram demonstrar que a concentração de proteína e DH podem influenciar significativamente essas propriedades.

Capítulo 6 - Rice protein hydrolysates as natural emulsifiers for an effective microencapsulation of orange essential oil by spray drying: Em nosso estudo anterior (**Capítulo 5**), foram relatados os efeitos de diferentes níveis de DH (2, 6 e 10%) e concentrações da proteína de arroz nas propriedades físico-químicas e especialmente na oxidação lipídica das micropartículas de óleo de linhaça obtidas por *spray drying*, No entanto, mudanças na composição química na superfície das micropartículas utilizando hidrolisados de proteína de arroz como emulsificante, que também devem desempenhar um papel essencial no processo de microencapsulação do óleo essencial de laranja, não

foram investigadas. Neste estudo, examinamos a influência de hidrolisados de proteína de arroz na formação de um filme de proteína em superfícies das micropartículas de óleo de laranja obtidas por *spray drying*.

O tópico **Discussão geral** apresenta uma compilação dos resultados mais relevantes apresentados nos capítulos descritos acima, bem como uma discussão com base nos aspectos aos quais a tese contribuiu para o desenvolvimento tecnológico e científico. As principais conclusões da tese estão apresentadas no tópico **Conclusões gerais.**

CAPÍTULO 3 – SURFACE COMPOSITION AND MICROSTRUCTURE OF SPRAY-DRIED MICROPARTICLES: THEIR EFFECT ON FUNCTIONAL PROPERTIES

SURFACE COMPOSITION AND MICROSTRUCTURE OF SPRAY-DRIED MICROPARTICLES: THEIR EFFECT ON FUNCTIONAL PROPERTIES

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HIGHLIGHTS

- Functional properties of powders are affected their surface composition.
- Several mechanisms of interactions may occur among microparticles components.
- Interfacial accumulation of protein at the droplets is preserved during spray-drying.
- Competitive surface migration of proteins influences the powder recovery.
- Diffusion rates of different components influence the surface composition.

ABSTRACT

Many functional properties of spray-dried microparticles are affected by the presence and composition of the materials used in the matrix and on the surface. The characteristics of these materials are an essential factor because any damage or alteration on the microparticle surface can affect the protection and retention of the bioactive-loaded delivery system and influence its interaction with the environment. There is a real gap between scientific concerns from the field and accessible reviews on the subject. This review presents a detailed description of the surface modification of spray-dried powders for improving the powder quality, efficiency of the microencapsulation, and microencapsulation process design in food industries. Additionally, the theoretical foundation, mechanisms, and methods used to understand the surface modification of spray-dried food powders are highlighted. Therefore, for a better understanding of the mechanisms involved in the particle surface formation, the application of advanced microscopy techniques and x-ray photoelectron spectroscopy (XPS) used together have offered very precise and detailed results under different conditions at the molecular level. These findings will contribute to further research improvement about of surface composition of spray-dried microparticles and how they influence on their functional properties.

Keywords: surface composition; spray drying, food powders, functional properties, surface modification, surface characterization

3. INTRODUCTION

Many of the characteristics of spray-dried microparticles are influenced by the operating conditions of the process and the composition of the carrier agents (wall materials). One of them is the surface composition that plays an important role for powder functional properties. Understanding the surface formation mechanisms and the final microstructure of spray-dried microparticles is essential for reducing technological problems associated with the drying process and powder use (storage, flow, agglomeration, dispersion, solubilization, etc.) in addition for modulating the barrier properties.

Particle surface composition has been studied rather extensively, as reported for spray-dried food protein and biopolymer formulations (Drapala et al., 2017; Jayasundera, Adhikari, Howes, et al., 2011; Nuzzo et al., 2015) and dairy-like emulsions (Ho et al., 2021; Murrieta-Pazos, Gaiani, Galet, & Scher, 2012; Xu et al., 2012, 2013). All these authors observed that the surface composition is extremely different from the bulk composition. Indeed, when a surface-active component was present in the feed solution (such as a protein), the powder surface was mainly covered by that component (Adhikari et al., 2007; Tamm et al., 2015). From these results, some authors suggested that the diffusion and precipitation rates of different components (e.g., lipids, proteins, and carbohydrates) influenced the surface composition (Fäldt & Bergenståhl, 1994; Kentish et al., 2005; E. H. J. Kim et al., 2003, 2009a, 2009b, 2009c; Sadek et al., 2016; Wang & Langrish, 2009b). Evaluating the effect of the molecular size of proteins on spray drying in sugar-rich solutions, Jayasundera et al. (2011) observed that sodium caseinate migrated faster to the air-water interface than the pea protein isolate, resulting in more sodium caseinate on the particle surface. This can be

attributed to the compositional and structural difference between these two proteins, for instance, the lower molecular weight and greater solubility of sodium caseinate.

Some other authors also showed that the surface distribution of the components could be influenced by a multitude of factors related to the process, such as the operating conditions and physical configuration of the dryer (K. Fyfe et al., 2011; Gaiani et al., 2010; Nikolova et al., 2015).

To provide a better understanding of the mechanisms involved in the particle surface formation, some techniques, such as X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM), offer accurate and detailed results under several molecular-level conditions. Moreover, there is a range of imaging modalities that help to assess the surface composition, such as transmission electron microscopy (TEM), scanning electron microscopy (SEM), and confocal laser scanning microscopy (CLSM). These combined techniques show possibilities for an in-depth understanding of surface formation behavior. This review highlights how the surface of different microparticles is influenced by the modification of wall materials and process conditions. Some experimental techniques that allow the characterization of powder surfaces are presented in this review, including the description of some works that applied these techniques to explain the functional properties of particles.

3.1.FACTORS AFFECTING POWDER SURFACE PROPERTIES

3.1.1. Surface composition

The functional characteristics of multicomponent microparticles are substantially affected by their chemical surface composition. Therefore, it is important to know the composition and structure of the carrier agents and core materials, as well as the interaction between these components before and after the drying process. The

utilization of carrier agents with a charged surface is essential to develop microparticles with desired functionality (including controlled release of the core), which is expected to be maintained during storage. In many cases, characteristics of the carrier materials (i.e., surface activity) remain on the microparticle surface, leading to excellent powder functionality (Lu et al., 2019).

However, there are some cases where the surface activity is overcome by another component due to several factors. For instance, Porras-Saavedra et al. (2018) investigated the microencapsulation of paprika oleoresin using blends of soy protein isolate (SPI), maltodextrin (MD) and gum arabic (GA) as wall materials. They observed that SPI, component with surface activity, was overcome by the presence of oil on the particle surface conferred hydrophobicity to the powder surface. Consequently, powder wettability was reduced, and the powder surface becomes sticky, reducing flowability. A strong correlation was found between lipid on particle surface and the caking ability, as reported by Nijdam and Langrish (2006). A high caking (>90%) was observed by the author when surface lipid on milk powders ranged from 5% to 30%. In contrast, caking was notably reduced (<60%) when the lipid content was lower than 3%. These works demonstrated how the particle surface composition can affect functional properties of powders and the importance of understanding the mechanisms involved in this phenomenon.

One of the reasons for differences in the surface composition is related to the diffusivity coefficient and solubility of compounds. Once a solidified crust is formed on the droplet surface during spray drying, the precipitated material no longer diffuses; thus, a lower solubility could lead to a higher concentration near the microparticle surface. Other mechanisms involved in surface composition were reported for particles containing oily phase (Nijdam & Langrish, 2005). When the fat is in a molten state

during spray drying due to exposure to high temperatures, a rich-fat surface is formed due to the convection of oil to the particle surface through a network of pores and cracks, driven by capillary forces or overpressure of internal vacuoles.

Few studies have focused on the influence of particle surface composition on encapsulation efficiency. For instance, Munoz-Ibanez et al. (2016) elucidated how the breakup of sunflower oil droplets during the atomization step in the spray-drying process influenced the localization of ingredients (maltodextrin, acacia gum, and sunflower oil) on the microparticle surface. During spray drying, the authors showed a segregation of some components using confocal Raman microscopy, once the particle surface composition differed from the bulk composition. Such surface composition depends on some variables (e.g., the emulsion size and process conditions) that must be carefully controlled and studied, because it directly affects functional properties of powder. The authors reported an improvement on oil retention due to the protective layer of the acacia gum (>0.5 μm) formed on the particle surface. In some cases, if the particle surface has high amounts of protein this can have negative effects. For example, interactions between hydrophilic groups of native casein micelles deposited on the particle surface formed an interlinked network between the aggregated powders, decreasing their ability to disperse and penetrate water (Anema et al., 2006; Baldwin & Truong, 2007).

Surface composition plays also an important role in the spray drying process, as reported for example by Foerster et al. (2016b). These authors observed how the surface composition of milk droplets pulverized into spray dryer chamber affected on droplets drying time and the water evaporation resistance, as well as droplet shrinkage behavior. According to the authors, the droplet shrinkage was lower for higher-fat emulsion and slower evaporation rate. This can be explained due to the deposition of a hydrophobic

lipid film on the droplet surface immediately after being generated, which remained until the drying process was completed. On the other hand, for low-fat emulsion, the surface droplets had a stoichiometric amount of fat relative to the bulk composition. In addition, the absolute concentration of fat on droplet surface was lower than when compared with protein concentration. An increase in the protein concentration on the droplet surface during drying presumably occurred by diffusion due to its surface activity. Consequently, this protein-rich film conferred a more hydrophilic and flexible characteristic on the microparticle surface, making the surface of the low-fat model emulsion droplets more susceptible to shrinkage and more permeable to moisture evaporation.

3.1.2. Molecular forces and interactions

The constituents of the continuous matrix of spray-dried microparticles, also known as wall material, interact with each one and the core material in a complex manner, influencing the structure, quality, and process of many food powders. Usually, these interactions may occur during feed solution preparation, consisting of the homogenization of wall materials and active compounds, or/and during the drying of atomized droplets in the dryer chamber. In this case, water molecules are replaced by other components via different interactions, modifying or improving the quality of microparticles (Soltanizadeh et al., 2014). The interactions among these components can occur by different mechanisms, such as electrostatic interactions, van der Waals, hydrogen bonding and hydrophobic interactions. A series of studies was undertaken to understand how molecular forces influence the particle structure and surface (Firoozmand & Rousseau, 2015; Grabowska et al., 2014; Sarbon et al., 2015).

Proteins and polysaccharides could form complexes by covalent bonds and/or non-covalent bonds in two ways, as showed by Ji et al. (2015). Complexation by covalent bonds, also known as Maillard reaction, is a mechanism that occurs mainly due to the nonenzymatic glycosylation reaction between the free amino groups of proteins and the aldehyde groups of reducing sugars. This reaction usually involves thermal denaturation of protein and the addition of polysaccharide as a Maillard cross-linking agent (Consoli et al., 2018). Augustin, Sanguansri, and Bode (2012) confirmed that Maillard reaction products obtained by heating a mixture of protein sources and carbohydrates had a positive influence on encapsulation efficiency. Non-covalent bonds include hydrogen bonds and electrostatic attraction. Generally, uncharged polysaccharides can form complexes with proteins mainly by hydrophobic interactions, whereas in ionic polysaccharides, the complexes are formed by electrostatic interactions. Some other authors hypothesized that carbohydrates replace water molecules bonded to protein to maintain the secondary protein structure.

Studies have shown that proteins usually possess a high binding capacity in active compounds and consequent encapsulation efficiency (Baranauskiene et al., 2006; Pierucci et al., 2006; Rosenberg & Young, 1993). Pierucci et al. (2006) noted that the retention of ascorbic acid in pea protein/carbohydrate microparticles was influenced by electrostatic interactions between core material and proteins.

On the other hand, Carpenter and Crowe (1989) reported that the interaction between dried proteins and carbohydrates via hydrogen bonds preserved the labile proteins during drying. This is because after eliminating water, the formation of hydrogen bonds between proteins and carbohydrates stabilizes proteins during drying or freezing (Broadhead et al., 1992; Imamura et al., 2003; Labrude et al., 1989). Moreover, higher protein content on the particle surface increased the wetting time (Carpenter &

Crowe, 1989). The hydrophobic interactions resultant from protein-protein association can be responsible to reduce solubility (Murrieta-Pazos et al., 2011). Additionally, the presence of proteins in the outer shell favored elasticity. Such particle characteristics may be favorable in requiring longer times to release the microencapsulated bioactive compound.

Evaluating microparticles of anhydrous milk fat encapsulated with whey protein, Rosenberg and Young (1993) observed a softening of the microparticle wall. According to the authors, this result can be linked to the relatively high hydrophobicity of whey proteins that permitted the interaction with the apolar core material. Thus, it is vital to focus on the interactions among the wall materials and core materials in multicomponent systems during spray drying, since these interactions affect the surface composition of powders.

3.1.3. Spray drying conditions

Recent studies have demonstrated that drying conditions can influence the surface powder composition. The outlet drying air temperature (Tout) could potentially influence the surface powder composition in different ways, as reported in the literature: (a) by interrupting the diffusion of proteins and phospholipids to the particle surface during drying at Tout>110°C before equilibrium, changing the powder surface composition (Gaiani et al., 2010); (b) by increasing drying rates which accelerate solidification (crusting) of the drying droplet surface, impairing molecular segregation (Nikolova et al., 2014, 2015) . In this latest study, the authors reported a decrease in the carbon-oxygen ratio on the particle surface of spray-dried of skim milk when the outlet air temperature increased, causing a more hydrophilic particle surface due to the presence of less carbon and more oxygen.

In contrast to the works cited above, Masum et al. (2020) reported that inlet-outlet temperature combinations (180-80, 180-90, 180-100, 200-80, 200-90, and 200-100) did not influence the chemical surface composition of infant milk formula powders. The particle surface was covered mainly by lipid (41-44%), followed by protein (31-33%) and lactose (24-26%), irrespective of the inlet-outlet temperature combinations. So, it becomes evident the difference in the powder surface composition can be also attributed to the characteristics of the components of feed solution, not just the temperature, (e.g., surface-active, differences in density, and capacity to diffuse through the powder matrix), which caused the segregation during spray drying.

A synergistic effect between air temperatures with feed solids content on surface particle composition can occur. Higher feed solid contents increase the viscosity, while higher temperature caused a rapid crust formation, reducing the redistribution of components within the atomized droplet. For the spray drying of skim milk and whole milk (E. H. J. Kim et al., 2009b) higher drying temperature and feed solid content resulted in less protein and lipid on the powder surface. In this same work, the authors also reported an opposite relation between the degree of homogenization and the quantity of fat on the surface particle, because higher degree of homogenization decreased the fat droplet size.

The change in surface composition of the milk protein particles was also related to differences in the type of atomization systems, affecting some techno-functional properties of the powders (Augustin et al., 2012; K. Fyfe et al., 2011; Nuzzo et al., 2017). There are several reasons for these differences, e.g., the pilot and laboratory spray dryers used a two-fluid nozzle, producing finer particles than the rotary atomizer used in commercial dryers; the extent of mechanical shear that contributed to lipid particle disruption and spreading; and the number of drying stages in the individual

drying systems, thus influencing the surface composition of powders. Depending on the scale of the dryer, the protein/lactose ratio, and the stress caused by feed atomization affect fat globule rupture, spreading fat on the particle surface.

Several researchers have used conventional 2-fluid nozzle (2FN) spray drying for the encapsulation of compounds. On the other hand, the novel 3-fluid nozzle (3FN) spray drying that involves the use of three concentric channels in which two different fluids are flown through two separate channels, has been used and presented differences in relation to surface composition and morphology of powders (Nimbkar et al., 2023). The development of sodium alginate and carboxy methyl cellulose microparticles for co-delivery of curcumin and resveratrol through conventional two fluid nozzle (2FN) and three fluid nozzle (3FN) spray drying showed difference in the morphology, release, and consequently surface composition. Overall, earlier shell formation in the 3FN process as compared to the 2FN process resulted from increased Péclet number (where evaporative flux dominates diffusion rate), controls diffusion of inner feed resveratrol to the surface and thus densifies resveratrol at the core. Whereas, in the 2FN process, the bioactive diffuses throughout the solid particle due to low Péclet number resulting from balanced evaporation and diffusion rates (Maria Leena et al., 2020).

3.2.CHARACTERISTICS OF PARTICLE SURFACE MORPHOLOGY

The surface morphology of spray-dried microparticles is affected by various parameters, such as the drying rate, the content of solids in the feed solution, and the viscoelastic and composition properties of the adsorbed surface film of the drying droplet. The correlation between surface particle composition and morphology has been studied rather extensively for spray-dried dairy-like emulsions (Murrieta-Pazos, Gaiani, Galet, & Scher, 2012; Nikolova et al., 2014; Wu et al., 2014) and biopolymer

formulations and other food protein (Elversson & Millqvist-Fureby, 2006; Fäldt & Bergenståhl, 1995; Munoz-Ibanez et al., 2016; Nuzzo et al., 2015).

Porras-Saavedra et al. (2015) reported the effect of the surface distribution of components through a descriptive model and consequent microparticle structure. In this study, the particle surface of gum Arabic (GA) was smooth, while powders samples of soy protein isolate (SPI) and maltodextrin (MD) were rough and dented. In the case of the SPI/MD/GA blend, the surface was changed, and the particles tended toward spherical shapes with a smooth surface. The authors also proposed that microparticles encapsulated with SPI blends and high maltodextrin content presented a characteristic of a folded rough surface with narrow crests. As the protein has higher surface activity, it tends to migrate more quickly and precisely to the surface of the particle. Thus, these results can be due to the formation of superficial protective film on the droplets in the initial drying stage, followed by film (Porras-Saavedra et al., 2018). Proteins were bonded by hydrophobic interactions and showed low lateral mobility evidencing this rough surface.

Nuzzo et al. (2015) investigated how the formulation composition and properties of wall material components influence the morphology of spray-dried particles. A set of surface-active polymers, hydroxypropyl methylcellulose (HPMC), bovine serum albumin (BSA), and copolymer poloxamer, combined with lactose, were analyzed for their influence on particle structure and their dynamic surface characteristics in formulation. The particles at low concentrations of BSA were spherical with a few dented and ridged wrinkles, and as the protein concentration increased, the morphology presented dents and ridges increasingly. Fäldt and Bergenståhl (1994) indicated that the surface chemical composition of the microparticles was due to the presence of a protein

that seems to cause dents. Regarding the morphology of particles with poloxamer, no effect of concentration was observed, and the particles were spherical.

Different plant protein (soybean protein isolate, sunflower protein, pea protein isolate, hemp protein, and brown rice protein) used as carrier agents for the encapsulation of sunflower oil by spray drying affected significantly the microparticle morphology and surface regularities (Le Priol et al., 2019). Some works suggest that the surface-active compounds are overrepresented on the powder surface, since these components modify the morphology/structure and adsorb to the surface of droplets during spray-drying (Chew et al., 2015; Nikolova et al., 2015). It can be concluded that the characteristics of the macromolecular compounds are vitally important for the surface structure and morphology of spray-dried particles.

3.3.MECHANISM OF THE POWDER SURFACE FORMATION

Various mechanisms have been studied and discussed on particle surface formation and, consequently, how they affect its composition (Drusch et al., 2012; Kentish et al., 2005; E. H. J. Kim et al., 2003, 2009a, 2009b, 2009c; Sadek et al., 2016; Wang & Langrish, 2009b). Three main hypotheses originating from experimental and theoretical studies have been proposed to explain them (Charlesworth & Marshall, 1960; Fäldt & Bergenståhl, 1994; Meerdink & van't Riet, 1995). All these hypotheses provide key answers and are complementary to explain how components are driven to the interface. These findings were also summarized in a review published by Wang and Langrish (2009a) and Chen et al. (2011). According to the authors, at the beginning of drying, droplet drying is controlled by the diffusion of molecules water from the surface toward air, while peptides/proteins are progressively concentrated at interface (air-liquid), forming a protein-rich film. Although this mechanism has been described for

dairy products, there is little doubt that such mechanisms are generic and can be applicable for other microencapsulated products.

This diffusion process can almost instantly precipitate proteins upon initial heating at the air-liquid interface, causing a decrease in the local protein concentration near the droplet surface. Then, the concentration gradient causes the diffusion of proteins from the internal region to the surface of the droplet. It also corroborates the hypothesis suggested by Fäldt and Bergenståhl (1994), in which surface active compounds dominate the surface composition of the final microparticle and accumulate at the interface (air-liquid). The authors, therefore, proposed that preferential accumulation should occur at the surface regarding the active surface characteristics of each molecule, leading to higher protein concentration on the particle surface compared to less active surface components (e.g., fat and lactose) and the bulk composition. As the molecular diffusivity decreases as molecular weight increases, substances with low diffusivity also display low solubility. Thus, the outermost layer of particles is dominated by small and initially soluble molecules. In an attempt to quantitatively assess the mechanisms mentioned above, Wang and Langrish (2009b) and Wang (2011) concluded that the surface activity of proteins and molecular diffusion exerted the most outstanding effects on component segregation.

The investigation of low-fat model emulsion behavior during drying of single droplet also provided a better understanding into the protein diffusion/migration phenomenon (Foerster et al., 2016b). In the feed emulsion containing 0.5% v/v fat, 41.8% v/v protein and 57.7% v/v lactose, the fat component did not control the particle surface. On the other hand, a significant increase in protein surface concentration during the drying process was observed. According to Foerster et al. (2016a), the protein migration was probably dominated mainly by diffusion since the time for caseinate

molecules to diffuse possibly is within the range of the single droplet drying time scale. Some phenomena could have caused this diffusion. First, the radial moisture gradient from the internal part of the droplet toward the surface, induces the diffusion of protein, fat, and lactose in a opposite direction (toward the droplet center).

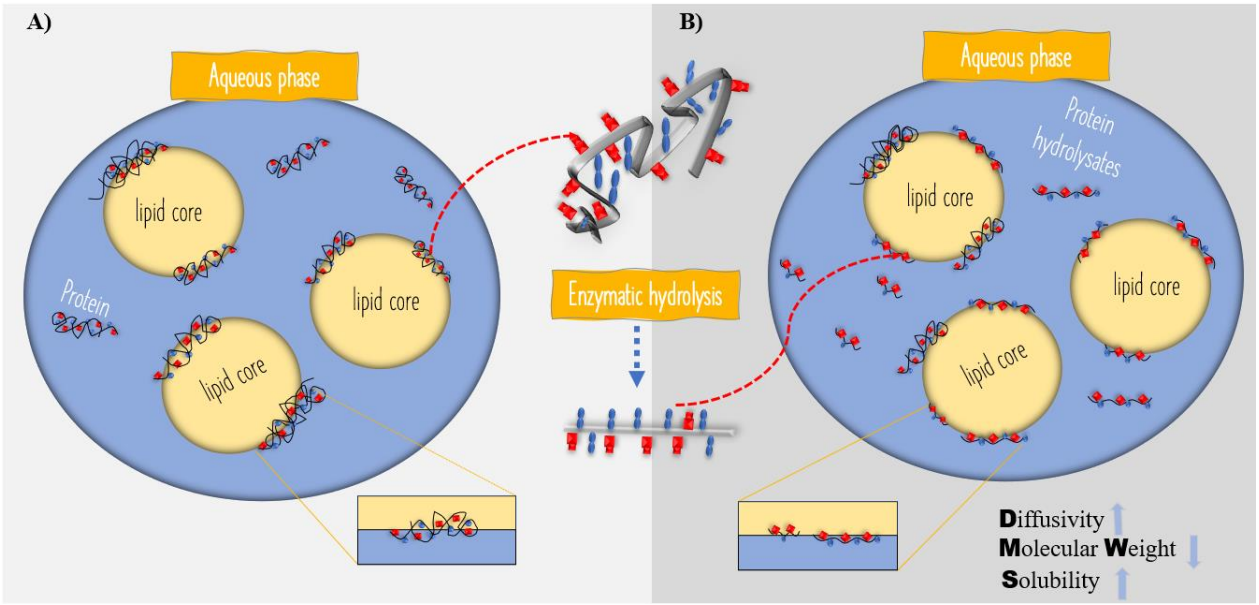
As suggested by several authors (Grasmeijer et al., 2016; Grosshans et al., 2016; Meerdink & van't Riet, 1995; Porowska et al., 2016), component segregation can be explained by several transport velocities that depend on the diffusion coefficients and concentration of each component. The diffusivity rate of lactose exceeded that of caseinate by approximately two orders of magnitude. Compared to the lactose concentration, it might have led to the increase of protein in almost all outer droplet regions. Second, caseinate molecules are surface-active adsorb at the interface (air-water) of the droplets (Fäldt & Bergenståhl, 1994). Thus, this protein adsorption at the surface decreased the free protein responsible for diffusion movement toward the droplet center.

3.4.SURFACE MODIFICATION OF SPRAY DRIED FOOD BY USING DIFFERENT TYPE OF PROTEIN

Protein/surfactant layers adsorbed on the interfacial area effectively stabilize thin films between emulsion droplets due to their hydrophobicity, electric charge, high elasticity, and thickness. Figure 1 shows changes in the conformation of the protein caused by enzyme action may affect its diffusion rate, and the amphiphilicity of protein/peptides on an oil–water interface. This balance of hydrophilic and hydrophobic groups allows a favorable intermolecular interaction between the protein and the phases in the colloidal system (e.g., lipid), indicating its faster diffusion towards the oil/water

interface and remaining on the microparticle surface (Gomes & Kurozawa, 2023). Besides that, this treatment increases the protein solubility.

Figure 1. Mechanisms for emulsion stabilization using hydrolysates protein. A) A depiction of globular proteins migrating to the water–oil interface B) The peptides reorientation and film formation on the interface influenced by molecular weight, diffusivity, and solubility. The red dots represent hydrophobic moieties found in proteins whereas blue strands represent hydrophilic. Aqueous phase represents water and maltodextrin.



Several studies reported that an interfacial accumulation of protein/surfactant on the atomized droplets is preserved during spray-drying (Elversson & Millqvist-Fureby, 2005; Fäldt & Bergenståhl, 1994; Landström et al., 2000; Munoz-Ibanez et al., 2016). After stabilization of the protein at the oil/water interface, it remains during drying. Furthermore, during the drying process, proteins that are still dispersed in the solution/emulsion can migrate to the liquid-air interface. It is known that the protein coverage on spray dried particle surface depends not only on the amount of compounds adsorbed but also on their types and structures and the other surface-active compound present in the formulation (Fang et al., 2013). Several studies investigated the influence

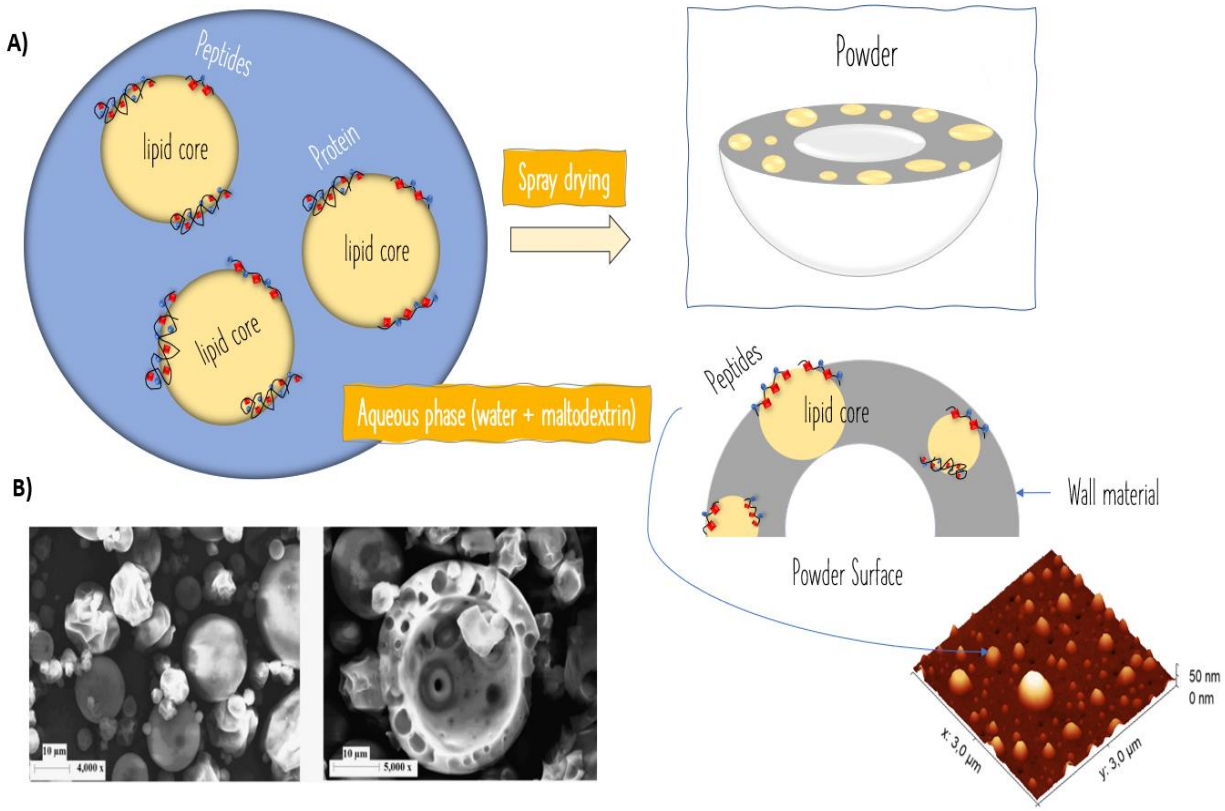
of protein molecules and small-molecule surfactants (i.e., Tween, Span, etc.) on spray drying of sugar-rich foods and found that the presence of surfactants significantly reduced the surface coverage of proteins (Adhikari, Howes, Wood, et al., 2009; Adler et al., 2000; Jayasundera, Adhikari, Adhikari, et al., 2011; Jayasundera, Adhikari, Howes, et al., 2011). Surfactant molecules often displace proteins at the interface due to their low molecular weight, ability to fit in the interface, and strong affinity toward the air/water interface (Dickinson, 2011; Diftis & Kiosseoglou, 2004; Jayasundera, Adhikari, Adhikari, et al., 2011; McClements & Jafari, 2018).

For example, Adhikari et al. (2009) studied the influence of competitive surface migration of protein molecules (sodium caseinate and hydrolyzed WPI) and small-molecule surfactant Tween-80 on powder recovery of sticky sugar-rich food. The authors verified a significant reduction on powder recovery (from ~85% to zero) when sodium caseinate and hydrolyzed WPI were replaced by Tween-80 These results indicated that surfactant molecules partially displaced the proteins from the droplet surface. This is because during the spray drying of solutions and emulsions containing proteins, the initial formation of glassy protein films on the particle surface minimizes the attachment of sprayed droplets and powder to the spray dryer walls (i.e., increases powder recovery). Although surfactants are efficient in stabilizing emulsion droplets and reducing interfacial tension, they cannot form a glassy film on spray dried particle surface, reducing the protection of microencapsulated compounds and conferring poor technological properties to the powders.

In addition to the improvement on powder recovery, the glassy protein film on particle surface prevented flavor release, minimized the permeation of both oxygen in the particle and water from the surrounding environment, and reduced the adhesive interactions of the particles on the drying wall (Adhikari et al., 2007; Tamm et al.,

2015). Figure 2 represents a structural arrangement of carrier agents and core material on the surface powder. The AFM analysis helped to understand hydrolyzed protein's role in forming microparticle surfaces, as observed by Gomes and Kurozawa (2024). The scheme considers the structure formed immediately after spray drying and after a possible reorganization of RPH on the particle surface, characterized by a "sharp" aspect.

Figure 2. Schematic distribution of components on the surface of microparticles obtained by spray drying. A) How the protein remains on the microparticle surface after the spray drying process and observed by AFM; B) External (on the left) and internal (on the right) microstructures of powders produced with Maltodextrin:Hi-Cap and the core material linseed oil (Carneiro et al., 2013).



The improvement in the interfacial mobility or molecular flexibility of a protein or surfactant tends to improve its ability to displace or exchange with another protein. Recent progress in understanding the interactions and competitive adsorption of mixed

systems of surfactants and proteins at fluid interfaces has been reported by several authors (Day et al., 2010; Dickinson, 2011; Kotsmar et al., 2009). For example, some works reported higher protein content on spray dried particle surface when hydrolyzed proteins (peptides) were used in the solution formulations (Drusch et al., 2012; Tamm et al., 2015).

This behavior can be attributed to reducing peptide size. Xu et al. (2012) reported that hydrolyzed whey proteins presented lower surface activity (measured by surface tension) when compared with intact protein. This means that peptide tends to have higher surface activity and preferential migration to the surface of microparticles, forming a thin protective layer (Figure 1). As the residence time of atomized droplet during drying process is very short, the emulsifier surface adsorption is adopted to be mainly diffusion-controlled (Landström et al., 2000). Thus, an increase in the content of small peptides results in higher nitrogen content on the particle surface.

Many proteins have been used as alternatives to conventional carrier agent on spray drying. One of the most used are maltodextrins. Once this carrier agent is used in a large amount (often in total solids >35%), it alters the risks consumer disapproval and powder quality. To reduce the stickiness problem caused by sugar and organic acid-rich foods, small quantities of protein in the sucrose solution were enough to overcome the sticky interactions of the spray drying microparticles on the dryer chamber wall (Adhikari, Howes, Bhandari, et al., 2009). In this study, 0.5% (dry basis) sodium caseinate or whey protein isolate (WPI) was sufficient to increase the powder recovery for sucrose to over 80%. The efficiency of maltodextrin and WPI in overcoming the stickiness problem during drying of bayberry juice was compared. As a result, a smaller amount of protein (1%) was sufficient to obtain higher powder recovery (>50%) when compared to maltodextrin (>30%), both were used separately (Fang & Bhandari, 2012).

This is attributed to two reasons. First, protein preferably migrates to the droplet-air interface driven by its surface activity. Second, a thin and glassy protein-rich film is formed soon after contacting the drying air. The film minimizes both the particle-to-particle and particle-to-wall chamber dryer stickiness by raising the glass transition temperature of the surface layer.

3.5.MICROSCOPY TECHNIQUES FOR CHARACTERIZING THE PARTICLE SURFACE

The term 'microscopy' typically refers to techniques enabling the visualization of substances invisible to the naked (normal) eye. There is a range of imaging modalities to assess the morphology/surface and microstructure of microparticles; some are more versatile and efficient, such as transmission electron microscopy (TEM), scanning electron microscopy (SEM), atomic force microscopy (AFM), and confocal laser scanning microscopy (CLSM). Optical microscopy is the most uncomplicated technique for assessing the surface microstructure and morphology of microparticles of various compounds and carrier agents compared to other types of microscopes. Figure 3 shows the benefits and shortcomings of each microscopic technique to assess the properties and characterization of microparticles.

Figure 3. Comparison between different microscopy techniques.

	MICROSCOPY TECHNIQUES			
	SEM	TEM	AFM	CLMS
	Utilization of high-energy electron beams		Application of a scanning probe	Optical
Sample preparation	Easy to difficult	Easy to difficult	Easy	Ease of application at ambient conditions
Cost	Medium	High	Low	Low
Time for image	(0.1 - 1 min)	(0.1 - 1 min)	(1 – 5 min)	Immediately
Field of View	1nm	100nm	100nm	The resolution limit is highly dependent on specimen or specimen preparation (µm)
Depth of Field	Good	Good	Poor	
Sample environment	Vacuum	Vacuum	Vacuum/Air/Liquid	-
Maximum magnification	100,000×	1,000,000×	It is highly dependent on sample, imaging mode, environment, etc	100× (objective lens)

Generally, more than one microscopy imaging technique is essential to reach a complete set of information on any given scientific question. The techniques are complementary as they provide information on different magnification and from distinct areas of the compounds. Table 1 presents a summary of combining complementary methods to understand the development of particle microstructure during the spray-drying process, impacting the design of high-quality food, drugs, and biotechnological products.

Table 1. Combinations of different microscopy techniques to characterize bioactive-loaded micro/nanocarriers and powders.

Combined Microscopy techniques	Compounds	Comments	References
AFM and SEM	β-carotene, maltodextrin DE20, and gum arabic	✓ The SEM was used to show that microparticles obtained with major protein content on the surface has been associated with the formation of wrinkled particles and increased dents; ✓ Through the AFM, it was observed the emulsification technique modifies the topographic surface of microparticles, specifically through the formation of pores.	(Villalobos-Castillejos et al., 2021)
SEM and CLSM	Infant milk formula powders	✓ SEM was used to assess qualitatively changes in the microstructure of powders; ✓ CLSM was used to verify the surface composition of powders related to the fat and protein distribution.	(Phosanam et al., 2020)
SEM and AFM	Blend of chia oil with soy protein isolate and maltodextrin DE20	✓ It was possible to observe that rotor-stator homogenization generated broken particles through SEM; ✓ The information exhibited by the 3D plots obtained by AFM showed smooth surface samples, and samples displayed rough surfaces with nanopores with diameters ranging from 50 to 100 nm and depths from 10 to 50 nm.	(Pereyra-Castro et al., 2018)
SEM and CLSM	Milk powder stabilized with λ-carrageenan	✓ SEM showed the particle surfaces obtained from emulsions without carrageenan or with low concentration thereof featured convex bumps of 2-4 μm in diameter; ✓ CLSM was used to observe the protein and fat distributions on the spray-dried particle surface. Overall, particles were covered by a fat layer, and the thickness of the fat surface layers varied greatly based on the carrageenan content.	(Foerster et al., 2017)
SEM and CLSM	Blends of soy protein isolate, gum arabic and maltodextrin DE20	✓ SEM and CLSM were used to analyze the internal structure of microparticles by applying mechanical force to break them. It was possible to observe compact or hollow particles with a continuous matrix wall, also the internal zone of the hollow microparticles, and the wall	(Porras-Saavedra et al., 2015)

		thickness.	
SEM and optical microscopy	Sunflower oil, maltodextrin DE12 and Acacia gum	✓ The optical microscopy of particles showed the overall morphology and the presence of vacuoles in the particles. More detailed morphology studies were conducted using SEM, whereby the internal distribution of the oil droplets was observed in sectioned particles.	(Munoz-Ibanez et al., 2016)
SEM and TEM	Cocoa powder	✓ SEM images were used to evaluated cocoa powders during storage and revealed the present a smooth surface and a significant quantity of surface fat. Pores were also observed by TEM, indicating the possibility of fat release from the core to the surface during powder storage.	(Jacquot et al., 2016)
SEM and AFM	Micellar casein powders	✓ SEM captured the particle structure and surface topography. Particle sizes were heterogeneous, and their surface appeared smooth despite the presence of dents. Fresh and aged powders presented similar surface topography by SEM. ✓ However, AFM topographical measurements revealed increased surface roughness during powder aging. These small variations were not discernible on SEM observation because perhaps the iridium coating may have overlay differences in surface profiles in fresh and aged powders.	(Burgain et al., 2016)
SEM and CLSM	Soybean oleosomes with maltodextrin DE of 16.5–19.5	✓ Highly agglomerated particles were observed by SEM that are partially merged to undefined shaped structures and, in some cases, there are spherical particles with crinkled surfaces that exhibit a large degree of size distribution, ranging from 1 to 14 μm; ✓ Since SEM is restricted to the powder surface, CLSM was used to examine the bulk microstructure and obtain information about the powder composition, particularly regarding the location and amount of the encapsulated oil phase.	(Maurer et al., 2015)
SEM and AFM	Skim and whole milk powders	✓ SEM was used to observe the surface microstructure of the powders. Some particles presented a uniform surface, whereas other particles were characterized as a "brain type" surface with some deep and shallow folds. ✓ Concurrently, AFM was performed on the same powders to enhance the analysis of the particle surfaces. The AFM images obtained by 3-D projection completely agreed with those obtained by	(Murrieta-Pazos et al., 2011)

		SEM.	
SEM and CLSM	Reconstituted milk powders (skim milk, whole milk, and milk protein concentrate powders)	✓ SEM was used to show structures of skim milk, milk protein concentrate, and whole milk powders. They were quite different and there were some minor surface morphological differences among dryer types; ✓ CLSM image showed the fat was in the liquid state while drying, causing the spreading of liquid fat-oil (nanometer-scale thin film) on the surface.	(K. Fyfe et al., 2011)
SEM and AFM	Milk protein concentrate (MPC)	✓ After storage for 30 days at 25 °C and 66%RH, MPC powders were analyzed for surface electrostatic forces and/or hydrophobic forces responsible for the adhesion of particles to mica and graphite surfaces using AFM; ✓ SEM investigated the MPC microstructure.	(K. N. Fyfe et al., 2011)
SEM and CLSM	Gum Arabic and maltodextrin DE11 with some additives such as decaglycerin mono- laurate (surfactant), ethanol, gelatin, and D-limonene.	✓ Powder morphology was observed by using CLSM and SEM to investigate the effects of inlet-outlet air temperature and formulation on the particle morphology; ✓ Using SEM, the location of D-limonene emulsion can be clearly identified in the wall matrix; ✓ CLSM images quickly identified the vacuoles in the particles.	(Paramita et al., 2010)

3.5.1. Electron microscopy: Classical SEM and TEM

SEM has been widely used to obtain information about the particle surface topography and morphology of particles as a potent surface imaging equipment (Jayasundera et al., 2009; E. H. J. Kim et al., 2009c; Shamaei et al., 2017; S. Yang et al., 2012; Zhang et al., 2014, 2015). Briefly, the SEM apparatus uses an electron beam instead of light to form an image. In several situations, it is essential to cover the particles with a layer of gold or carbon to make them assume conductive properties. It is important to emphasize that the application of an electron beam occurs in a high vacuum environment since the presence of some gas molecules in the particle environment induces electron scattering leaving the beam.

The internal structures of microparticles can be evaluated by SEM; however, mechanical force, for instance, must be applied to break them. Carneiro et al. (2013) observed that microparticles of flaxseed oil presented void spaces and that the active material was incorporated as tiny droplets in the wall material matrix. The SEM technique was also used by Porras-Saavedra et al. (2015) to visualize the thickness of the wall and the internal structure of hollow microparticles within a continuous matrix wall. The distribution of the compounds (oil droplets) and the internal particle structure was observed in broken particles by SEM (Munoz-Ibanez et al., 2016). The authors showed that the oil droplets are not close to the surface but distributed throughout the entire thickness of the shell. This type of microscopy can exceptionally demonstrate a highly great field of view with high resolutions down to a few nanometers, making it an indispensable tool. Despite the benefits, SEM is not a good method for characterizing the internal microstructure of the microparticles powder and possesses lower resolution than techniques such as TEM and AFM (Sarabandi et al., 2020).

TEM technique operates on the same principles (e.g. light microscope) but uses an electron source, while other techniques use of a light source. Its application and utilization in food powders is not very extensive compared to SEM due to some disadvantages, such as a time-consuming and costly process, and difficulties in sample damage, preparation, and structural changes of samples during the preparation, and the relatively limited field of vision (approximately 3 mm in diameter). Despite these disadvantages, TEM technique has been used to verify particle morphology and surface structure (J. Yang & Ciftci, 2017) and changes in particle microstructure during digestion (Park et al., 2019).

3.5.2. Atomic force microscopy (AFM)

Atomic force microscopy (AFM) investigates sample surfaces without using lenses or photons and operates using mechanical scanning and atomic forces, in which the basic idea is not to collect data for images by "looking" but "feeling". AFM has been applied in chemistry, material science, biological science, and recently food science (Lin et al., 2017; Oymaci & Altinkaya, 2016; Sow et al., 2017). This method obtains three-dimensional (3D) topographic images and structural details of the samples using a cantilever with a tip scanning a surface. According to the region of the force field between the sample surface and the tip during AFM, and the operation modes, it can be classified into three types (contact, noncontact, and tapping modes). Based on the three primary AFM modes, many modes have been used to study biomolecules and are widely reported in the literature, but there is still much research focused on enhancements in AFM electronics, experiments with cantilever size, and data interpretation (Zhong, 2011; Zhong & Yan, 2016).

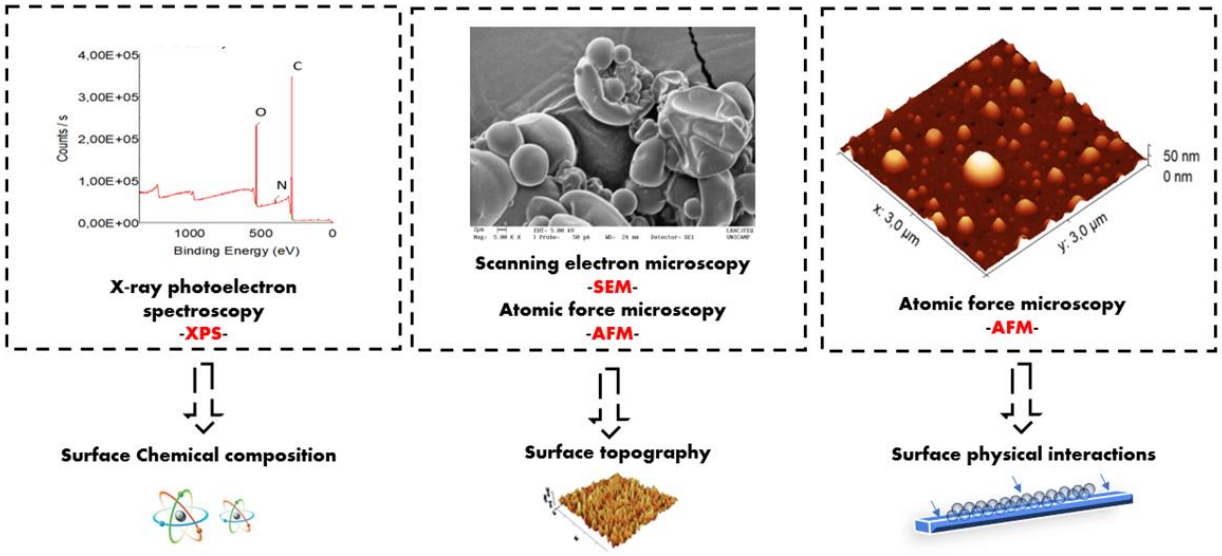
AFM provides an excellent opportunity to know and study the microstructural and physical characteristics of the microparticle powders. This technique presents several advantages when compared with other microscopy imaging techniques (e.g., SEM and TEM), including the following: it is a nondestructive and high-resolution imaging tool; it is not needing complex sample preparation; structural accuracy at the molecular level; and it can operate in liquid or air while obtaining useful information on a wide range of properties from the sample. The unique preparatory requirement is to fix the samples firmly to a supporting surface, avoiding the removal of the sample by the probe (Liu & Wang, 2011).

Different studies have applied this technique to evaluate particle size, surface topography, fissures, or breakages of particles due to these mentioned benefits of microscopy (Pereyra-Castro et al., 2018; Preetz et al., 2010). Through AFM imaging, Pereyra-Castro et al. (2018) observed that particles of chia oil encapsulated with maltodextrin and soy protein isolate exhibited a rough surface containing nanopores with diameters ranging from 50 to 100 nm and depths from 10 to 50 nm. Such particle characteristics influence functional and technological aspects (Acosta-Domínguez et al., 2016). Murrieta-Pazos et al. (2011) evaluated the surface of two dairy powders presenting low and high-fat surface coverage by measuring the average surface roughness of 306 nm and 146 nm for skim milk and whole milk powders, respectively. AFM topographic measurement (contact mode) showed changes in surface roughness during powder aging, which was not possible by SEM observation. This was because the iridium coating applied to the sample for the SEM assay was thicker than 5 nm, which can have caused overlay differences in the surface profiles of fresh and aged micellar casein powders. In contrast, AFM is highly sensitive to very fine topographical deviations (Burgain et al., 2016). AFM imaging was also able to accurately show the

topography of spray-dried skim milk particles, identified by small edges dispersed irregularly and that looked like tiny crystals (Nikolova et al., 2014).

AFM is also a powerful tool used to measure molecular forces and interactions and the previously mentioned benefits (K. N. Fyfe et al., 2011; Liu & Wang, 2011; Murrieta-Pazos, Gaiani, Galet, Calvet, et al., 2012). Some researchers have found that during storage of milk protein concentrate (MPC) powders, the surface powders become more hydrophobic due to interactions between proteins, thus decreasing the powder functionality (e.g. rehydration properties) (Havea, 2006; Mimouni et al., 2010). Using the AFM technique, Fyfe (2011) showed that the bonding state of components, mainly protein, in the region close to the surface indicated an increase in hydrophobicity at the surface, associated with an increase in nonpolar bonds. In Figure 4, is presented an example of a multiscale surface investigation.

Figure 4. Techniques for surface and morphology characterization at different levels



3.5.3. Confocal laser scanning microscopy (CLSM)

Confocal laser scanning microscopy (CLSM) belongs to the family of high-resolution optical microscopy. It has been used to visualize distribution components on

particles, using conjugate focusing technology to process various points on the focal plane of the fluorescent marker specimen. The main advantage of CLSM is that it is a nondestructive technique that enables observation of the internal structure of particles without fragmentation. The capability of CLSM to noninvasively visualize a wide range of micro- or nanoparticles and liquid systems help to accurately characterize the some attributes of these samples, such as microencapsulation efficiency, particle powder deformation, stability, surface roughness, particle size and distribution, and the release behavior of loaded bioactive substances (Drusch & Berg, 2008; Falsafi et al., 2020). Moreover, CLSM allows characterization and visualization of the internal microstructure of the samples, provided that the material is sufficiently transparent and can be fluorescently labeled.

Some research has been conducted to evaluate the distribution of fat and protein on particle surfaces. Recently, Sarkar et al. (2016) used CSLM to observe the close packing of fat droplets in spray-dried emulsions with ultra-high oil content and cross-linked whey protein as an emulsifier and matrix material. The impact of infant milk formula powder storage on the surface composition was evaluated using CLSM (Phosanam et al., 2020). In this study, the lipid components were initially embedded in the powder shell and tended to migrate toward the particle surface when stored at higher RH and temperature. Drusch and Berg (2008) showed how emulsion droplets are distributed in powders obtained by spray drying using CLSM. The authors found that particles with a higher fat content had higher levels of surface fat and fat droplets near the surface than particles with a lower fat content. It is worth mentioning that the protocol for component labeling involves using water or solvents processing steps that can influence the particle integrity. In addition, CLSM does not distinguish between substances of similar dyeing properties, for instance polysaccharides, and choosing

proper lasers of efficacious fluorophore excitations and appropriate objective lenses and their working distances are also significant challenges to the CLSM approach.

3.6. SURFACE COMPOSITION OF FOOD POWDERS BY ELECTRON SPECTROSCOPY FOR CHEMICAL ANALYSIS (ESCA)

Over the last few decades, variable analytical techniques have been developed to increase the investigation of particle surfaces. X-ray photoelectron spectroscopy (XPS), also called electron spectroscopy for chemical analysis (ESCA), is a technique that provides information on the surface elemental composition (atomic percentages) and chemical (binding percentages), whereby only atoms present at depths of up to 10 nm can be detected. In short, an X-ray source with known energy is emitted on the sample surface, and the beam emission of produced photoelectrons from the sample surface is collected. Percentage of the main components present at the powder surface can be determined by their atomic values (protein, lactose, and lipids). The prediction of these percentages can be performed with the help of a matrix formula composed of the relative amount of the substances in the sample, the relative amount of certain compounds in the pure component, and relative coverage of the macromolecules (Fäldt & Bergenståhl, 1995; E. H.-J. Kim et al., 2002; Rafiee et al., 2020).

To understand the distribution of substances during microcapsule formation, Porras-Savedra et al. (2018) quantified the concentrations of nitrogen (N), carbon (C), and oxygen (O) on microparticles surface through XPS. The authors reported that the protein distribution was attributed to its surface affinity, making the protein migrate to the droplet surface during spray drying. Already low molecular weight compounds (e.g carbohydrates) tend to migrate rapidly into the inner layers of the microparticles during drying, decreasing the C concentration. The XPS was also used to investigate changes in

surface chemical composition during powder storage by Fyfe et al. (2011). The bonding state of the components in the near-surface region, mainly protein, showed an increase in nonpolar bonds, which is associated with an increase in hydrophobicity at the powder surface. Furthermore, XPS was also used to characterize the particle surface related to its functional properties, such as flowability (E. H. J. Kim et al., 2005, 2009c), wetting (Gaiani et al., 2007; E. H.-J. Kim et al., 2002; E. H. J. Kim et al., 2009c)**,** stickiness (Adhikari, Howes, Wood, et al., 2009; Fang et al., 2013)**,** and solubility (Jayasundera, Adhikari, Howes, et al., 2011). It is important to mention that in all these studies, the surface chemical composition of sample powders is different from their bulk composition due to different diffusion and precipitation rates of each component, e.g., protein, carbohydrate, and lipid, indicating that there is redistribution during particle formation.

3.7.CONCLUSIONS AND FUTURE DIRECTIONS

Several studies have demonstrated that proteins play an essential role in food powder surfaces. The mechanisms associated with protein migration affect the formation of the particle surface, which controls some essential functional properties, such as wetting, dispersibility, stickiness, and flowability.

Understanding the surface formation, surface, and morphology of particle powders is essential for improving their storage and functionality. Several techniques have been developed for characterization to predict these variables successfully, and a significant amount of work is available in the literature showing how this can help in the behavior of food powder storage, this knowledge empowers better design of formulations and processes used in encapsulation.

Thus, spray drying products requires making decisions as to wall materials, processing method and operating parameters, and evaluation methods to achieve the desired performance in final food or beverage applications. More collaborated efforts between academia and the food industry are needed to bridge the gap and to mesh wall materials and techniques with specific active ingredients to achieve the desired functionality and performance in the target food application.

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CAPÍTULO 4 – PERFORMANCE OF RICE PROTEIN HYDROLYSATES AS A STABILIZING AGENT ON OIL-IN-WATER EMULSIONS

PERFORMANCE OF RICE PROTEIN HYDROLYSATES AS A STABILIZING AGENT ON OIL-IN-WATER EMULSIONS

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HIGHLIGHTS

- Functional properties of rice protein were improved by enzymatic hydrolysis.
- Enzymatic hydrolysis (6 and 10%) enhanced the protein solubility.
- O/W emulsions were produced with low levels of protein hydrolysates.
- Rice protein hydrolysates reduced the interfacial tension between O/W.
- Emulsions contained hydrolysates were more physically stable than rice protein.

ABSTRACT

Rice protein isolate (RPI) has been receiving increasing attention from the food industry due to its performance as an emulsifier. However, it is possible to enlarge its field of applications through enzymatic hydrolysis. Therefore, this work aimed to investigate the effects of the controlled enzymatic hydrolysis (degree of hydrolysis DH as 2, 6, and 10%) using Flavourzyme on the physicochemical properties of rice protein and to identify the minimum concentration of these hydrolysates (0.5, 1.0, and 1.5%) to form and stabilize oil/water emulsion. The physicochemical, interfacial tension (IT), and surface characteristics of RPI and their hydrolysates (RPH) were determined. Even at a lower protein concentration (1.0%), protein hydrolysate presented lower IT when compared with RPI at a higher protein concentration (1.5%). The interfacial tension decreased from 17.6 mN/m to 9.9 mN/m when RPI was hydrolyzed. Moreover, enzymatic hydrolysis (DH 6 and 10%) enhanced the protein solubility by almost 20% over a pH range of 3-11. The improved amphiphilic property of RPH, supported by the results of IT and solubility, was confirmed by the higher emulsion stability indicated by the Turbiscan and emulsion stability indexes. Emulsions stabilized by RPH (DH 6% and 10%) at lower protein concentrations (1%) exhibited better physical stability than RPI at higher protein concentrations (1.5%). In this work, we verified the minimum concentration of rice protein hydrolysate required to form and stabilize oil-in-water (O/W) emulsions.

Keywords: Rice protein hydrolysates; Emulsion; Interfacial tension; Functional properties.

4. INTRODUCTION

Besides their nutritional benefits, proteins are widely used in the food industry for food formulations due to their functionality, many of which are used as emulsifiers. Among different proteins available for emulsion formulation, plant proteins have been increasingly studied because of their environmental friendliness compared to animal proteins. In addition, the search for plant proteins is also linked to religious issues, veganism, and especially consumer health concerns (Karaca et al., 2015).

Rice (*Oryza sativa* L.) is one of the world's most valuable staple food sources globally with approximately 496.4 million tons of worldwide production in 2019/2020 (USDA, 2021). For decades, there was little interest in the processing of rice proteins, mainly because of the relatively low protein content of rice, the low solubility of rice proteins in water, and the limited commercial opportunities for such protein-enriched ingredients.

However, over the years, researchers and industries have shown interest in using rice protein as a substitute for animal-based proteins in new product formulations, especially as an emulsifier. Its use is growing and gaining attention as the industry pushes to find value-added opportunities (Gomes & Kurozawa, 2020; Z. Yang et al., 2022). It is worth mentioning that the emulsifier concentration can affect the final products obtained from emulsions (Francisco et al., 2020).

For example, small amounts of proteins can occupy the small pores in the matrix formed by maltodextrin during microencapsulation by spray drying, resulting in better protection of the active compound and preventing the oxidation process during storage (Drusch et al., 2012). However, stabilizing emulsions using plant protein is challenging when compared to animal protein. In addition, the proper application of a protein as an emulsifier depends on how its structural, conformational, surface, and functional

properties are affected by processing parameters, which directly influence the behavior of proteins in food systems during production, storage, and consumption (Akharume et al., 2021; Paulo et al., 2020).

Several researchers have attracted attention due to their research on partial enzymatic hydrolysis that allows improved emulsification abilities of protein by increasing its solubility, reducing its molecular weight, exposing buried hydrophobic groups, and increasing its surface hydrophobicity (García Arteaga et al., 2020; Gomes & Kurozawa, 2021; Singh et al., 2021). These possible modifications can considerably reduce the amount of protein used as an emulsifier. In addition, hydrolyzed proteins can stabilize a larger interfacial area, and their diffusional transport and affinity for adsorption to the oil-water interface are accelerated (Mokni Ghribi et al., 2015; O'Regan & Mulvihill, 2010). Gomes and Kurozawa (2020) emphasized that, depending on the enzyme used, the degree of hydrolysis (DH) has to be carefully controlled to maintain desirable peptide functionality. Although in some cases, hydrolysates with high DH may possess enhanced interfacial diffusivity and emulsifying capability, they have shown poor stabilizing ability in conventional emulsion systems, impairing long-term storage (Mokni Ghribi et al., 2015; Yust et al., 2010).

Recently, many studies have focused on understanding the interfacial behavior of adsorbed hydrolyzed proteins and the relationship between the interfacial properties and physicochemical properties of emulsions (Mokni Ghribi et al., 2015; Singh et al., 2021; Zang et al., 2019). For instance, Klost and Drusch (2019) reported that interfacial films formed by pea protein hydrolysate, compared to intact pea protein, displayed an increase in film strength while maintaining a high proportion of elastic properties. Likewise, the formation of an interfacial film with improved viscoelasticity was obtained when protein hydrolysates were used in the emulsion, as observed by Tamm et

al. (2016). Besides, depending on degree of hydrolysis, some emulsion droplets stabilized by peptides presented a higher surface charge and coverage of a greater interfacial area in comparison to the nonhydrolyzed protein (O'Regan & Mulvihill, 2010). However, the literature has yet to report protein hydrolysate concentrations below 2.0% to verify the stability of feeding emulsion before spray drying (Gomes & Kurozawa, 2020; Mota da Silva et al., 2021; Tamm et al., 2016). Usually, maltodextrins are used in combination with proteins as wall materials, because they provide good oxidative stability to encapsulated oil due to their capacity to form a continuous film (Amagliani et al., 2017).

Therefore, the overall goal of this research was to study the effect of enzymatic hydrolysis of commercial rice protein isolate on interfacial and functional properties and to investigate the minimum concentration of rice protein hydrolysate required to form and stabilize oil-in-water (O/W) emulsions that would be used in spray drying processes.

4.2.MATERIAL AND METHODS

4.2.1. Material

The rice protein isolate (RPI) (90% dry weight-protein concentration) from commercial rice flour was kindly donated by Gramkow (Joinville, Brazil). Maltodextrin (MD) 10 DE Mor-Rex® 1910 was kindly donated by Ingredion (Mogi Guaçu, Brazil). Linseed oil was used as dispersed phase (Vital Âtman, São José do Rio Preto, Brazil). The commercial protease Flavourzyme was purchased from Sigma-Aldrich (St. Louis, Mo., USA). The other reagents used were analytical grade.

4.2.2. Enzymatic hydrolysis of rice protein

Enzymatic hydrolysis of RPI was conducted as described by Gomes and Kurozawa (2020), using the pH-stat method (Adler-Nissen, 1986). RPI solutions (5 wt%) were submitted to the enzymatic hydrolysis using Flavourzyme enzyme to obtain protein hydrolysates with degrees of hydrolysis (DH) as 2, 6, or 10%, in which the DH were chosen based in our previous report (Gomes & Kurozawa, 2020). The process was carried out under optimum enzyme conditions: 50 °C and pH 6.0. In this work, the rice protein hydrolysates (RPH) will be abbreviated as RPH(Y), as Y defines the degree of hydrolysis.

4.2.3. Evaluation of rice protein isolate and its hydrolysates

4.2.3.1.Thermal property

The thermal characteristics of RPI and freeze-dried RPH were evaluated by differential scanning calorimetry (DSC) (DSC1 Mettler Toledo, Schwerzenbach, Switzerland). Approximately 8 mg of a sample was placed in an aluminum hermetic pan (40 μL), and an empty aluminum pan was used as a reference. The samples were heated from 25 to 130 °C, at a 10 °C/min heating rate and submitted to a nitrogen gas flow of 50 mL/min to detect the protein denaturation. Denaturation peak temperature (T_d) and onset temperature (T_o) were computed from the DSC thermograms using the equipment software.

4.2.3.2.Protein solubility

Protein solutions (RPI or RPH) were prepared by dispersing the samples in water (4.5% w/v), and the pH was adjusted at different values ranging from 3.0 to 11.0 by 0.1 M NaOH or 0.1 M HCl. Dispersions were magnetically stirred for 30 minutes at room temperature (~ 25 °C) and centrifuged at 5000 rpm for 15 minutes. Protein solubility

was calculated as the ratio of the amount of solubilized protein in the supernatant and the amount of protein in the dispersion. The protein content in the supernatant was determined by the Dumas method, using a 5.61 nitrogen conversion factor (Sosulski & Imafidon, 1990).

4.2.3.3.Surface charge (zeta potential) measurements

The surface charges for RPI and RPH samples were measured using a Zetasizer Nano Series (Malvern Instruments, Worcestershire, UK). RPI and RPH solutions containing 0.1% protein (w/w) were prepared in deionized water, and the ζ-potential was determined as a pH function (adjusted by 0.01 N HCl or 0.01 N NaOH) ranging from pH 3.0 to 9.0, at 1.0 intervals. Measurements were performed in triplicate.

4.2.3.4. Dynamic interfacial tension

The effect of the protein concentration and the DH on decreasing the interfacial tension between aqueous and oily phases was evaluated at room temperature using a Tracker-S tensiometer (Teclis, France) employing the pendant droplet method. Based in our previous study in which used 2.0% protein concentration (Gomes & Kurozawa, 2020), dispersions containing less and different concentrations of RPH or RPI (0.5, 1.0, and 1.5% w/w) and MD (33.5, 34.0, and 34.5% w/w) were used as the aqueous phase, and linseed oil was used as the lipid. Measurements were performed in triplicate.

4.2.4. Preparation and evaluation of oil-in-water emulsions

Linseed oil (10%) was emulsified in water and stabilized by maltodextrin at concentrations of 33.5, 34.0, and 34.5% (w/w) and RPI or RPH at protein concentrations (considering soluble and insoluble fractions) of 0.5, 1.0, and 1.5% (w/w) (Gomes &

Kurozawa, 2020; Tamm et al., 2016). The emulsions stabilized by RPI and RPH will be referred to hereon as a whole (soluble and insoluble fraction).

Two homogenization methods were used to prepare the oil-in-water emulsions. The first method involved mixing the solutions using a rotor-stator homogenizer (Ultra-Turrax IKA T18 Basic, Wilmington, USA) operating at 15,600 rpm for 2 min, obtaining macroemulsions.

The second method used the previously prepared macroemulsions and subjected them to a high-pressure homogenization process (Panda 2K NS1001L, Niro Soavi, Italy). The pressure was 300 MPa, and the samples were subjected to two passes through the homogenizer. Emulsions with greater stability were evaluated regarding droplet size and stability by Turbiscan.

4.2.4.1.Emulsion activity (EAI) and stability (ESI) indexes

The emulsifying activity (EAI) and stability indexes (ESI) of all emulsions were determined in triplicate by the turbidimetric method described by Pearce and Kinsella (1978). An aliquot of 50 µL of emulsion sample was immediately removed from the bottom of the tube at 0 and 10 minutes and dispersed into 7.5 mL of 10 mM sodium phosphate buffer (pH 7.0) containing 0.1% sodium dodecyl sulfate (SDS). The solution was vortexed for 10 s. The sample absorbance was measured at 500 nm immediately at 0 and 10 minutes. EAI and ESI were calculated by equations (1) and (2).

$$EAI\ (m^2/g) = \frac{2 \times 2.303 \times A_0 \times N}{C \times \varphi \times 10000} \quad (1)$$

$$ESI\ (min) = \frac{A_0}{\Delta A} \times 10 \quad (2)$$

where, A_0 is the absorbance of the diluted emulsion immediately after homogenization; N is the dilution factor (×150); C is the protein weight per volume (g/mL); φ is the oil

volume fraction in the emulsion; ΔA is the change in absorbance from 0 to 10 min (A_0–A_{10}).

4.2.4.2.Emulsion stability

The emulsion stability was evaluated in duplicate during 24 hrs. at room temperature by the Turbiscan ASG optical scanning instrument (Formulaction, l'Union, France). Emulsion destabilization was analyzed using backscattering (BS) and the Turbiscan stability index (TSI) profiles with scans at 880nm. This technique allows determining the intensity of multiple light scattering throughout the heights of glass tubes containing the emulsions.

4.2.4.3.Emulsion droplets: ζ-potential

The ζ-potential was determined in triplicate using a Zetasizer Nano Series (Malvern Instruments, Worcestershire, UK). 100 µL of each emulsion was diluted in 19.9 mL of deionized water after being produced for determination.

4.2.4.4.Mean droplet diameter and size distribution

The mean oil droplet diameter and the droplet size distribution were determined in triplicate by light scattering (Mastersizer 2000, Malvern Instruments Ltd, UK). The mean droplet diameter was expressed as the volume-surface mean diameter (Sauter diameter - $D_{3,2}$), and the polydispersity of droplet size was determined as the Span.

4.2.5. Statistical Analysis

The data results were submitted to an analysis of variance (ANOVA) and a Tukey's ($p<0.05$) *post-hoc* test using Statistical 7.0 software.

4.3.RESULTS AND DISCUSSION

4.3.1. Thermal properties

The thermal characteristics of the RPI and RPH could provide information about behavioral changes including glass transition, denaturation, or other reactions during several industrial processing operations, which can be useful for future strategies. Denaturation temperature (T_d) could be obtained from thermograms, indicating thermal stability, in which higher T_d is usually associated with higher thermal stability (Table 1).

Table 1. Thermal properties (denaturation temperature T_d and onset temperature T_o) of rice protein isolate (RPI) and its hydrolysates (RPH) with a degree of hydrolysis as 2, 6, and 10%.

Sample	Thermal properties	
	T_d (°C)	**T_o (°C)**
RPI	68.51	56.53
RPH(2)	57.08	50.14
RPH(6)	59.25	51.53
RPH(10)	59.39	51.60

Only one thermal event was observed for the RPI sample, where the major peak occurred at 68.51°C (T_{onset}= 56.53 °C) (Fig. S1, supplementary material). Regardless of the process conditions employed to obtain RPI or RPH and their moisture content, the T_d value was similar for rice bran, which ranged from 69 to 72 °C (Capellini et al., 2020). However, the literature reported T_d for rice protein as approximately 80 °C (Ju et al., 2001; Paraman et al., 2006; Singh et al., 2021). This variation can be due to genotypic differences in the raw material or the effect of protein extraction and isolation conditions. Moreover, sample preparation and instrument operation can influence the

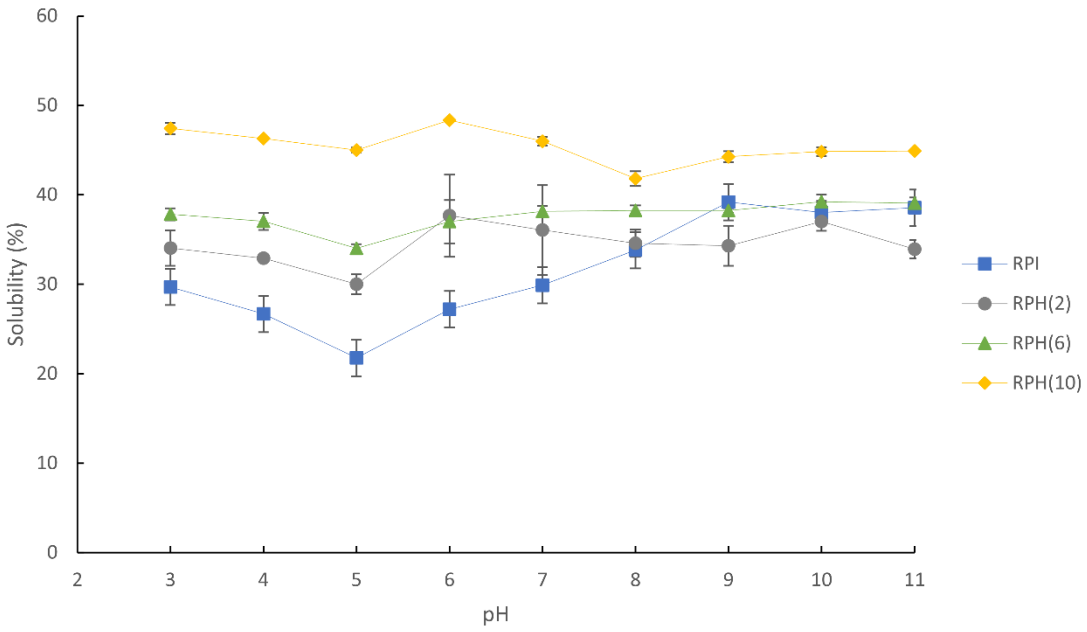
results; therefore, data comparison obtained from different DSC studies of protein denaturation/aggregation is often complex.

Also, we notice a T_d decrease when RPI (68.51°C) was hydrolyzed. Similar values were observed between the protein hydrolysates with different DH (~59°C) (Fig. S1, supplementary material). Thermal stability is usually associated with the denaturation temperature or degradation of a globular protein. Higher thermal stability is due to the greater proportion of β–sheets which are the extent of ordered protein structure. In our previous study (Gomes & Kurozawa, 2020), we observed reducing conditions by gel electrophoresis, the disruption of intermolecular bonds caused by protease action; thus, the compact structure characteristic of RPI was lost. Singh et al. (2021) also showed that enzymatic hydrolysis of rice bran protein decreased β–sheets content and, consequently, denaturation temperature. Otherwise, we could suggest this difference between Td could also be associated with the moisture content of the samples (Kitabatake et al., 1989)

4.3.2. Influence of DH on protein solubility and potential zeta

Enzymatic hydrolysis usually significantly improves protein solubility, as shown in several studies (Meinlschmidt et al., 2016; Noman et al., 2018; Singh et al., 2021; Yust et al., 2010; Zang et al., 2019). In our study, the rice protein solubility was evaluated as a function of pH since this is an important protein parameter considering potential industrial applications. All hydrolysates, especially RPH(10), were more soluble than intact rice protein in the pH range of 3.0-7.0 (Figure 1).

Figure 1. Protein solubility profiles as a function of the pH of rice protein isolate (RPI) and its hydrolysates (RPH) with a degree of hydrolysis as 2, 6, and 10%.

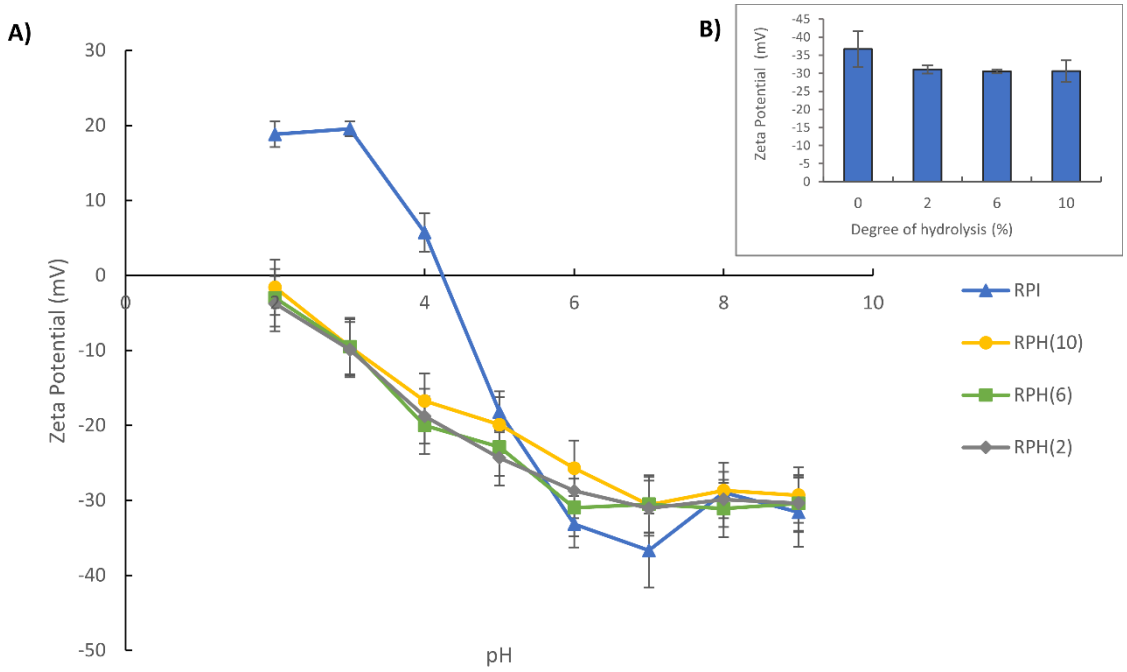


The 27% increase in the solubility for RPH(10) sample regarding RPI was due to the decreased molecular size of the rice protein (as reported in our earlier study (Gomes & Kurozawa, 2020)), releasing smaller and more flexible peptides and increasing charged groups. Polar amino acid groups buried inside protein molecules could have been exposed on the surface of protein molecules after hydrolysis. Consequently, they may interact with water molecules through hydrogen bonds and electrostatic interactions, improving protein solubility. Interestingly, no significant differences on solubility between RPI and RPH(2) or (6) were observed at alkaline pH. Some authors reported a considerably increase in the solubility at ~pH 4; however, at neutral or alkaline pH, a negative or little effect of hydrolysis on protein solubility was observed for lentil, pigeon pea, chickpea, and cowpea (Segura-Campos et al., 2012; Vogelsang-O'Dwyer et al., 2023; Xu et al., 2021). Enzymatic hydrolysis may have resulted in more exposed hydrophobic groups, leading to protein aggregation and subsequent reduced solubility at this pH region (Vogelsang-O'Dwyer et al., 2023).

The lowest protein solubility was observed at ~ pH 5 except for RPH(10) due to the proximity to the isoelectric point. Similar results were obtained by Cao et al. (2009) and Romero et al. (2012), who reported the isoelectric point of rice proteins as pH 4.5. The minimum protein solubility of RPI (21.76%) at the isoelectric point was due to the high protein-protein interaction and minimum net electrostatic molecule charges. Furthermore, in a pH range of 4-5, the hydrolysates RPH(2) and RPH(6) presented low solubility even after hydrolysis. The newly formed peptides were probably still bounded by unhydrolyzed peptides through interactions, like disulfide bonds, or self-associated through inter–peptide hydrophobic interaction. In any case, the solubility for all samples was lower than 50 wt% in the whole studied pH range, which may be probably due to a significant protein denaturation during the extraction and isolation of protein from rice flour. There are several works in the literature that reported the effect of extraction and isolation of proteins on protein solubility (Gao et al., 2020; Romero et al., 2012).

The electrostatic attractive and repulsive forces between the protein hydrolysates, indicated by zeta potential, were determined for enhanced understanding of the protein solubility results. In an appropriate pH value, proteins play an important role in solubility, emulsifying, foaming properties, and gel network structure (Figure 2A).

Figure 2. The surface charge for rice protein isolate (RPI) and its hydrolysates (RPH) as a function of: (A) pH and degree of hydrolysis as 2, 6, and 10% and (B) degree of hydrolysis at pH 7.



The pH influenced the surface charge in all samples (Figure 2A). The pH above the isoelectric point and low H^+ concentration cause the carboxyl groups ($-COO^-$) and amino groups ($-NH_2$) to be non-protonated, leading to a net negative charge. For instance, at pH 7, the intact and hydrolyzed rice protein (RPH10) had a net negative charge of -36.7 mV and -30.59 mV, respectively (Figure 2B). The electronegativity of the RPI is associated with charged amino acid side chains on the protein molecule surface, such as arginine, lysine, aspartic acid, and glutamic acid, which, even after hydrolysis, the net charge maintained as electronegative above IP.

The results also pointed out that the enzymatic hydrolysis affected the zeta potential of the rice protein in some pHs (Figure 2A). As shown in other studies (Avramenko et al., 2013; Mahmoud et al., 1992; Mokni Ghribi et al., 2015), peptide bonds are cleaved by the proteolytic enzymes, more ionizable carboxyl and amino groups are released,

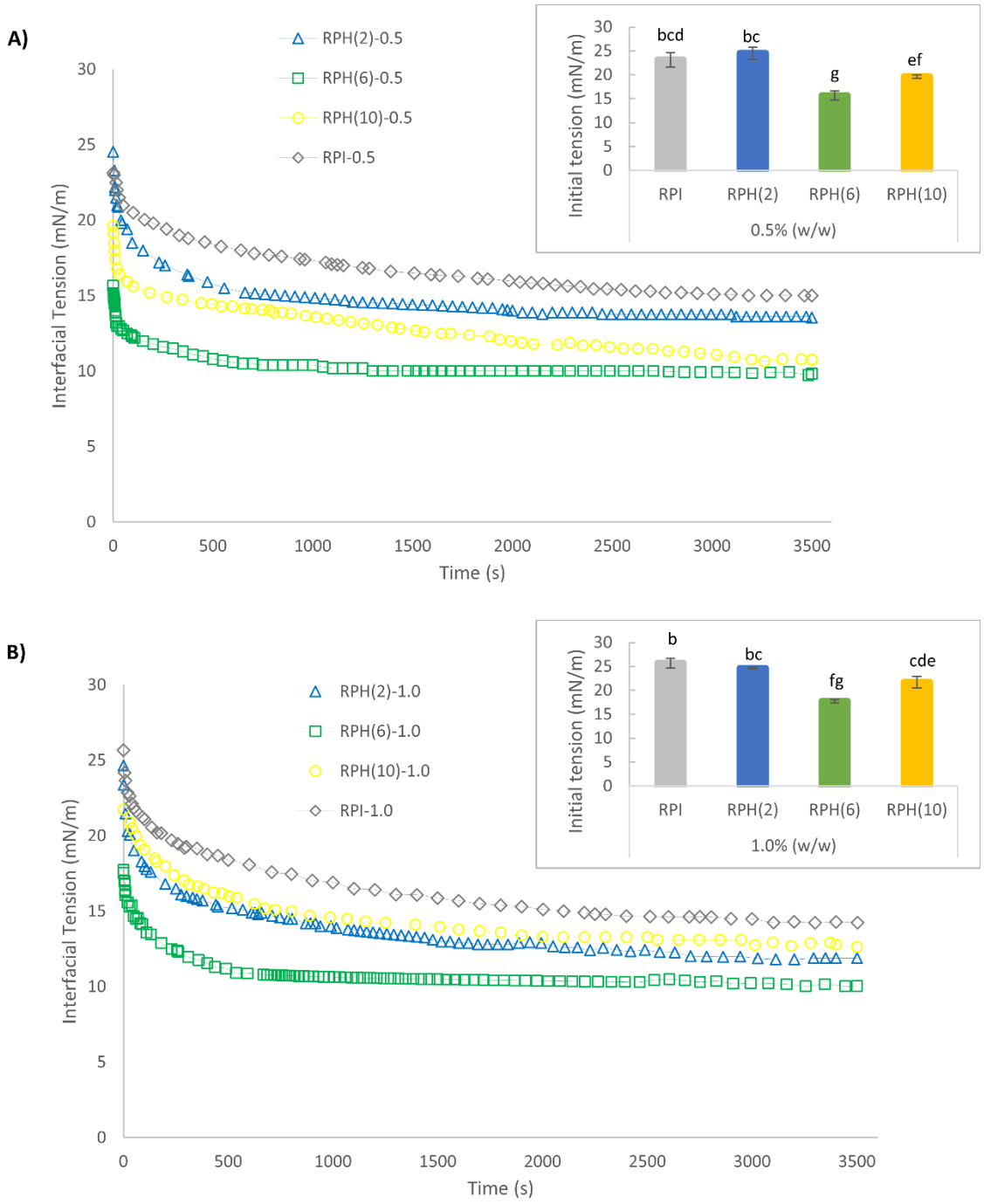
thus increasing the negative charge of the protein hydrolysates. Under these circumstances, the hydrolysis enhances intra- and inter-molecular electrostatic repulsion, reducing protein–protein aggregation and increasing protein–water interactions. This kind of reaction could increase the solubility and improve the functional properties. Overall, all hydrolysates presented a satisfactory surface charge in all pH ranges, demonstrating their potential in acidic food systems, such as sports beverages and acidified sauces.

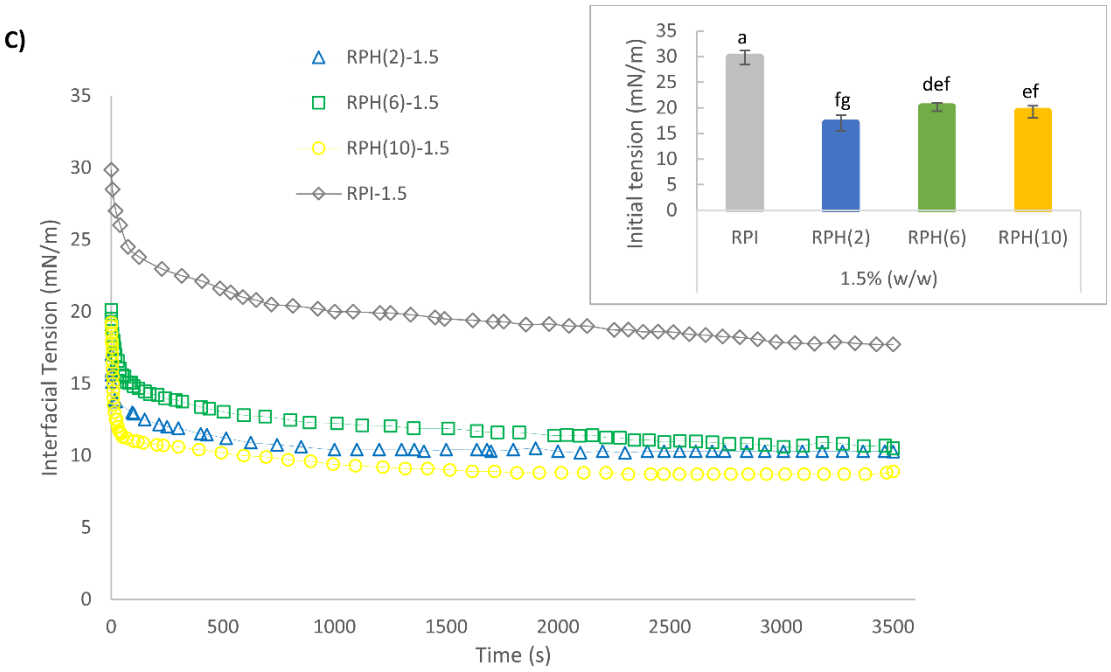
The use of protein as an emulsifier provides an indirect measurement of a protein's surface activity. The amphipathic nature induces its adsorption at the fat globules' surface, reducing interfacial tension. This fact facilitates the breakdown of the oily phase into small and spherical droplets, decreasing the surface energy and influencing the interface's electrostatic balance. As a result, the stability of emulsions against coalescence is improved through electrostatic repulsive and steric forces.

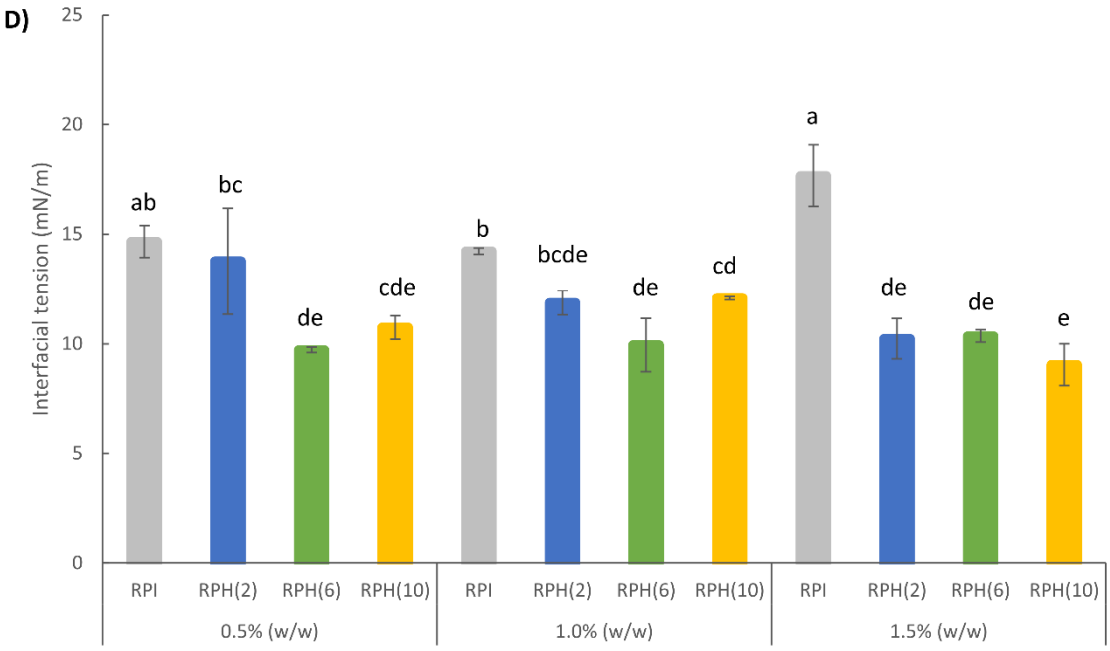
4.3.3. Effect of DH on the interfacial tension

As a function of protein concentration, the interfacial tension of RPI and RPH with different DHs decreased over time (Figure 3). As expected, the interfacial tension at the linseed oil-water interface was greatly affected by the structure modification of RPI by enzymatic hydrolysis. However, as the protein concentration increased, it seemed that the interface reached saturation since the interfacial tension was no longer affected by adding protein. In some cases, even at lower protein concentrations, the hydrolyzed samples RPH6 and RPH10 showed strong adsorption due to the high proportion of surface-active components.

Figure 3. Comparative evolution of interfacial tension vs. time for rice protein isolate (RPI) and its hydrolysates (RPH) at different protein concentrations: (A) 0.5%; (B) 1.0%; (C) 1.5% and D) Equilibrium interfacial tension at linseed oil-water interface as a function of protein concentration at pH 7 and 20 °C.







Initially, there was a sudden reduction in interfacial tension, followed by its stabilization until reaching a constant value, known as equilibrium interfacial tension (Figure 3B). In general, this initial decay leads to a less smooth curve as the degree of hydrolysis increases, which may be attributed to the time required for accumulating the amphiphilic molecules at the interface. The changes in the conformation of the rice protein caused by enzyme action may have affected its diffusion rate, as reported for

sunflower and chickpea proteins (Conde & Rodríguez Patino, 2007; Mokni Ghribi et al., 2015). In addition to protein size and conformation, Rahali et al. (2000) reported that the amphiphilicity of protein/peptides and amino acid sequence on an oil-water interface influences the interfacial tension. According to these authors, these factors play a greater role in interfacial tension than the peptide length.

RPH(6) and RPH(10) presented greater solubility (Figure 1) and possible higher hydrophobicity, as reported by several authors, in which protein hydrolysis increases the hydrophobicity of proteins (Jin et al., 2020; Zang et al., 2019), indicating higher protein amphiphilicity. The amphiphilic nature of proteins allows them to act as emulsifiers, lowering the surface tension between the phases. This balance of hydrophilic and hydrophobic groups allows a favorable intermolecular interaction between the protein and the phases in the colloidal system (e.g., lipid), indicating its faster diffusion towards the oil/water interface (J. Yang et al., 2018). Figure 3D shows the equilibrium interfacial tension of the linseed oil-water interface at pH 7.

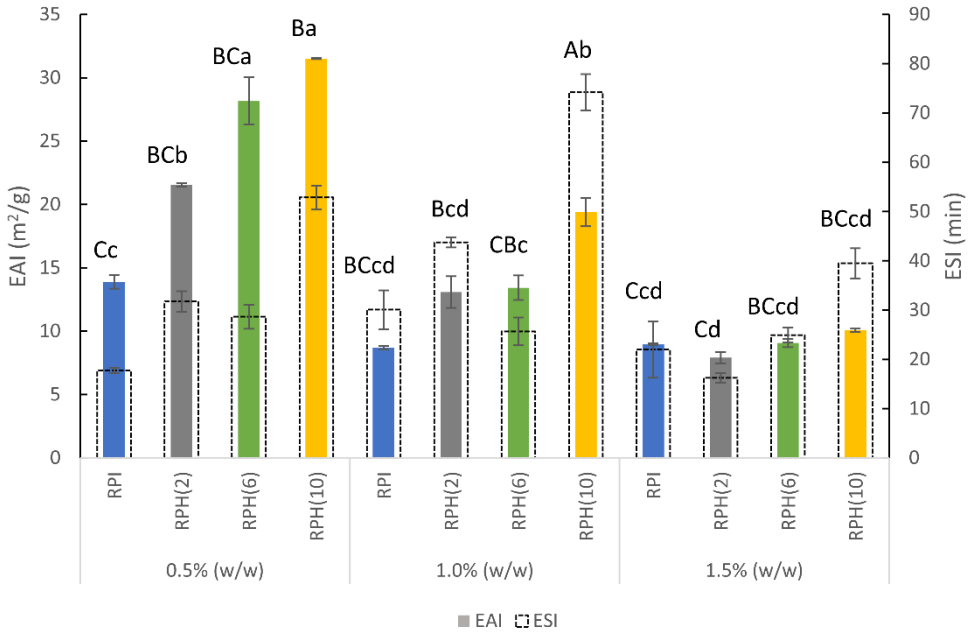
The equilibrium interfacial tension values for emulsions stabilized by protein hydrolysates were lower than for RPI. This result may be due to the ability of the hydrolyzed proteins to align more quickly and to integrate at the oil–water interface. Although intact protein can unfold and reorient at the interface to stabilize emulsions, the RPI showed a poor capacity to decrease the interface tension in this case. It is due to its tertiary and secondary structures, which require some time to unfold and lower the interfacial tension (Amagliani et al., 2017). RPI must undergo limited hydrolysis to unfold its secondary and tertiary structures partially, aiming to improve its emulsifying properties as long as its primary structure (or amino acid sequence) is preserved, as reported by Rahali et al. (2000).

4.3.4. Stabilization of the oil droplets

The protein concentration caused a negative effect on EAI, in which the highest values were obtained at 0.5% of protein for all hydrolysates (Figure 4). Probably in a lower protein concentration, the large area occupied per protein molecule facilitates its rearrangement and adsorption and results in ameliorative emulsifying capacities. On the other hand, high protein concentration means crowding protein molecules at the interface, impairing the complete unfolding or reorienting of the molecules. Moreover, additional proteins surround the film and form multilayers, which induce the small total surface area covered per gram of protein. Similar behavior was also reported by (Mokni Ghribi et al., 2015; Zhang et al., 2009).

DH positively affected EAI except for the emulsion containing 1.5% protein concentration (Figure 4). Specifically, RPH(10)-0.5% conferred the highest emulsifying activity value (31.5 m^2/g), while RPI-1.0% presented the lowest value (8.67 m^2/g). An increase in the EAI by DH was also observed for papain-treated Indian black gram protein isolates, trypsin-treated dehulled walnut proteins, and alcalase-treated chickpea protein isolates (Jin et al., 2020; Mokni Ghribi et al., 2015; Wani et al., 2015).

Figure 4. Emulsifying activity index (EAI) and emulsion stability index (ESI) of emulsions stabilized by rice protein isolate (RPI) or rice protein hydrolysates (RPH) at different degrees of hydrolysis (2%, 6%, and 10%) as a function of protein concentration.



Different lowercase letters above the bars indicate a statistical difference between the samples for EAI, and capital letters refer to ESI ($p < 0.05$).

Some researchers (Jin et al., 2020; Zheng et al., 2019) hypothesized that small peptides released by protein hydrolysis might diffuse rapidly and be absorbed on the surface of freshly formed oil droplets, enabling greater alignment at the oil–water interface. However, as reported in our previous work (Gomes & Kurozawa, 2020), the enzymatic hydrolyses produced peptides with high MW. Thus, the improvement of emulsifying properties of rice protein can be due to the changes in the surface hydrophobicity and the increase of hydrophilic carboxylic and amino groups, which contribute to the protein's amphiphilic nature. Employing a multiple regression predictive model for EAI, Karaca et al. (2011b) identified significant isolate factors (surface charge, hydrophobicity, and solubility) for several vegetable proteins (e.g.,

chickpea, fava or lima bean, lentil, and pea). The individual effects of solubility and surface hydrophobicity on EAI were positive. At the same time, their interaction had a negative effect, which emphasized the importance of balance between hydrophilic and hydrophobic interactions on emulsifying properties.

On the other hand, some studies showed the opposite behavior, in which EAI decreased by enzymatic hydrolysis (Achouri et al., 1998; Avramenko et al., 2013; Klompong & Benjakul, 2007; G. Zhao et al., 2011). For instance, Singh et al. (2021) reported that enzymatic hydrolysis of rice bran protein released small peptides, which thus drastically reduced the protein amphiphilicity. Although limited enzymatic hydrolysis produced peptides with high molecular weight, the specificity of the enzyme in producing peptides with more amphiphilic characteristics was essential for good stability. Flavourzyme contains both endoprotease and exopeptidase activities. The latter can selectively release hydrophobic amino acid residues from the protein molecules, increasing the amphiphilic characteristics of protein (Saha & Hayashi, 2001).

A high EAI does not necessarily represent a high ESI. A relationship between EAI and ESI of all RPH with different protein concentrations can be observed in Figure 4. ESI provides a measure of the ability of the protein to impart strength to the emulsion to resist changes in its structure (e.g., coalescence, creaming, flocculation, and sedimentation) during a defined period.

ESI of all the hydrolysates ranged from 16.27 ± 0.96 min to 74.22 ± 3.69 min, in which the highest value corresponded to the RPH(10)-1.0%. This behavior can be closely associated with the solubility and hydrophobicity of protein hydrolysates. Although RPH(10) may have increased capacity to stabilize emulsions, they were not able to improve the emulsifying capacity of rice protein, presenting low EAI value of

19.0 ± 11.1 m^2/g. Eckert et al. (2019) observed the same behavior where fava bean protein hydrolysates with DH varying from 9.5% to 16.9% showed low ESI values but high EAI values compared to intact protein.

4.3.5. Emulsion stability

Turbiscan backscattering data were obtained over time to verify the destabilization phenomena of emulsions. Some important dependent variables, including oil droplet mean size, span, and surface charges, were also analyzed to improve the characterization and understand these phenomena better.

The average diameter $D_{3,2}$ of emulsions containing different protein concentrations is summarized in Table 2. Droplet size and protein interactions are responsible for the microstructure of emulsions, which plays a key role in emulsion stability. In this work, using protein hydrolysate as an emulsifier resulted in reduced oil droplet size emulsions. For instance, the emulsion stabilized by RPH(6) 1.0% and 1.5% showed droplet size smaller than RPI 1.0% and 1.5% (10.15 ± 2.44 and 8.76 ± 0.92μm; 19.48 ± 4.62 and 16.20 ± 2.80 μm, respectively). On the other hand, no significant differences ($p < 0.05$) were observed among emulsions stabilized by other RPHs in different protein concentrations (Table 2). This improvement in emulsification properties associated with DH was related to a decrease in interfacial tension at the oil-water interface (Figure 3), along with a balance between hydrophilic and hydrophobic interactions of hydrolysates.

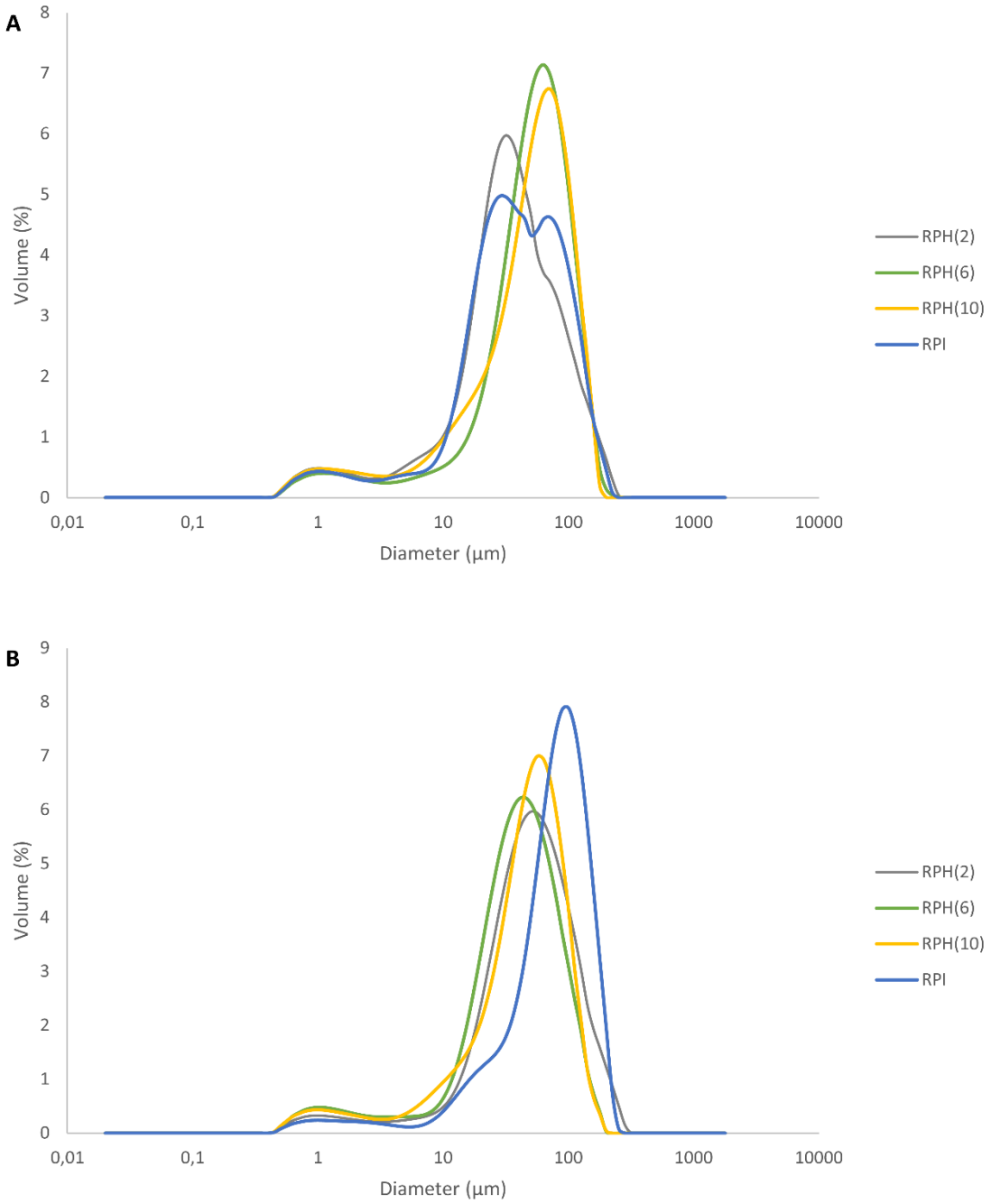
Table 1. Droplet mean size ($D_{3,2}$), span, and charge properties (ζ-potential) of linseed oil-emulsion stabilized by maltodextrin (MD) combined with rice protein isolate (RPI) or its hydrolysates (RPH) at different degrees of hydrolysis (2%, 6%, and 10%) based on protein concentration.

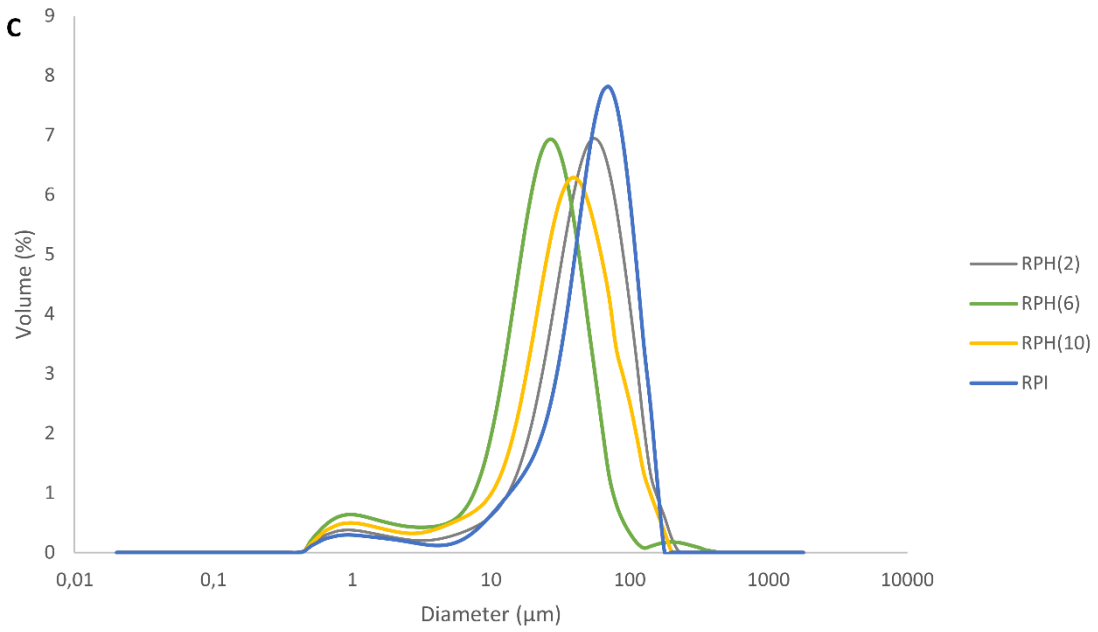
Continuous phase		**$D_{3,2}$ (µm)***	**Span***	**ζ-potential (mV)***	**$D_{3,2}$ (µm)****
MD (% w/w)	Protein (% w/w)				
33.5	RPH2-1.5%	14.70 ± 1.19^{abc}	1.73 ± 0.17^{a}	-17.67 ± 2.33^{b}	-
34.0	RPH2-1.0%	16.69 ± 3.07^{ab}	1.71 ± 0.13^{a}	-20.49 ± 1.49^{ab}	-
34.5	RPH2-0.5%	12.99 ± 0.94^{abc}	1.85 ± 0.20^{a}	-17.49 ± 0.64^{b}	-
33.5	RPH6-1.5%	8.76 ± 0.92^{c}	1.88 ± 0.39^{a}	-19.08 ± 0.70^{b}	7.13 ± 1.40^{ab}
34.0	RPH6-1.0%	10.15 ± 2.44^{bc}	1.71 ± 0.23^{a}	-26.84 ± 3.63^{a}	7.31 ± 0.19^{ab}
34.5	RPH6-0.5%	14.99 ± 1.74^{abc}	1.82 ± 0.06^{a}	-15.87 ± 1.77^{b}	8.08 ± 1.59^{ab}
33.5	RPH10-1.5%	11.30 ± 0.65^{bc}	1.87 ± 0.39^{a}	-17.50 ± 1.67^{b}	-
34.0	RPH10-1.0%	13.13 ± 1.63^{abc}	1.82 ± 0.34^{a}	-18.41 ± 2.32^{b}	-
34.5	RPH10-0.5%	12.46 ± 1.20^{bc}	1.96 ± 0.15^{a}	-22.16 ± 5.11^{ab}	-
33.5	RPI-1.5%	16.20 ± 2.80^{ab}	1.97 ± 0.09^{a}	-17.67 ± 1.79^{b}	9.51 ± 1.47^{a}
34.0	RPI-1.0%	19.48 ± 4.62^{a}	1.73 ± 0.15^{a}	-16.44 ± 2.46^{b}	7.43 ± 0.77^{ab}
34.5	RPI-0.5%	15.11 ± 3.29^{abc}	1.83 ± 0.18^{a}	-14.62 ± 3.76^{b}	4.87 ± 0.74^{b}

Different letters represent statistically significant differences ($p < 0.05$) between the concentration of protein and DH (Tukey's test). *emulsions obtained by mechanical homogenization; **emulsions obtained by mechanical homogenization followed by high-pressure homogenization.

When the droplet size distribution was also analyzed, no difference between samples was observed since they all showed a bimodal distribution. Commonly, emulsions stabilized by vegetable proteins present polydisperse behavior with a bimodal droplet size distribution (Figure 5) (Karaca et al., 2011a).

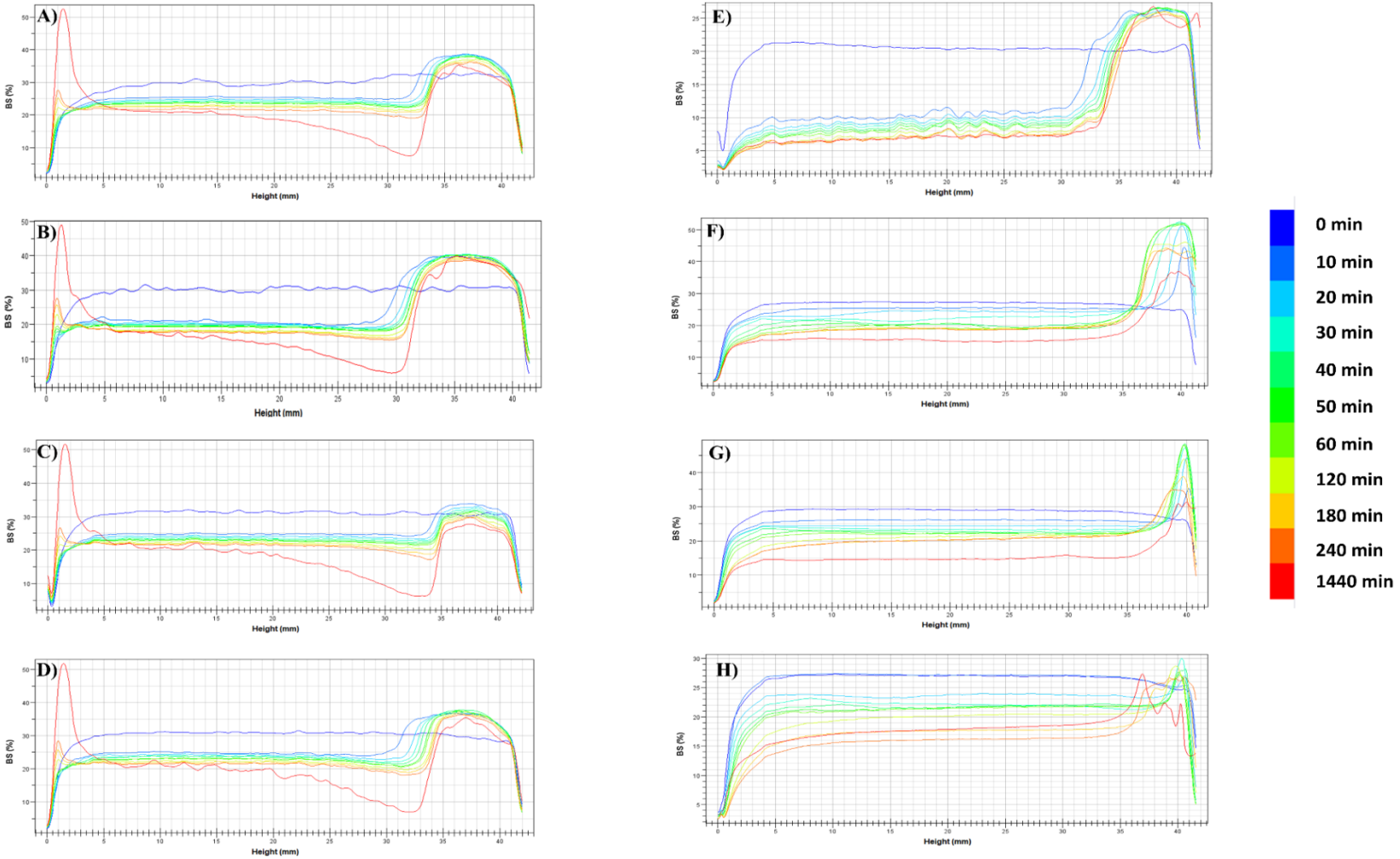
Figure 5. Oil droplet size distribution of emulsions stabilized by maltodextrin combined with rice protein isolate (RPI) or its hydrolysates (RPH) at different degrees of hydrolysis (2, 6, and 10%) at protein concentrations: (A) 0.5%; (B) 1.0%; (C) 1.5%.





For emulsion stabilized by RPI-1.0% (Figure 5B), the large peak observed may be attributed to coalescence phenomena associated with large droplet sizes. This statement is based on observing the backscattering BS (Figure 6E), where the BS signal increased at the top of the sample vial, suggesting that creaming occurred. Besides, the BS decreased during storage, suggesting flocculation or coalescence. On the contrary, the BS signal for emulsion stabilized by RPH(6)-1.0% (Figure 6G) decreased slowly during storage, proving to be more stable. This result complies with smaller oil droplet sizes (Table 2). As previously reported by Tcholakova et al. (2004), emulsifiers have displayed different abilities to stop coalescence and decrease droplet oil size depending on their adsorption at the oil-water interface.

Figure 6. Backscattering profiles of emulsions obtained by mechanical homogenization and stabilized by maltodextrin combined with rice protein isolate (RPI) or its hydrolysates (RPH) at different degrees of hydrolysis at different protein concentrations: A) RPI-1.5%; B) RPH(2)-1.5%; C) RPH(6)-1.5%; D) RPH(10)-1.5%; E) RPI-1.0%; F) RPH(2)-1.0%; G) RPH(6)-1.0% and H) RPH(10)-1.0%.



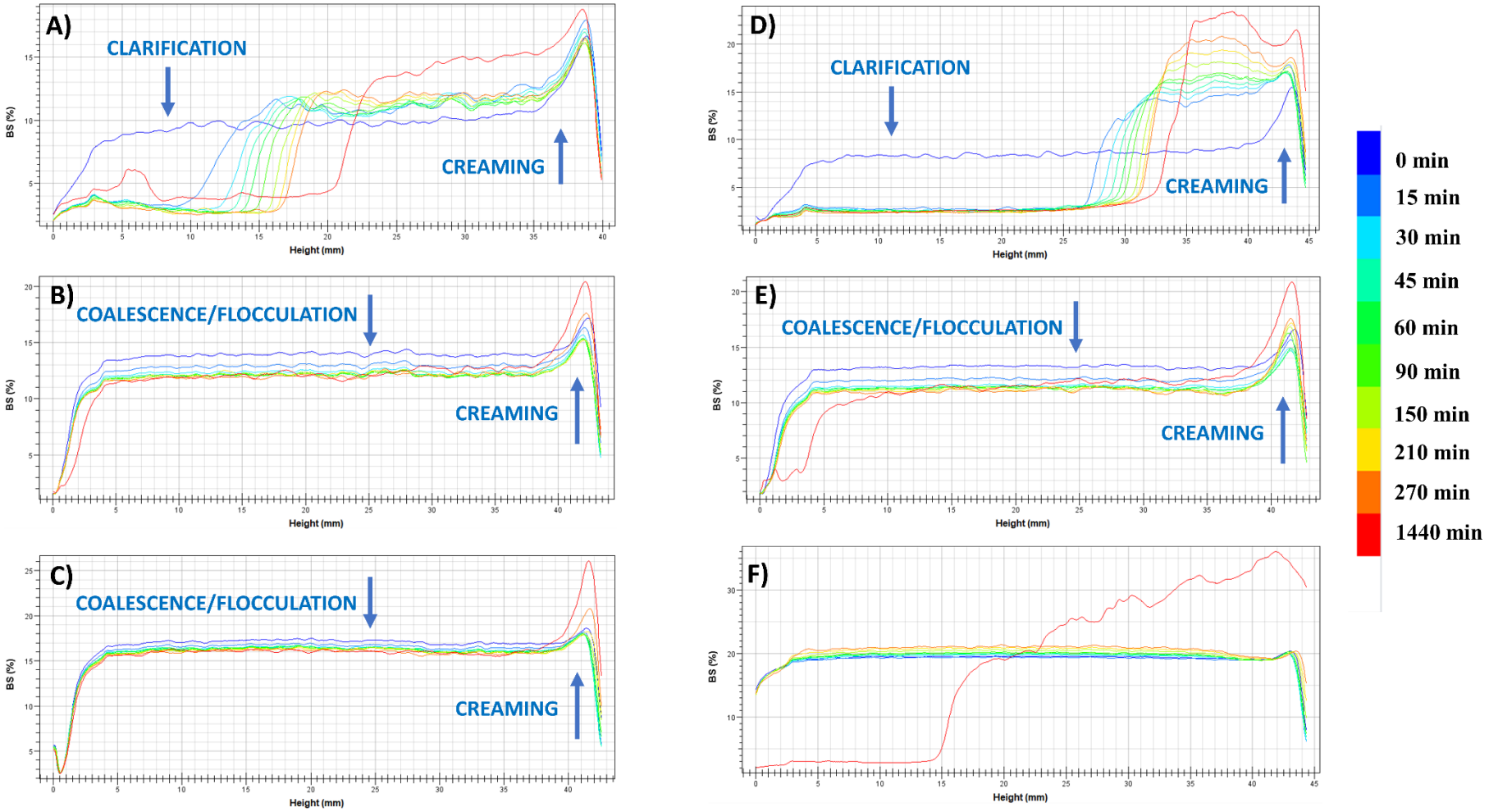
A high protein concentration (1.5%) was expected to prevent emulsion coalescence (Figure 6). However, emulsions stabilized by RPH with 1.0% protein (Figures 6F-6H) were more stable than those containing 1.5% protein (Figures 6B-6D), as also observed in the ESI analysis (Figure 4). This result could be related to the equilibrium between the proteins adsorbed at the interface and those dispersed in the continuous phase. A small change level may destabilize or stabilize the equilibrium and the concentration of proteins at the interface. Romero et al. (2011) supported and reported that protein concentration and process changes affected the interfacial tension at the oil–water interface and droplet size. Although the emulsions with low protein concentration (0.5%) have been able to adsorb at the interface showing high EAI (Figure 4), the same was not able to bear the instability of o/w emulsions, such as creaming, coalescence, or flocculation as observed by Turbiscan (data not shown).

As mentioned above, the surface charge of emulsions also indicates how these systems behave and are an important indicator of emulsion stability. Each protein behaves differently due to its characteristics after hydrolysis and concentration in the formulation. Thus, they do not show a linear behavior. For instance, RPH(6)-stabilized emulsion showed an increase in the ζ-potential (in a module) with protein concentration (0.5% to 1.0%), followed by a decrease with 1.5% of protein (Table 2). In an emulsion containing RPI, the ζ-potential increased (in a module) with protein concentration. Regarding RPH(10), an opposite behavior was observed. This result may be related to the preferential adsorption of certain proteins or peptides at the interface. Besides, the decrease in ζ -potential is a lesser extent result of protein and peptide unfolding or dense multilayer formation (increased surface coverage).

Based on the stability results (Figure 6) for emulsions obtained by mechanical homogenization, RPH(6)-stabilized emulsions showed the best stability compared to the

RPI and other RPH samples. Nevertheless, these emulsions showed a destabilization during storage. These emulsions obtained by mechanical homogenization were submitted to a high-pressure homogenization to increase their stability. The influence of pressure and the number of passes through the homogenizer on the structure of the emulsions were evaluated by droplet size (Table 2) and emulsion stability using the Turbiscan device (Figure 7).

Figure 7. Backscattering profiles of emulsions obtained by high pressure homogenization and stabilized by maltodextrin combined with rice protein isolate (RPI) or its hydrolysates (RPH) at different degrees of hydrolysis at different protein concentrations. A) RPI-0.5%; B) RPI-1.0%; C) RPI-1.5%; D) RPH(6)-0.5%; E) RPH(6)-1.0% and F) RPH(6)-1.5%.



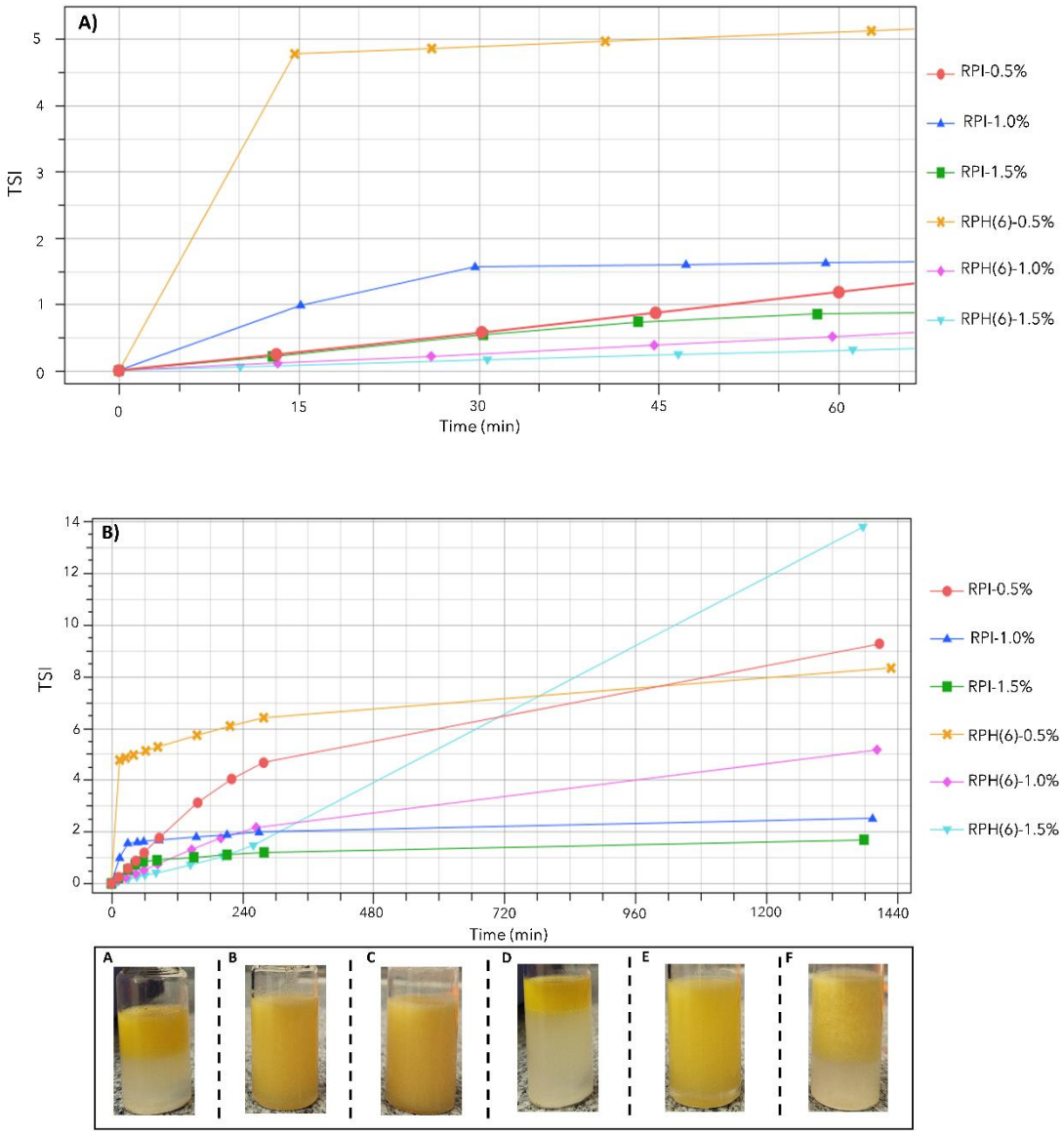
Overall, the high-pressure homogenization process significantly affected droplet size compared to mechanical homogenization. The droplet size of all samples decreased significantly but showed no significant difference between them ($p<0.05$) (Table 2). It is common to obtain reduced droplet sizes using a high-pressure homogenizer (Kuhn & Cunha, 2012).

As shown in Figure 7, a decrease in the protein concentration (0.5%) seems to promote a rapid coalescence of the emulsion droplets and, consequently, higher destabilization. The same behavior was observed in samples homogenized by mechanical homogenization (data not shown). Despite producing very small particles (Table 2), there is immediate re-coalescence, as observed by the increase in BS at the top (Figure 7), causing low emulsification efficiency under these homogenization conditions. The protein adsorption fraction in emulsions needs to be understood to comprehend the underlying cause of differences in physical stability and droplet size of all emulsions. During the high-pressure homogenization process, a protein may not cover some new droplets within a very short period during the deformation and disruption of the droplets caused by the homogenizer. Or part of the new interfaces may be incompletely covered, occurring a quick emulsion destabilization (Jafari et al., 2007; Kuhn & Cunha, 2012). There was enough protein for the emulsions containing 1 and 1.5% of protein to occupy the interface of the new formed drops.

RPH(6)-1.5% sample showed the highest stability, in which the most pronounced destabilization occurred after 24 hrs. (Figure 7F). At the end of the analysis, the droplets initially dispersed homogeneously in the emulsified system and became more concentrated at the top of the measuring cell, increasing the BS values. In contrast, BS values around 1% could be observed at the bottom of the measuring cell, indicating a clarification process. Meanwhile, the BS signal increased at the top of the RPI-1% and RPH(6)-1% samples vial, suggesting that concomitant creaming phenomena occurred.

Figure 8 compares all the Turbiscan stability index (TSI) curves for all the emulsions stabilized by RPI or RPH(6) with different protein concentrations. TSI indicates the destabilization of emulsions caused by creaming, coalescence, and/or flocculation. These data agreed with the previous statement, where lower TSI values represent higher system stability.

Figure 8. Turbiscan stability index (TSI) profiles during A)1 hr. and B) 24 hrs. Pictures after 24 hrs. of the emulsions stabilized by maltodextrin combined with rice protein isolate (RPI) and its hydrolysates at different degrees of hydrolysis: A) RPI-0.5%; B) RPI-1.0%; C) RPI-1.5%; D) RPH(6)-0.5%; E) RPH(6)-1.0% and F) RPH(6)-1.5%.



4.4.CONCLUSION

This study indicated a great influence of DH on the techno-functional properties of rice protein. Even at lower concentrations, the rice protein hydrolysates with 6 and 10% degrees of hydrolysis reduced the interfacial tension between linseed oil and water. They produced more stable oil-in-water emulsions than the intact protein, even at high concentrations. These results confirm the relevance of performing interfacial tension measurements to select the appropriate DH for assessing emulsion stability. However, our results suggest that the homogenization process can improve the kinetic stability of the emulsions. RPH-1.5% emulsion could resist coalescence and other destabilization mechanisms during almost all storage periods.

The RPH(10) and RPH(6) showed a slightly higher solubility than RPI over a wide range of pH, considering their potential in several industrial applications. Thus, these samples proved to be better suited for many food formulations. In conclusion, the present study showed that limited enzymatic hydrolysis might be a good method to improve the functionalities of rice protein. Consequently, this modified protein could be useful for several applications in the food industry.

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CAPÍTULO 5 - COMPOSITIONAL ASPECT AND MECHANISM OF SURFACE FORMATION OF SPRAY-DRIED MICROPARTICLES WITH SURFACE-ACTIVE RICE PROTEIN HYDROLYSATES

COMPOSITIONAL ASPECT AND MECHANISM OF SURFACE FORMATION OF SPRAY-DRIED MICROPARTICLES WITH SURFACE-ACTIVE RICE PROTEIN HYDROLYSATES

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HIGHLIGHTS

- The degree of hydrolysis exerted an influence on the distribution of protein on the surface.
- Differences in powder surfaces were characterized with AFM and XPS.
- RPH10 microparticles presented higher surface protein content than those containing 1.5%.
- The AFM helped to understand protein role in forming microparticle surfaces.
- The lowest peroxide value was found from the powder formulated with RPH10-1.5%.

ABSTRACT

Flavourzyme enzymatically hydrolyzed rice protein isolate to different degrees of hydrolysis (DH, 2, 6, and 10%) to stabilize formulated linseed oil emulsions with different protein concentrations (0.5, 1.0, and 1.5%). The emulsions were spray dried, and the microstructure, surface composition by X-ray photoelectron spectroscopy (XPS), and oxidative stability of the resultant microparticles were investigated. The physicochemical properties of powders were also evaluated. DH and protein concentration did not affect solubility, hygroscopicity, wetting time, and particle size. However, a strong and positive correlation was found between DH and oxidative stability. The lowest peroxide value (2.48 – 1.99 meq/kg oil) was found from the powder formulated with protein hydrolysate (DH 10%) and 1.5% of protein concentration during the entire storage study. Confocal laser scanning and atomic force microscopies also allowed identifying the effects of these variables on oxidative stability. Through XPS analysis, it was possible to observe that higher DH favored the surface accumulation of proteins at the powder surface. These results showed that the degree of hydrolysis exerted an influence on the distribution of components and the percentage of protein on the powder surface.

Keywords: Rice protein isolate, Enzymatic hydrolysis, Functional properties, XPS, Oxidative stability.

5.1.INTRODUCTION

The compositional aspect and mechanism of surface formation of microparticles obtained by spray drying need to be known and understood since they are extremely important to the quality and development of new products. Several studies have shown that the surface composition of spray-dried microparticles significantly influenced particle-liquid interactions (e.g., hygroscopicity and reconstitution properties of powders, such as dispersibility and wettability); particle-particle interactions (fluidity of particulate material, agglomeration); barrier and retention properties of volatile compounds; and drying performance (Adhikari et al., 2009; Jayasundera et al., 2011; McCarthy et al., 2013; Rosenberg and Sheu, 1996; Sadek et al., 2016).

In addition to the surface composition of the microparticle, another important phenomenon to be considered is the formation of pores in the particle wall during the drying process, which has been widely reported in the literature (Drusch and Berg, 2008; Edris et al., 2016; Wang et al., 2014). Although several studies have evaluated the microencapsulation process by encapsulation efficiency and powder recovery, pore formation analysis requires a more detailed approach. These pores serve as a path for oxygen to diffuse through the microparticle, react with the active material, and release volatile compounds inside the particle.

Modifying the surface properties of the droplets/particles by adding proteins to the particle wall formulation is an alternative for improving the microparticle barrier properties. Due to their excellent functional properties, animal proteins have been extensively researched and applied to various processes. However, the production of these proteins has a negative environmental impact, as data reported by the Food and Agriculture Organization (2018). Thus, the use of vegetable proteins in several processes has attracted considerable attention in recent years due to their nutritional

value, availability, low cost, and antimicrobial and antioxidant activity (Chang et al., 2016; Le Priol et al., 2019).

Despite this, it is interesting to emphasize that animal proteins are more effective as wall material than plant proteins in the microencapsulation process. However, some works in the literature showed that the functional properties of plant proteins could be improved by modifying their structure (Gomes and Kurozawa, 2021; Nesterenko et al., 2014; Tamm et al., 2016). Partial enzymatic hydrolysis of proteins, generally ranging from 1% to 10% of the degree of hydrolysis, can increase protein solubility and improve its emulsifying properties by exposing hydrophobic groups, thus, increasing its surface hydrophobicity. Furthermore, protein hydrolysates have a kinetic advantage by migrating more easily to the surface of the sprayed emulsion droplet during spray drying through the diffusion mechanism and possibly forming a surface film on the particles, providing a protective barrier (Jayasundera et al., 2011; Tamm et al., 2015). The influence of plant proteins on the barrier properties of films and particles is still poorly reported in the literature and needs further research.

Thus, this work aimed to study the influence of rice protein hydrolysates on forming a protein film on particle surfaces. In addition, we evaluated the role of the protein film in the oxidation barrier and how it influenced powder properties. Thus, the spray-dried microparticles were analyzed regarding surface composition by X-ray photoelectron spectroscopy (XPS), internal material distribution by fluorescence optical microscopy, and size and topography by Atomic Force Microscopy (AFM). These analyses were applied to verify how the hydrolyzed proteins can significantly influence the stability of the encapsulated compound (represented in this work by linseed oil) during spray drying and storage.

5.2. MATERIAL AND METHODS

5.2.1. Materials

Linseed oil was supplied by Vital Âtman (São José do Rio Preto, Brazil). The wall materials were maltodextrin 10 DE Mor-Rex® 1910 and rice protein isolate (90% dry weight protein concentration) were kindly donated by Ingredion (Mogi Guaçu, Brazil) and Gramkow (Joinville, Brazil), respectively. The commercial protease Flavourzyme, fluorescein isothiocyanate (FITC), and Nile red were purchased from Sigma-Aldrich (St. Louis, Mo., U.S.A). The other reagents used were analytical grade.

5.2.2. Enzymatic hydrolysis of rice protein

Rice protein isolate was hydrolyzed by the protease Flavourzyme action to obtain protein hydrolysates at a 2, 6, or 10% degree of hydrolysis (DH). Hydrolysis was performed under optimal enzyme conditions at 50°C and pH 6.0. A detailed description of the experiment was reported in our previous work (Gomes and Kurozawa, 2020). In that work, the rice protein hydrolysates (RPH) were abbreviated as RPH(Y)-Z%, with Y being the degree of hydrolysis and Z the protein concentration in the emulsion.

5.2.3. Preparation of emulsions and production of spray-dried linseed oil microparticles

Based on our previous work (Gomes and Kurozawa, 2023) on the performance of RPH on oil-in-water emulsions, the surface-active compound, and maltodextrin (MD) proportions were defined and displayed in (Table 1).

Table 1. Composition of linseed oil emulsion (g/100g emulsion) stabilized by protein hydrolysate (RPH) or rice protein isolate (RPI) combined with maltodextrin (MD).

Samples	Continuous phase		Dispersed Phase
	MD (% w/w)	Protein (% w/w)	Linseed oil (% w/w)
RPH2/RPH6/ RPH10/RPI	33.5	1.5	10
	34.0	1.0	
	34.5	0.5	

The total solid concentration, corresponding to the wall material solids (maltodextrin and protein) and linseed oil, was 45%. The oily phase concentration was fixed at 10%, and the wall material concentration was 35%. The emulsions were prepared using a rotor-stator homogenizer (Ultra-turrax IKA T18 Basic, Wilmington, USA) operating at 15,600 rpm for 2 min. After that, all emulsions were spray dried using a laboratory scale spray dryer (Lab Plant SD-06A, North Yorkshire, UK), consisting of a 500 × 215 mm dryer chamber and a 0.5 mm diameter double-fluid atomizer nozzle operating at 2.8 bar. The emulsion was pumped to the spray dryer nozzle through a peristaltic pump at a 485 mL/h feed rate at room temperature and dried at the inlet and outlet air temperatures of 180±5 ºC and 110±5 ºC, respectively (Gomes and Kurozawa, 2020).

5.2.4. Analysis of the surface composition of the microparticles by X-ray photoelectron spectroscopy (XPS)

The surface composition of the microparticles was quantified using a K-Alpha spectrometer (X-ray Photoelectron Spectrometer, Thermo Scientific, England). The samples, placed in a high vacuum (2×10^{-7} Pa), were irradiated with well-defined X-ray radiation (monochromatic AlK-α X-ray source (1487 eV)), resulting in the emission of

photoelectrons from the outermost surface layers. Survey and high-resolution spectra were collected using 200 and 20 eV pass energies, respectively. The quantitative analysis was made from high-resolution spectra averaged from three points in different areas on the sample surface.

5.2.5. Bulk tapped and true densities, and porosity

Bulk tapped density (ρ_{tapped}) was determined by gently adding 2 g of powder in a 10 mL graduated cylinder and compacting it by tapping it on the countertop five times. The powder particles' true density (ρ_{true}) was determined using a Pycnomatic helium pycnometer (AccuPyc 1330, Norcross, USA).

Porosity (ε) of the powder samples was calculated using the ratio between the tapped (ρ_{tapped}) and particle (ρ_{true}) densities of the powder (Eq. 1).

$$\varepsilon = \frac{(\rho_{true} - \rho_{tapped})}{\rho_{true}} \times 100 \quad (1)$$

5.2.6. Microencapsulation efficiency

The microencapsulation efficiency (EE, %) was calculated using Equation (2).

$$EE = \frac{(Oil_{total} - Oil_{surface})}{Oil_{total}} \times 100 \quad (2)$$

Where: Oil_{total} (g) was the amount of total oil, and $Oil_{surface}$ (g) was the amount of surface oil present in the microcapsules and quantified according to Bae and Lee (2008).

The total oil content was assumed to be equal to the initial oil content since linseed oil is non-volatile, and the oil losses while spray drying was considered negligible.

5.2.7. Powder recovery

Powder recovery (%) was measured for each treatment and expressed as the powder mass ratio obtained at the spray-dryer output and the solid mass in the feed solution.

5.2.8. Characterization of the microparticles

5.2.8.1. Moisture content and water activity

Powder moisture content was determined gravimetrically using a vacuum oven at 70°C until achieving constant sample weights. The microparticles' water activity (aw) was measured at 25 ºC using a Novasina thermoconstanter (Novasina AG Zürich, Switzerland). Both assays were carried out in triplicate.

5.2.8.2. Wettability (reconstitution test)

Powder wettability was determined as the method reported by Fuchs et al. (2006), but with a few modifications. About 0.1 g of powder was dispersed over a water surface in a Beaker containing 100 mL of ultrapure water without stirring at 25 ºC. The time spent immersing or wetting the last powder particle was used as a wettability response.

5.2.8.3. Solubility

With some modifications, powder solubility was evaluated according to Cano-Chauca et al. (2005). A 1 g amount of powder was added carefully to 100 mL distilled water under stirring conditions with a magnetic stirrer at 400×g for 4 min. The resulting solution was centrifuged at 3000×g for 4 min. Then, 25 mL of the supernatant was dried

in an oven at 105 °C for 3–5 h. The dried solid matter weight was used as the initial powder percentage to determine the water solubility.

5.2.8.4. Hygroscopicity

Two grams of the sample were placed in a saturated NaCl solution desiccator (relative humidity 75%). Samples were kept at room temperature for seven days. Powder hygroscopicity was determined as a gram of adsorbed moisture per 100 g of dry powder (Cai and Corke, 2000).

5.2.8.5. Particle size distribution

The particle size distribution was determined in triplicate using a laser light diffraction instrument (Mastersizer 2000, Malvern Instruments Ltd., UK). A small quantity of powder was dispersed in 99.5% ethyl alcohol and stirred. The particle size distribution was monitored during each successive measurement until the readings became constant. The mean diameter was expressed as Brouckere diameter ($D_{4,3}$).

5.2.9. Morphology of emulsion droplets and microstructural properties of spray-dried linseed oil powder

5.2.9.1. Morphology of emulsion droplets by fluorescence optical microscopy

The morphology of fluorescently labeled oil droplets in the emulsions stabilized by RPI or RPH10 was examined by an optical microscope (Zeiss microscope Axio Visio, Germany) equipped with a Filter Set 46 fluorescence filter. In brief, 0.1% (w/w, based on labeling materials) of Nile Red (for staining the oil) in acetone and FITC (staining the RPI and RPH) and 1 mL of emulsion were dissolved in ethanol, respectively. These labeled emulsions were observed using two lasers at the excitation

and emission wavelengths for Nile Red (excitation k = 530 nm; emission k = 635 nm) and FITC (excitation k = 633 nm; emission k = 740 nm).

5.2.9.2. Atomic force microscopy (AFM)

The powders were attached onto a freshly cleaved mica sheet using epoxy glue, and a compressed nitrogen stream was used to dry the sample, achieving a homogeneous distribution on the mica plate. After that, the mica plate was kept in a desiccator for 30 min. to ensure the complete drying of the sample. AFM images were recorded using an NX-10 instrument (Park Systems, Sunnyvale, US. The AFM system was equipped with model FMR cantilevers (NanoSensors), a resonance frequency of 75 kHz, and a spring constant of 2.8 Nm^{-1}. A 3×3 μm^2 section was analyzed in tapping mode for each particle. The AFM images of the powders were captured by Gwyddion v2.61 software.

5.2.9.3. Confocal laser scanning microscopy

The images were captured using a Confocal laser scanning microscope (LSCM VK-X200 – Keyence, Osaka, Japan) with a 408 nm wavelength, and images were taken at 50× and 150× magnifications.

5.2.10. Oxidative stability by peroxide value

The microparticles were sealed in a glass vial and stored for 30 days at 45°C to accelerate the oxidation process. The oil extraction was performed according to Partanen et al. (2008). The peroxide value was determined spectrophotometrically according to the IDF standard method 74A:1991. Measurements were carried out in triplicate. Hydroperoxide concentrations were obtained using a Fe^{+3} standard curve with

iron concentration varying from 1 to 24 μg, following the Shantha and Decker method (1994).

5.2.11. Statistical analysis

The results were submitted to the analysis of variance (ANOVA) followed by Tukey's ($p<0.05$) *post hoc* test using Statistical 7.0 software.

5.3. RESULTS AND DISCUSSION

5.3.1. Surface composition analysis by XPS

The XPS technique was applied to identify the DH effect and protein concentration on the distribution of chemical elements and functional groups on the microparticle surfaces (Table 2). The chemical composition was measured at three different particle surface depths. Each depth corresponds to level 1, the particle surface; level 2, an approximate depth of 0.2 nm; and level 3, a 0.3 nm depth.

Table 2. Effect of hydrolysis degrees (2, 6, and 10%) and protein concentration (0.5, 1.0, and 1.5%) on the surface composition obtained from XPS for the spray-dried linseed oil powders.

Sample	Level 1*			Level 2			Level 3		
	C (%)	O (%)	N (%)	C (%)	O (%)	N (%)	C (%)	O (%)	N (%)
RPH2-0.5%	77.77 ± 4.02	19.73 ± 3.28	1.12 ± 0.19	86.62±1.24	11.98±0.41	1.11±0.63	88.18±0.95	10.72±0.17	0.96±0.58
RPH2-1.0%	78.39 ± 3.21	19.39 ± 2.6	1.31 ± 0.16	79.80±4.53	19.13±5.13	0.89±0.81	81.31±5.34	18.00±4.67	0.53±0.12
RPH2-1.5%	78.73 ± 2.99	19.18 ± 2.71	1.22 ± 0.4	82.03±10.21	16.82±1.34	0.97±0.53	81.62±9.88	17.01±9.46	1.31±0.48
RPH6-0.5%	82.97 ± 4.68	15.75 ± 4.58	1.05 ± 0.06	88.50±3.36	10.76±3.29	0.64±0.03	89.99±2.69	9.45±2.69	0.41+0.02
RPH6-1.0%	79.60 ± 2.64	18.37 ± 2.46	1.39 ± 0.28	86.94±2.51	12.22±1.73	0.75±0.78	88.38±1.70	11.16±1.41	0.37±0.23
RPH6-1.5%	84.41 ± 0.6	14.81 ± 0.52	1.27 ± 0.66	87.15±0.86	11.95±0.71	0.88±0.18	88.63±0.23	10.76±0.22	0.53±0.12
RPH10-0.5%	78.88 ± 1.28	18.97 ± 0.82	1.55 ± 0.34	86.57±0.19	13.03±0.28	0.84±0.23	87.80±0.06	11.54±0.19	0.57±0.19
RPH10-1.0%	78.88 ± 1.28	18.97 ± 0.82	1.30 ± 0.28	84.90±0.86	13.62±0.31	1.16±0.73	85.91±0.03	12.42±1.06	1.48±1.09
RPH10-1.5%	79.38 ± 0.66	18.14 ±0.53	1.76 ± 0.16	85.00±1.99	14.08±2.13	0.90±0.17	86.04±2.86	13.02±2.47	0.79±0.39
RPI-0.5%	79.90 ± 2.39	19.02 ± 2.65	0.60 ± 0.28	86.63 ± 0.98	12.87 ±0.83	0.43 ± 0.08	88.16 ±0.64	11.08 ±0.64	0.60 ±0.02
RPI-1.0%	81.40 ± 1.70	18.23 ± 1.61	0.29 ± 0.08	88.10±1.04	11.29±0.95	0.40±0.06	89.05±0.57	10.56±0.63	0.35±0.03
RPI-1.5%	78.72 ± 1.23	20.44 ± 1.16	0.84 ± 0.03	86.48±1.18	12.68±1.34	0.70±0.04	87.75±0.11	11.64±0.07	0.45±0.13

RPI is rice protein isolate, and RPH is rice protein hydrolysate;

* XPS measurement depth. Level 1 corresponds to the particle surface; level 2 is an approximate 0.2nm depth; and level 3 is a 0.3 nm depth

In all cases, a carbon element was predominant (77.77-84.41 wt.%), followed by oxygen (14.81-20.44 wt.%) and nitrogen, with the lowest content range (0.29-1.76 wt.%). As N indicates the presence of proteins, the distribution of these macromolecules was evaluated based on the concentration of N.

A clear influence of DH was observed on the concentration of N on the external surface of microparticles, comparing the results obtained for RPH10 and RPI samples. Even at a lower protein concentration (0.5%), RPH10 microparticles presented higher surface protein content than those containing 1.5% RPI. These results are due to differences in protein structure, whereas the RPH10 shows a more flexible nature compared to the RPI globular structure. The ability of the protein hydrolysate to efficiently reduce the interfacial tension of the oil-in-water emulsion, as reported previously by our research group (Gomes and Kurozawa, 2023), can influence these results. The hydrolysates protect the oil droplet while drying, thus forming a surface film on the formed particles. Although some hydrolysates, for example, RPH2-0.5%, showed no difference in decreasing interfacial tension compared to RPI, they may have undergone structural and conformational rearrangements after adsorption, as that implies a greater number of N on the particle surface. A peptide's solubility, molecular size, and amino acid sequence contribute to this conformational rearrangement. Besides, protein hydrolysates have a kinetic advantage as they migrate more easily to the surface of the sprayed emulsion droplet while spray drying through the diffusion mechanism and possibly forming a surface film on the formed particles. These minimal differences in the N content on the particle surface between RPH microparticles suggest fast protein diffusion to the particle surface, saturating the external surface.

It is also necessary to discuss the distribution of chemical elements (C, N, and O) in the wall (or shell) thickness of linseed oil microparticles (Table 2). These variable

observation scales enabled a better understanding of how the components were distributed from the particle surface to the bulk matter. A smaller amount of protein is observed as we go deeper into the protein levels with higher surface activity. For example, microparticles RPH10-1.5% and RPH6-1.5% show higher surface activity and lower amounts of protein at levels 2 and 3. Thus, depending on the nature of the proteins, different results can be found on the particle surface and, consequently, inside the particle. Gaiani et al. (2010) reported that casein presents lower lipids surface enrichment than whey proteins due to its lower surface tension at the air-water interface. Tamm et al. (2015) observed a decrease in microparticles on the surface when whey protein hydrolysates were used in the formulation.

Several mechanisms for forming the surface composition were discussed in the literature (Fäldt and Bergenståhl, 1994; Kentish et al., 2005; Kim et al., 2009a, 2009b, 2009c, 2003; Sadek et al., 2016; Wang and Langrish, 2009). One of their concerns was that particle drying was controlled at the beginning of the process by water diffusion from the droplet surface toward the surrounding air. At the same time, the proteins are progressively concentrated at the air-liquid interface, quickly forming a monolayer. Although this mechanism has been described for dairy products, there is little doubt that such mechanisms are generic and can also apply to microencapsulated products. This diffusion process can almost instantly precipitate proteins on initial heating at the air-liquid interface, causing a decrease in the local protein concentration near the droplet surfaces. Then, this concentration differential causes protein diffusion from the interior to the droplet surfaces. Since diffusivity decreases as the molecular weight increases, substances with low diffusivity also have low solubility. Thus, the outermost layer of the particle is dominated by small and soluble molecules.

Moreover, such results show how the distribution of chemical elements was affected by the encapsulating agent proportion and DH, which controls the diffusivity and spreading of the surface-active proteins on the surface, surface area–to–volume ratio of the particles (particle size), and encapsulation efficiency.

5.3.2. Particle density and porosity

The formation of glassy protein films on the particle surface can prevent flavor release and minimize oxygen penetration while spraying drying solutions and emulsions containing proteins (Adhikari et al., 2007; Tamm et al., 2015). This penetration of oxygen or release of volatile compounds in the matrix can be affected by the true density of particles. The decrease in true density reflects an increase in air inclusion within the spray-dried particle, which is less protective against oxidation (Drusch et al., 2007).

Overall, a positive correlation between DH and the true particle density of the spray-dried powder was observed, being more pronounced for microparticles containing protein hydrolysates with DH 10% (Table 3). Variations in the properties of the surface film could possibly explain the differences in particle density, as Elversson and Millqvist-Fureby (2005) observed. Film-forming polymers are likely to form a dense ("gas-resistant") shell during spray drying and as less gas reaches the interior void, decreasing true particle density and consequential porosity. Limited hydrolysis, represented by DH, improves functional protein properties, including their ability to migrate to the particle surface to form a protective layer. This statement is corroborated by the results of XPS, in which samples containing RPH10 showed higher protein levels on the particle surface than those formulated with RPI. Pearson's correlation coefficient also observed a positive and strong correlation between the amount of nitrogen on the

particle surface and true density (r =0.81). Another fact that supports this statement is the stronger protective effect of these particles formulated with hydrolysates observed by the high oxidative stability (Figure 6).

Table 3. Influence of enzymatic hydrolysis and protein concentration on the microparticle properties and powder recovery**.**

Sample	Continuous phase (% w/w)		True density (g/cm^3)	Bulk tapped density (g/mL)	Porosity (%)	ME (%)	Recovery (%)
	MD	Protein					
RPH(2)	34.5	0.5	1.178 ± 0.003^{c}	0.454 ± 0.022^{abc}	63.41 ± 0.81^{ab}	82.2 ± 3.0^{abc}	34.7 ± 0.8^{ab}
	34.0	1.0	1.151 ± 0.001^{g}	0.492 ± 0.013^{a}	62.29 ± 1.08^{ab}	79.0 ± 2.1^{c}	31.9 ± 1.2^{ab}
	33.5	1.5	1.148 ± 0.002^{g}	0.486 ± 0.007^{a}	66.28 ± 1.92^{a}	81.3 ± 0.4^{abc}	34.1 ± 3.2^{ab}
RPH(6)	34.5	0.5	1.161 ± 0.001^{f}	0.435 ± 0.015^{bcd}	62.53 ± 1.30^{ab}	83.7 ± 1.7^{abc}	34.0 ± 1.3^{ab}
	34.0	1.0	1.166 ± 0.000^{e}	0.459 ± 0.004^{ab}	60.65 ± 0.32^{bc}	80.7 ± 0.7^{abc}	35.0 ± 2.4^{ab}
	33.5	1.5	1.172 ± 0.000^{d}	0.432 ± 0.013^{bcd}	63.11 ± 1.16^{ab}	84.7 ± 2.3^{ab}	36.9 ± 0.8^{b}
RPH(10)	34.5	0.5	1.236 ± 0.000^{a}	0.431 ± 0.009^{bcd}	63.26 ± 1.79^{ab}	85.6 ± 0.3^{a}	35.9 ± 0.8^{b}
	34.0	1.0	1.149 ± 0.000^{g}	0.492 ± 0.013^{bcd}	57.17 ± 1.14^{c}	78.9 ± 0.9^{abc}	29.2 ± 1.2^{a}
	33.5	1.5	1.199 ± 0.001^{b}	0.387 ± 0.21^{d}	58.89 ± 0.46^{c}	83.1 ± 1.9^{abc}	31.6 ± 0.3^{ab}
RPI	34.5	0.5	1.068 ± 0.000^{i}	$0.458 + 0.009^{cd}$	62.04 ± 1.86^{ab}	83.7 ± 0.4^{abc}	33.1 ± 1.9^{ab}
	34.0	1.0	1.076 ± 0.002^{h}	0.449 ± 0.007^{d}	62.84 ± 2.98^{ab}	79.1 ± 2.5^{c}	34.4 ± 0.8^{ab}
	33.5	1.5	1.023 ± 0.000^{j}	0.420 ± 0.039^{d}	62.34 ± 1.86^{ab}	79.8 ± 1.9^{bc}	32.9 ± 1.7^{ab}

[a-d] Values in the same column followed by different letters are significantly different ($p < 0.05$); Results reported as mean ± standard deviation

5.3.3. Encapsulation efficiency

High microencapsulation efficiency (ME), ranging from 78.91 to 85.68%, was obtained for all RPH and RPI used as emulsifiers (Table 3). The state-of-the-art reports that many studies present a positive correlation between protein hydrolysis and ME,

leading to the belief that structural modification could improve entrapment efficiency (Gomes and Kurozawa, 2020; Tamm et al., 2016). However, the statistical analysis showed a non-significant impact of enzymatic hydrolysis and protein concentration on ME results. This behavior was not expected, considering that protein hydrolysates, specifically in RPH6 and RPH10, presented greater emulsion stability and solubility, in addition to their ability to decrease interfacial tension compared to RPI, as discussed in our previous work (Gomes and Kurozawa, 2023). Besides that, there was an increase in the content of N in the particle surface formulated with RPH10. In this case, a glassy film formed at the beginning of drying was expected to trap/encapsulate more oil than the RPI.

Overall, the intact protein, despite being less efficient in reducing interfacial tension than RPH, resulted in high microencapsulation efficiency (Table 3). These results could be explained by some phenomena that may have occurred during spray drying. As reported by Adhikari et al. (2007), some proteins display very rapid film-forming properties when subjected to air drying. Another hypothesis may be related to the location of the oil inside the particles. Since the intact protein is further inside the particle, it retained the oil, presenting encapsulation efficiency close to the microparticles formulated with protein hydrolysates.

We can hypothesize that the encapsulation process was efficient through the XPS results. It is worth mentioning that XPS and oil extraction provide information on different aspects of surface oil. We can observe that the deeper the layers are (Table 2), the higher the amount of C. Linseed oil is, which is a good source of ω-3. That contains more than 50% of α-linolenic acid (ALA) and high contents of monounsaturated oleic acid (21.2%) and linoleic acid (LA, 13.96%), both groups consisting of C (Bakry et al., 2016). This result indicates effectiveness in keeping a significant load of linseed oil

inside the particle during spray drying. It is an important aspect that further dictates the powder shelf life.

Additionally, XPS quantifies the coverage at the outermost layer of the particles. This property is relevant for particle-particle, particle-air, or particle-water interactions, such as wetting and dispersion. On the other hand, oil extraction quantifies the accessible oil, including that through cracks and pores (Munoz-Ibanez et al., 2016).

5.3.4. Powder recovery

Several research and review papers have reported that using a small amount of protein increased powder recovery (Fang et al., 2013; Jayasundera et al., 2011, 2009). In our work, the increase in protein concentration from 0.5% to 1.5% on a dry solid basis did not further improve the recovery, which ranged from 29% to 39% (Table 3). Besides that, a positive influence of DH was expected on the powder recovery since the hydrolysates protein migrates preferentially to the surface of droplets-particles, reducing adhesive behavior between particles and the dryer wall.

5.3.5. Characterization of particles

The physicochemical characteristics of microparticles produced using different protein concentrations and degrees of hydrolysis are presented in Table 4. Usually, spray-dried particles are nonhomogeneous particulate systems due to process conditions and wall materials. They can influence some powder properties (e.g., hygroscopicity, wettability, solubility, particle size ...) and, consequently, its storage stability.

Table 4. Physicochemical properties of the linseed oil microparticles formulated with maltodextrin (MD) and rice protein isolate (RPI) or rice protein hydrolysate (RPH).

Sample	Continuous phase		Hygroscopicity	Solubility	Wettability	Moisture	Water	*$D_{4,3}$ (μm)
	MD (% w/w)	Protein (% w/w)	(g H_2O/ 100g)	(%)	(s)	(% w/w)	activity (a_w)	
RPH(2)	34.5	0.5	8.07 ± 0.67^{abc}	83.79 ± 2.19^{c}	51 ± 12^{a}	1.64 ± 1.89^{a}	0.12 ± 0.02^{a}	38.35 ± 7.53^{a}
	34.0	1.0	7.94 ± 0.15^{abc}	88.45 ± 2.67^{abc}	50 ± 9^{ab}	0.54 ± 1.04^{a}	0.13 ± 0.02^{a}	19.85 ± 5.45^{c}
	33.5	1.5	8.58 ±0.24^{a}	87.42 ± 0.63^{abc}	52 ± 9^{ab}	0.58 ± 0.19^{a}	0.09 ± 0.00^{a}	25.14 ± 2.31^{bc}
RPH(6)	34.5	0.5	8.06 ± 0.08^{abc}	90.08 ± 1.72^{ab}	66 ± 10^{a}	0.52 ± 0.34^{a}	0.15 ± 0.02^{a}	26.09 ± 2.53^{bc}
	34.0	1.0	8.08 ± 0.11^{ab}	87.18 ± 1.73^{abc}	60 ± 11^{a}	0.50 ± 0.37^{a}	0.13 ± 0.02^{a}	20.38 ± 0.10^{c}
	33.5	1.5	8.08 ± 0.08^{abc}	88.85 ± 1.12^{abc}	53 ± 3^{ab}	0.32 ± 0.30^{a}	0.11 ± 0.00^{a}	23.31 ± 4.93^{bc}
RPH(10)	34.5	0.5	8.10 ± 0.03^{ab}	84.78 ± 1.06^{bc}	43 ± 5^{ab}	0.45 ± 0.20^{a}	0.12 ± 0.01^{a}	31.7 ± 4.49^{ab}
	34.0	1.0	7.33 ± 0.42^{cd}	87.82 ± 1.44^{abc}	58 ± 2^{ab}	0.83 ± 0.49^{a}	0.18 ± 0.01^{a}	32.66 ± 1.07^{ab}
	33.5	1.5	6.89 ± 0.14^{d}	91.16 ± 1.34^{a}	37 ± 4^{b}	0.53 ± 0.21^{a}	0.17 ± 0.02^{a}	22.26 ± 3.14^{bc}
RPI	34.5	0.5	7.77 ± 0.06^{bc}	88.83 ± 2.08^{abc}	65 ± 11^{a}	0.42 ± 0.32^{a}	0.15 ± 0.01^{a}	24.47 ± 2.23^{abc}
	34.0	1.0	8.25 ± 0.19^{ab}	87.11 ± 2.92^{abc}	56 ± 5^{a}	0.42 ± 0.15^{a}	0.14 ± 0.03^{a}	28.43 ± 2.65^{abc}
	33.5	1.5	8.30 ± 0.08^{ab}	86.83 ± 1.79^{abc}	43 ± 8^{ab}	0.34 ± 0.27^{a}	0.11 ± 0.02^{a}	27.84 ± 1.28^{bc}

$^{a-d}$ Values in the same column followed by different letters are significantly different ($p < 0.05$); Results reported as mean ± standard deviation

*Particle size ($D_{4,3}$)

The determination of hygroscopicity and moisture content is an important factor in the microencapsulation process since the amount of water can influence oxidative stability, powder flowability, dispersibility, and caking. When the powders get wet, the physical structure of microparticles can undergo significant changes, such as collapse, stickiness, and caking, losing their individual structure and function (Boonyai et al., 2004). The powder hygroscopicity and moisture content varied from 6.89 and 8.58% to 0.32 and 1.64%, respectively, values typically found in products obtained by spray drying (Paulo et al., 2021). However, lower hygroscopicity values were not expected due to the presence of protein hydrolysates in the microparticles. According to Rennie et al. (1999), the polarity of surface structural compounds defines the amount of water that can be adsorbed and bound into the powder matrix (Rennie et al., 1999). After RPI enzymatic hydrolysis, the peptide molecules can have imbalanced charges on the microparticle surface; thus, other molecules can adsorb onto its surface to find equilibrium. In this case, the microparticles can condition the number and availability of hydrophilic groups capable of binding water through hydrogen bond formation. However, in this current work, the peptides present on the surface microparticles may have interacted with each other or with the maltodextrin molecules, leaving no group available to bind/interact with water.

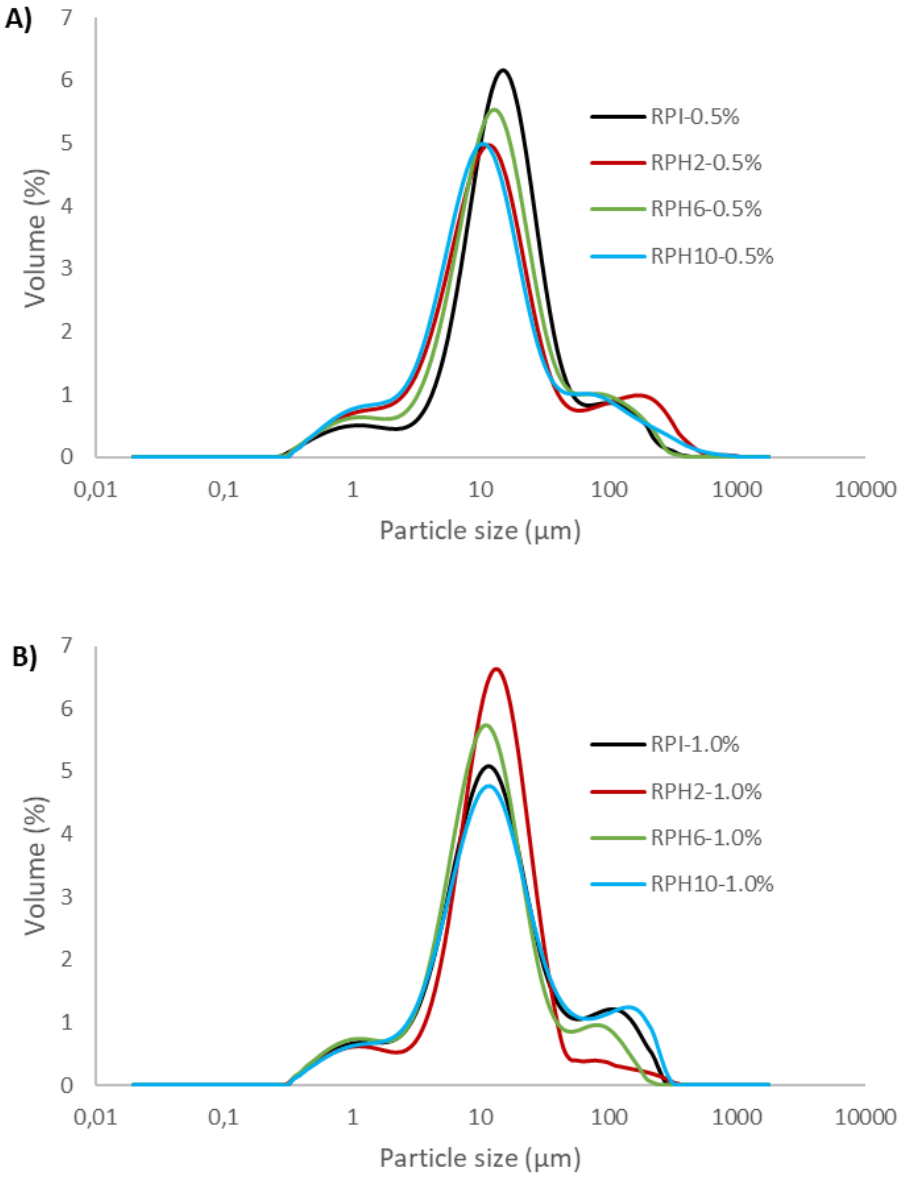
The powder solubility ranged from 83 to 91%, and it was not affected by the degree of hydrolysis and protein concentration ($p<0.05$) (Table 4). It is well known that solubility is affected by powder surface composition. Higher solubility results were expected for particles encapsulated with protein hydrolysates. After protein hydrolysis, hydrophilic groups are exposed, which increases the protein solubility and, consequently, the particle solubility. However, particle solubility was governed by the amount of maltodextrin present since it was higher than the protein concentration (Table

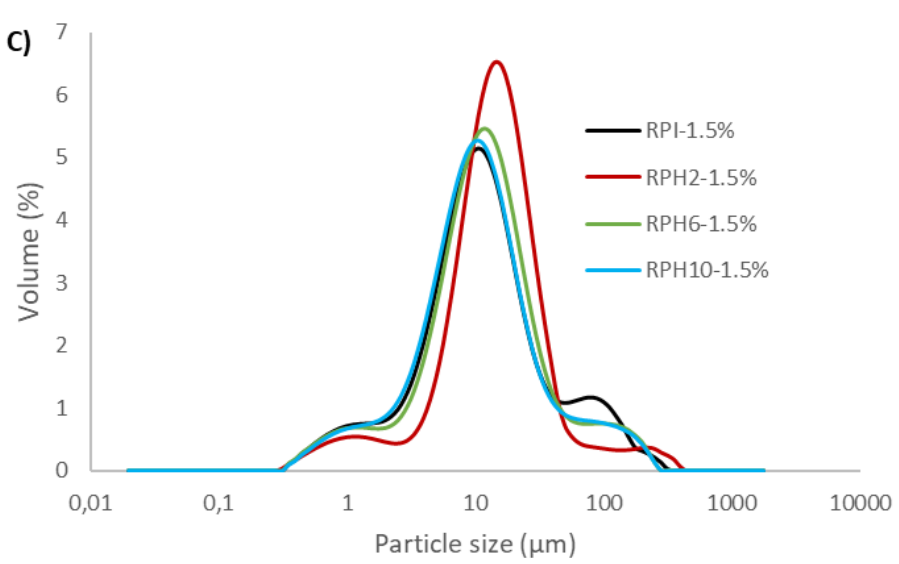
2). Maltodextrin is a carrier agent widely used in spray drying because of its physical properties, such as high-water solubility. The high powder solubility obtained in the current work may also be related to high encapsulation efficiency since the presence of lipids on the powder surface decreases its solubility (Thomas et al., 2004).

Higher protein surface powder impaired wetting properties, as reported by some authors (Crowley et al., 2015; Fyfe et al., 2011; Murrieta-Pazos et al., 2011). These authors stated that the wetting time was affected due to the protein non-polar bonds present on the particle surface. However, in this work, it is believed that the hydrophobic bonds were responsible for binding to the oil, favoring the encapsulation efficiency and, consequently, did not affect the wetting time. The wetting time was less than 70 s for all samples. A correlation was also not observed between the protein surface content and the powder wetting time. Interestingly, the surface protein coverage is not the only factor to be considered to explain the powder wettability properties. Generally, they depend on particle size, density, porosity, surface charge, surface area, the presence of amphipathic substances, and particle surface activity.

There was no effect of protein concentration and DH on the particle size of spray-dried microparticles ($p<0.05$) (Table 4). Overall, a trimodal particle size distribution was obtained, with three distinct peaks (Fig 1), whereas the particle diameters varied from ~1 to 1000 μm.

Figure 1. The particle size distribution of linseed oil microparticles containing different protein concentrations: A) 0.5%; B) 1.0%; C) 1.5%.



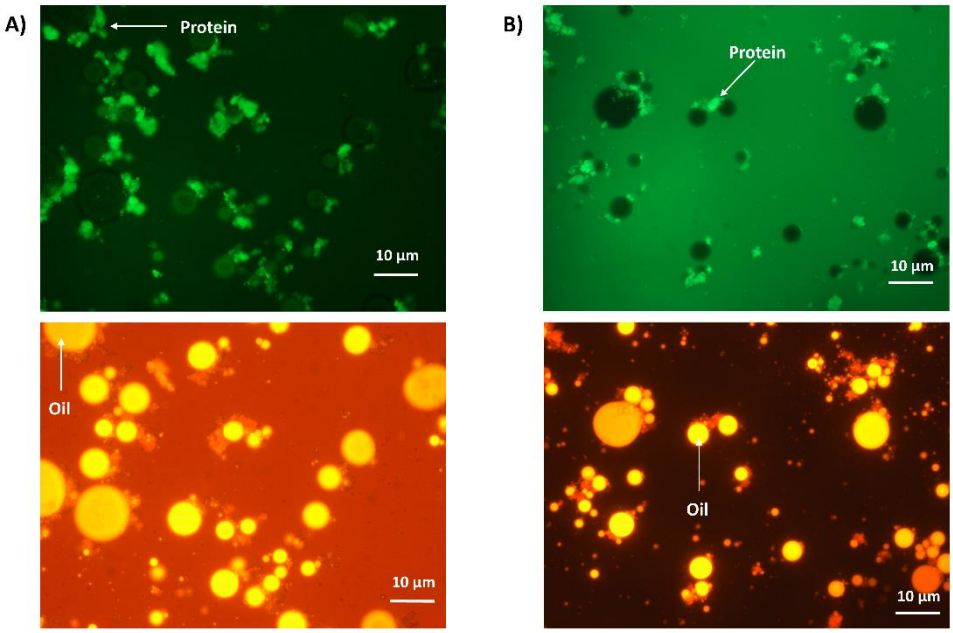


Several variables can influence particle size; one is the proportion of solids in the feed solution and feed composition. In this work, the small amount of protein related to the total solids in the different formulations could not affect the particle size. Gong et al. (2018) noted a minimum protein concentration in the feed solution, which can directly influence the size of spray-dried particles.

5.3.6. Morphology of emulsions and microparticle microstructural properties

Microscopy techniques could compare the surface composition of the spray-dried microparticles to their size and shape to improve understanding of the influence of feed composition and process conditions on particle structure. Microstructures of some emulsions using CLSM were illustrated in Figure 2 to compare with the XPS and AFM microparticle results, allowing a qualitative insight into the internal component distribution. Protein and fat were clearly distinguishable in the CLSM analysis, whereas the green regions represent the protein distribution in the droplet surface, while the red regions confirm the oil location.

Figure 2. Fluorescence optical microscopy of the emulsions stabilized by: A) rice protein hydrolysate with 10% degree of hydrolysis; B) rice protein isolate, both at 1.5% protein concentration. The upper and lower images correspond to samples stained with FICT (staining protein) and Nile Red (staining oil).

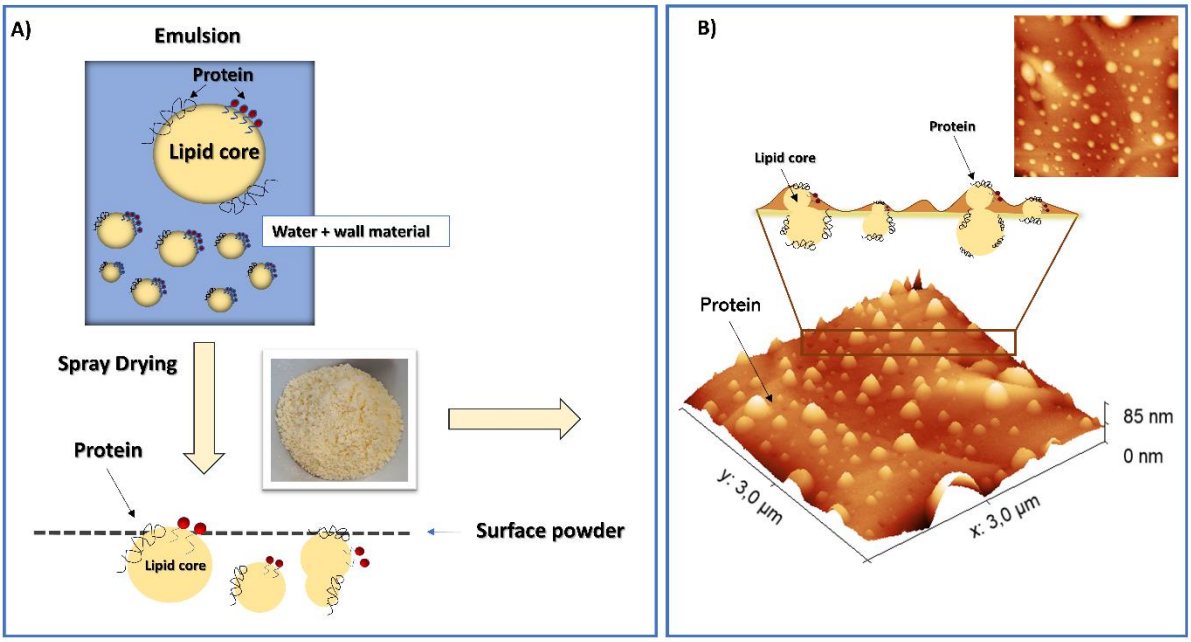


As presented in our previous study (Gomes and Kurozawa, 2023), RPH10-1.5% showed a more active surface than RPI-1.5%; even so, analyzing Figure 2, it was possible to observe that the emulsifier was insufficient to cover the entire surface of the oil droplets. Although RPH10-1.5% did not completely cover the oil droplet, it remained on the particle's surface after drying. This can lead to an improved protective effect of the protein on the microparticles and less fat leakage on the surface of the powder compared to RPI microparticles. When a surface active component was present in the feed solution (such as a protein), the powder surface was mainly covered by that component (Adhikari et al., 2007; Tamm et al., 2015). This agrees with the XPS observation, where there were higher amounts of nitrogen on their surfaces than those formulated with intact protein.

The AFM analysis helped to understand hydrolyzed protein's role in forming microparticle surfaces. Up to now, the description of mechanisms behind these

observations was never proven using AFM. A scheme representing how RPH10-1.5% remains on the particle surface and is observed by AFM is shown in Figure 3. The scheme considers the structure formed immediately after spray drying and after a possible reorganization of RPH on the particle surface.

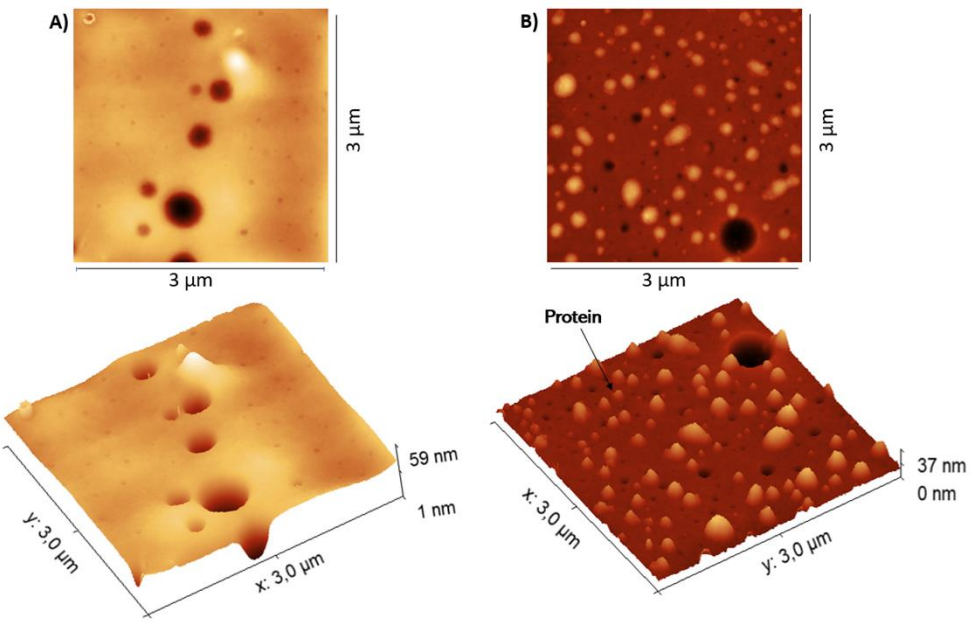
Figure 3. A) Schematic representation of how the protein remains on the microparticle surface after the spray drying process; B) RPH10-1.5% distribution on particle surface observed by AFM.



AFM imaging allowed the visualization of the topography of surface powders, which was characterized by a "sharp" aspect obtained from the RPH10 sample. Such lumps can represent the oil droplets located closer to the microparticle surface and recovered by protein molecules, forming the protein surface film, as can be seen in Figure 3.B. From a mechanical point of view, this fact results in an increased elastic modulus, reflecting a harder surface for powders (Burgain et al., 2016). Proteins with excellent surface-active properties have been associated with changing the microparticles' surface topographic and morphology (Fäldt and Bergenståhl, 1994;

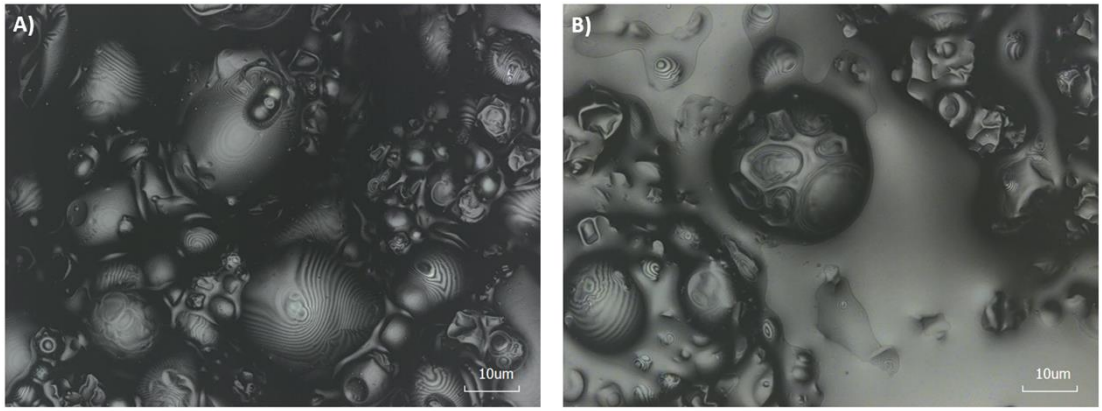
Porras-Saavedra et al., 2015; Wang and Langrish, 2010). The surface area of the selected sample represents small oil droplets, as shown in Figure 3. We can assume flocculation of oil droplets may have occurred during emulsification and subsequent drying. In contrast, the larger droplet remained on the inside, and the smaller droplet remained on the surface (Figure 3.B). On the other hand, the microparticles stabilized with RPI showed nano-pores on their surface, indicating that the intact protein was not able to form a protein film (Figure 4.A).

Figure 4. AFM topographic images 3D (3 x 3µm) of linseed oil microparticles stabilized with (A) RPI-1.5% and (B) RPH10-1.5%.



It was impossible to identify differences between the AFM images in both samples because the surface roughness did not allow us to identify differences due to the small area selected for observation (3 x 3µm). Thus, the morphology of microparticles was also evaluated by Confocal laser analysis (Figure 5). Generally, the microparticles presented a smooth surface with some wrinkled microparticles caused by the drying and subsequent cooling process.

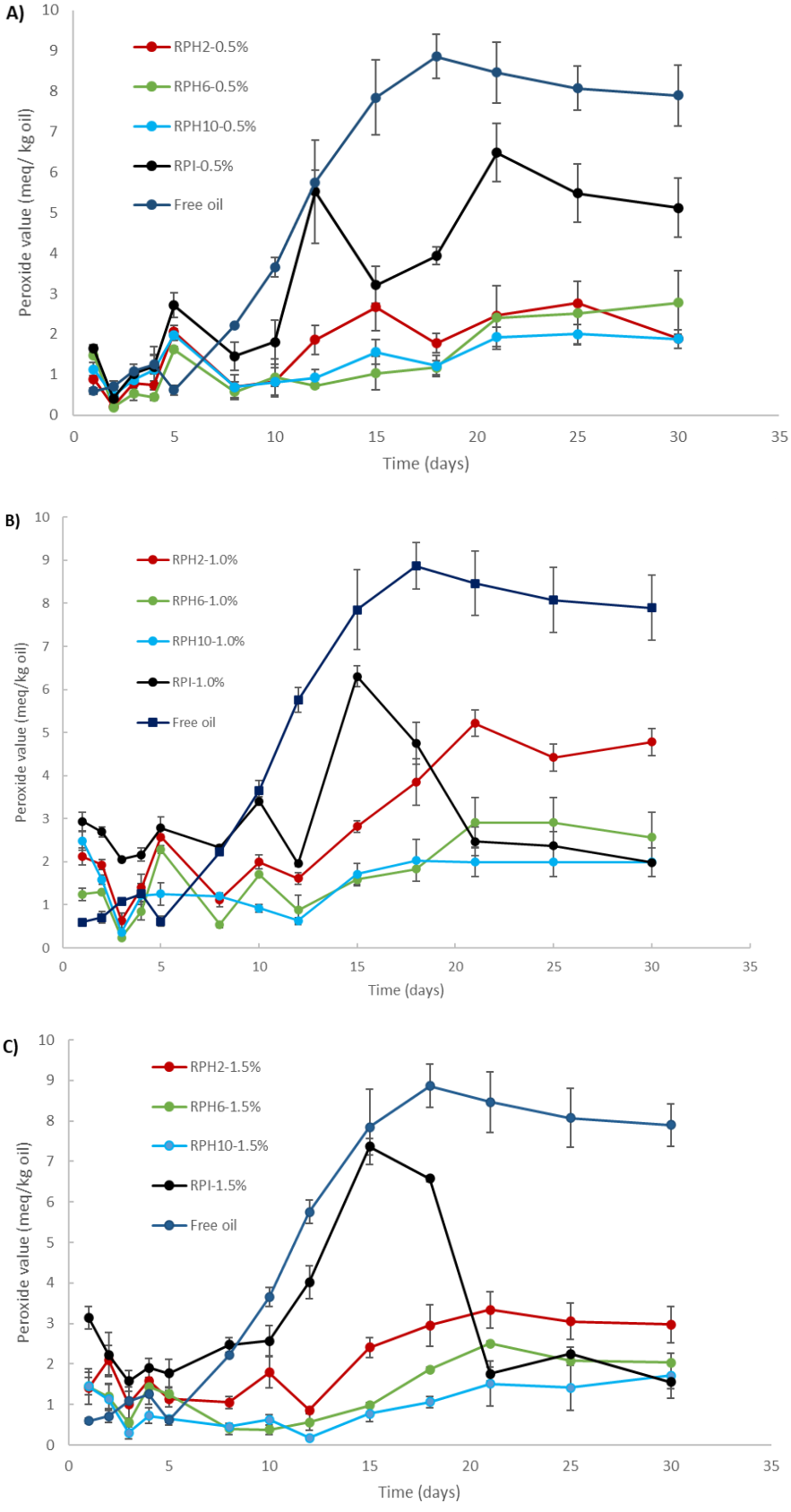
Figure 5. Morphology of the linseed oil microparticles A) RPH10-1.5% and B) RPI-1.5% by confocal laser scanning microscope. Micrographs at 150× magnification.



5.3.7. Oxidative Stability

To assess the influence of DH and protein concentration on the oxidative stability of linseed oil microparticles, an accelerated storage test of peroxide index was carried out. This analysis is considered a quality indicator of fatty food products. It is the most widely used index to determine the oxidation state of microencapsulated oils. The oxidative stability of the microencapsulated linseed oil increased for all protein concentrations through DH (Figure 6).

Figure 6. Lipid oxidation was evaluated by the peroxide value of free linseed oil and microencapsulated with rice protein isolate (RPI) or protein hydrolysates (RPH) at different protein concentrations: A) 0.5%; B) 1.0%, and C) 1.5%.



A positive correlation between protein concentration and stability was also observed, except for particles stabilized by RPH2. All microparticles stabilized by RPH during the first five days of storage displayed similar behavior, whereas all samples' PV content increased slightly. After that, the most pronounced protective effect stabilized the microparticles with RPH6 and RPH10, and RPI particles at a lesser degree for all protein concentrations. The PV of RPH10-1.5% particles did not exceed 1.71 meq/kg, while the RPI-1.5% reached values close to 7.36 meq/kg oil during storage. The PV-free oil varied from a minimum value of 0.56 meq/kg to a maximum of 8.86 meq/kg. This indicates that lipid oxidation in microencapsulated linseed oil may be considerably retarded using partially hydrolyzed rice protein in 6% and 10% DH, as reported recently (Gomes and Kurozawa, 2021). Besides that, it is evident that there is no need to increase protein concentration value since the hydrolysates can provide this protective effect regarding the concentration.

The stability of powders in this study was probably due to the occurrence of significant slight changes in the surface composition. The formation of the surface protein film generates denser (Table 3) and continuous structures during the formation of the microparticles, which may prevent oxygen transfer through the system and thus retard oil oxidation. The delayed particle crust formation and the absence of surface protein film in RPI microparticles, can be responsible for higher PV than other RPH microparticles, as observed by XPS results and AFM images. Based on our previous study, we can also confirm this increase in PV due to the low antioxidant capacity of the RPI than RPH (Gomes and Kurozawa, 2020). It is known that the surface protein coverage depends not only on the quantities of materials adsorbed but also on their types and structures and the other surface-active compound present in the formulation. Protein hydrolysates provide good functional properties and can also fill parts of the interfacial area that are not covered by large proteins. In this case, protein hydrolysates

layers adsorbed on the interfacial area effectively stabilize thin films between emulsion droplets due to their electric charge, hydrophobicity, thickness, and high elasticity, as observed in our previous work (Gomes and Kurozawa, 2023). Several studies reported that the interfacial accumulation of protein in oil droplets is preserved during spray-drying (Elversson and Millqvist-Fureby, 2005; Fäldt and Bergenståhl, 1994; Landström et al., 2000; Munoz-Ibanez et al., 2016). The recent correlation between the antioxidant capacity of RPI hydrolysates is another important issue tested in vitro, using three different assays and the formation of peroxides in spray-dried linseed oil emulsions stabilized with these hydrolysates (Gomes and Kurozawa, 2021, 2020). Thus, the RPI hydrolysates' antioxidant properties are important to understand the enhanced protective effect.

5.4. CONCLUSION

The knowledge of the effect of rice protein hydrolysates and protein concentration on the emulsions and, consequently, the microencapsulation by spray drying improves microparticles' protection and functional properties. The analysis of microparticles by XPS technique with AFM revealed that the protein concentration and DH might significantly influence these properties.

Overall, it was observed that the positive effects are most evident for microparticles stabilized with RPH6 and RPH10, which showed the highest percentage of surface protein. It is believed that due to its high diffusivity and pronounced characteristic of surface activity, such effects were more pronounced, thus resulting in a more protective effect. In some cases, there seemed to be a saturated state of protein concentration (about 1.0 and 1.5%) in the emulsion, as higher protein concentrations did

not significantly increase the microparticle protection. Meanwhile, a porous surface was formed when RPI was used, reducing the protective effect of microparticle.

Therefore, this study could help formulate microparticles with controlled surface composition in the future, allowing enhanced control of powder functionalities and protective effects.

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CAPÍTULO 6 - RICE PROTEIN HYDROLYSATES AS NATURAL EMULSIFIERS FOR AN EFFECTIVE MICROENCAPSULATION OF ORANGE ESSENTIAL OIL BY SPRAY DRYING

RICE PROTEIN HYDROLYSATES AS NATURAL EMULSIFIERS FOR AN EFFECTIVE MICROENCAPSULATION OF ORANGE ESSENTIAL OIL BY SPRAY DRYING

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ABSTRACT

The surface powder composition depends on the physicochemical properties of the dispersed phase and their interaction with the continuous phase. Rice protein hydrolysates (RPH) (degree of hydrolysis DH of 2, 6 and 10%) were added in emulsions to reduce the interfacial tension between dispersed and continuous phases and stabilize the oil droplets. The dispersed and continuous phases were represented by orange essential oil and maltodextrin solution, respectively. This work aimed to identify the influence of DH on the formation and stabilization of droplets of the volatile oil and verify how they affects the distribution of components on the surface and physicochemical properties of the spray dried microparticles. In general, RPHs were able to reduce the interfacial tension (3.78-4.04 mN/m) when compared to RPI (4.74 mN/m). However, no significant difference was observed between RPH samples. The correlation between the reduction of interfacial tension value and the decrease of droplet size was also observed, in which RPH emulsions showed 8.29-8.66 μm while RPI 9.06 μm. It was clear the influence of DH on the surface composition of the spray-dried RPH emulsions. RPH10 powder showed a greater presence of protein (3.3%) on the surface and consequently exhibited high oil retention (49.97%). In contrast, this sample presented high hygroscopicity, attributed to the percentage of protein on the surface of microparticles.

Keywords: Emulsions; Rice protein hydrolysates; X-ray photoelectron spectroscopy XPS; Surface composition; Spray drying

6.1.INTRODUCTION

The increase in value-added products has substantially affected global market trends. However, the incorporation of some substances into a given product becomes unfeasible due to technological limitations. Microencapsulation via spray drying, a method that keeps the active compound as a dispersed phase, is one of the most used tools to transform a wide variety of liquid products into powder form and is considered one of the solutions for substance stabilization, technological feasibility and financial in obtaining products (Dias et al., 2015; Favaro-Trindade et al., 2010).

Some problems related to the structure, functionality and protection of the active compound are due to the wall material used (Botrel et al., 2014). Plant protein-stabilized o/w emulsions synergized with polysaccharide is emerging as a “green” substantial step for the microencapsulation. This current scenario has attracted the attention of industry and academia to explore the use of new natural and more sustainable sources of emulsifiers. Plant proteins are some of the most promising natural emulsifiers that have been studied as a substitute for animal proteins and synthetic emulsifiers (Drusch et al., 2021; Hinderink et al., 2019). Plant proteins present inferior emulsifying properties than animal proteins; however, several studies have successfully applied enzymatic modification of vegetable proteins to increase their emulsifying and functional properties for the encapsulation of lipophilic compounds through different encapsulation techniques (Gomes & Kurozawa, 2020, 2021; Tamm et al., 2016).

Rice protein has already been reported as a good nutritional source and antioxidant components (Han et al., 2014); however the results for some techno-functional properties are not very satisfactory (Amagliani et al., 2017). Gomes and Kurozawa (2023) showed that rice protein hydrolysates have better emulsifying capabilities than non-hydrolyzed protein. Enzymatic hydrolysis offers a viable means to

improve the functionality of previously degraded proteins caused by extraction and isolation conditions (Eckert et al., 2019). This process comprises a reaction between proteolytic enzymes and proteins, submitting these protein substrates to a structural modification by cleaving the peptide bonds in the protein chain, resulting in short-chain peptides with lower molecular weights, exposed hydrophobic groups, and enhanced solubility and emulsifying properties.

The approach based on enzymatic modification of plant proteins confers the microparticles unique characteristics; however, there is scarce information on the relationship between the technique and the distribution of elements on the surface of microparticles, representing a gap in this research area (Data not published). The study, analysis, and application of knowledge on the surface composition of the microparticles can be used to improve the formulation of encapsulated products.

To the best of our knowledge, research studies on changes in the functional properties, particularly rehydration, are affected by particle surface composition (Ho et al., 2021; Murrieta-Pazos et al., 2011). In our previous study (Data not published), the effects of different DH levels (2, 6 and 10%) on the physiochemical properties of spray-dried linseed oil powders, especially on lipid oxidation during storage, were reported. However, changes in chemical composition on the surface of the powders using rice protein hydrolysates as emulsifier, which is also expected to play an essential role in the microencapsulation process of essential orange oil, have not been investigated. In this study, we examined the influence of rice protein hydrolysates on forming a protein film on particle surfaces of spray-dried orange oil powder. The changes in surface chemical composition of the microparticles were determined by X-ray photoelectron spectroscopy (XPS), followed by an evaluation of the correlation between these results and the powder recovery (as an efficiency and economic factor in spray-drying process),

hygroscopicity (as a physical stability index during preservation), particle size (as a determining factor in packaging conditions) and efficiency of microencapsulation.

6.2. MATERIAL AND METHODS

6.2.1. Materials

Orange essential oil was supplied by Citrosuco S/A Agroindústria (Matão, Brazil). The wall materials maltodextrin 10 DE Mor-Rex® 1910 and rice protein isolate (90% dry weight protein concentration) were kindly donated by Ingredion (Mogi Guaçu, Brazil) and Gramkow (Joinville, Brazil), respectively. The commercial protease Flavourzyme was purchased from Sigma-Aldrich (St. Louis, Mo., U.S.A). The other reagents used were of analytical grade.

6.2.2. Enzymatic hydrolysis of rice protein

Rice protein isolate was hydrolyzed by the action of the protease Flavourzyme to obtain protein hydrolysates with degree of hydrolysis (DH) of 2, 6 or 10%. Hydrolysis was carried out under optimal conditions of the enzyme, corresponding to 50°C and pH 6.0. A detailed description of experiment was reported in our previous work (Gomes & Kurozawa, 2020). In this work, the rice protein hydrolysates (RPH) are abbreviated by RPHX, with X being the DH.

6.2.3. Study of the formation of oil droplets: interfacial tension

The influence of different DH on the interfacial tension at the interface of the phases essential orange oil-water was measured at 25 °C using a tensiometer Tracker-S (Teclis, Longessaigne, France), by the rising (O/W) drop method. The system was assembled with the aqueous phase (1.5% of protein and 33.5% of MD w/w) in the syringe and the lipid phase in the cuvette. Measurements were performed in triplicate.

6.2.4. Preparation of emulsions

Based on our previous work (Gomes & Kurozawa, 2023) on the performance of RPH on oil-in-water emulsions, the proportions of solids corresponding 45% the total solids were: 10% of essential orange oil, 1.5% RPI or RPH, and 33.5% of maltodextrin. The emulsions were prepared using a rotor-stator homogenizer (Ultra-turrax IKA T18 Basic, Wilmington, USA) operating at 15,600 rpm for 2 min.

6.2.4.1. Droplet size distribution and mean droplet size of emulsions

The oil droplet size distribution and mean droplet size, expressed as the Sauter mean diameter ($D_{3,2}$) and polydispersity index (PDI), were measured in triplicate by the laser diffraction technique (Malvern Mastersizer 2000, Malvern Instruments Ltd., UK).

6.2.5. Production and characterization of spray dried microparticles of orange oil

Spray drying of all emulsions was carried out using a laboratory scale spray dryer (Lab Plant SD-06A, North Yorkshire, UK), consisted of a dryer chamber of 500 × 215 mm and a double-fluid atomizer nozzle of 0.5 mm diameter operating at 2.8 bar. Emulsion was pumped to the spray dryer nozzle through a peristatic pump at a feed rate of 485 mL/h at room temperature and dried at inlet and outlet air temperatures of 180±5 ºC and 110±5 ºC, respectively (Gomes & Kurozawa, 2020).

6.2.5.1. Powder recovery

Powder recovery (%) was measured for each treatment and was expressed as the ratio of the mass of powder obtained at the spray- dryer output and the mass of solids in the feed solution.

6.2.5.2. Moisture content and water activity

Powder moisture content was determined gravimetrically using a vacuum oven at 70°C until constant weight of samples. Water activity (a_w) of the microparticles was measured at 25 ºC using a Novasina thermoconstanter (Novasina AG Zürich, Switzerland). Both assays were carried out in triplicate.

6.2.5.3. Hygroscopicity

Two grams of sample was placed in a desiccator containing saturated NaCl solution (relative humidity 75%). Samples were kept at ambient temperature for 7 days. Powder hygroscopicity was determined as gram of adsorbed moisture per 100 g of dry powder (Cai & Corke, 2000).

6.2.5.4. Particle size distribution

The particle size distribution was determined in triplicate using a laser light diffraction instrument (Mastersizer 2000, Malvern Instruments Ltd., UK). A small quantity of powder was dispersed in 99.5% ethyl alcohol with stirring. The particle size distribution was monitored during each successive measurement until the readings became constant. The mean diameter was expressed as the Brouckere diameter ($D_{4,3}$).

6.2.5.5. Oil retention

The oil retention on the microparticles was determined by Eq. 2.

$$Oil\ retention\ (\%) = \frac{Actual\ oil\ content_{(total\ oil)}}{Theoretical\ oil_{(initial\ oil)}} \times 100 \quad (2)$$

Total oil was determined by hydro-distillation in a Clevenger apparatus according to the method described by (Jafari et al., 2007) with some modifications.

About 3 g of microparticles were dissolved in 150 mL of deionized water in a 500 mL round bottom flask. The flask was manually shaken for 2 min to aid in the dissolution of the microparticles. The Clevenger apparatus was placed on the top of the flask, and a condenser with water circulating at 5 °C was placed on the Clevenger. Distillation was performed for 2 h, and the volume of distilled oil was directly read in the Clevenger and multiplied by the orange essential oil's density (0.843 $g.cm^{-3}$) in order to calculate the mass of recovered oil. The theoretical oil content was taken as the mass of oil expected in the microparticles based on the emulsion formulation on a dry basis.

6.2.5.6. Analysis of the surface composition of the microparticles by X-ray photoelectron spectroscopy (XPS)

The surface composition of the microparticles was quantified using a K-Alpha spectrometer (X-ray Photoelectron Spectrometer, Thermo Scientific, England). The samples, placed in high vacuum (2×10^{-7} Pa), were irradiated with well-defined X-ray radiation (monochromatic AlK-α X-ray source (1487 eV) resulting in the emission of photoelectrons from the outermost surface layers. Survey and high-resolution spectra were collected using pass energies of 200 and 20 eV, respectively. The quantitative analysis was made from high resolution spectra averaged from three points located in different areas on the sample surface.

6.2.6. Statistical analysis

The results were submitted to the analysis of variance (ANOVA) followed by Tukey's ($p<0.05$) *post-hoc* test using Statistical 7.0 software.

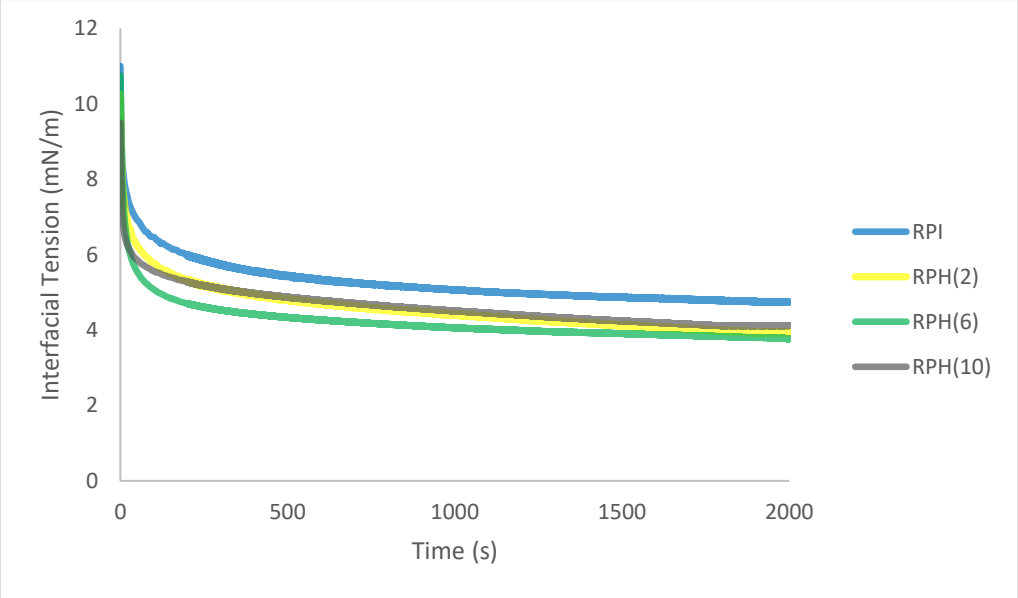
6.3. RESULTS AND DISCUSSION

6.3.1. Influence of interfacial tension on the droplet size

The interfacial tension gives an indication of the surface activity of protein at the oil/water interface, in which is impacted by the type of adsorbed protein at the interface. As the result shown in Figure 1, interfacial tensions for different DH were measured to investigate the ability of RHP spreading around the oil droplets. The initial interfacial tension at water–linseed oil interface was slightly lower (9.5–10.74 mN/m) for the RPH systems, where the lowest initial interfacial tension founded was for RPH10 system and the highest for RPI (11.00 mN/m).

The decrease in the interfacial tension can be related to the affinity of RPH for interfaces, since protein hydrolysis exposes more hydrophobic and hydrophilic sites, increasing the number of molecules absorbed at the oil/water interface. The same behavior was observed to lentil protein hydrolysate when compared to non-hydrolyzed protein (Avramenko et al., 2013). Different interactions must dominate in hydrolyzed samples, but hydrophobic and electrostatic interactions can influence film formation and interfacial tension decrease (Klost & Drusch, 2019). Assuming an increasing hydrophobicity after hydrolysis (Xu et al., 2016), the protein increases the number of contact points with the interface oil, which causes proteins to orientate more readily their polar groups towards the aqueous phase and their hydrophobic parts towards the oil phase. Besides, hydrolysis resulted in a significantly increased surface adsorption kinetic and enhanced conformational flexibility; as consequence, there is a reduction in equilibrium interfacial tension (Gomes & Kurozawa, 2023). The equilibrium interfacial tensions were around 3.78-4.04 mN/m for all RPH which were slightly lower than RPI (4.74 mN/m). Regarding RPI, this would correspond to a slow unfolding and molecular rearrangement such as intermolecular association or aggregation of the individual unfolded protein molecules at the interface, that in general, the systems containing RPH took less time to reach equilibrium interfacial tension than the system with RPI.

Figure 1. Interfacial tension at the interface between orange oil and RPI or RPH at 1.5% w/w prepared by enzymatic hydrolysis.

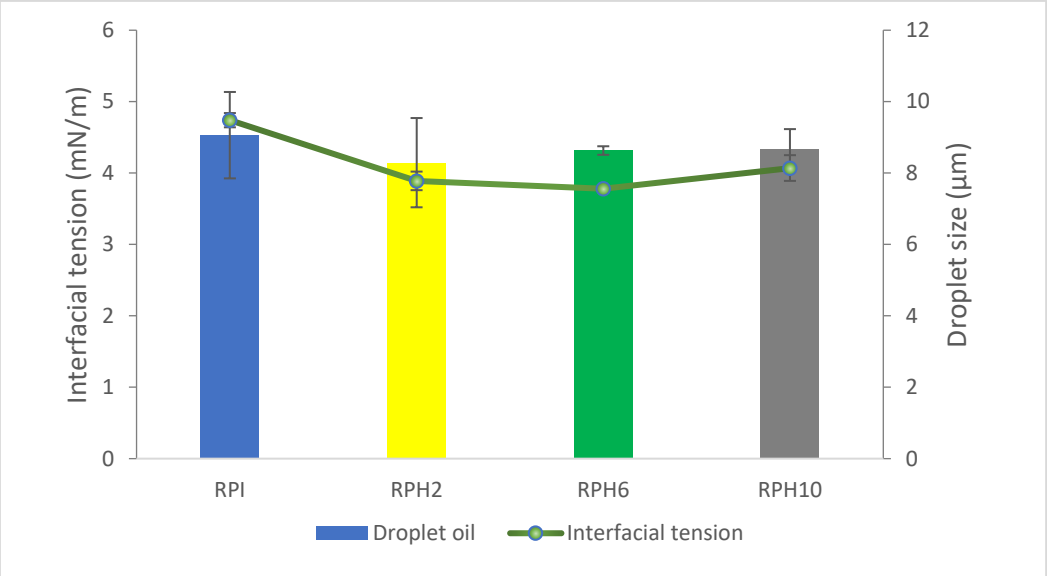


However, the decrease of interfacial tension does not depend only on protein type. Based in our previous study (Gomes & Kurozawa, 2023), the size of the fatty acids chain and essential oil compounds as well as the ratio of polar/non-polar groups of the oil interacting at the interface may have played an important role in the stabilization mechanisms**.** In our previous study, using linseed oil as lipid phase, the interfacial tension at equilibrium found for RPH was 9.88±0.72 mN/m, which was much higher than found in this work. Linseed oil is primarily composed of long-chain unsaturated fatty acid produces a bend in the molecule, decreasing the structural flexibility of the carbon chain (Singh et al., 2011). Thus, both hydrophobicity degree and energy that are required to break linseed oil into oil droplets are higher than the orange essential oil. The essential oils are composed primarily of small molecules (mono-terpenes) that tend to be more hydrophilic than fatty acids (Zhang et al., 2015). As results, there is a higher partition in the water phase that decreases the interfacial tension and facilitates the

formation of the oil droplet. The same behavior was observed by Barbon et al. (2020), in which they also verified the influence of the oil type on the interfacial tension.

For all, a high and positive correlation between interfacial tension and droplet size was observed by Pearson's correlation coefficient of +0.844. Since there was no difference between interfacial tension for all RPH, no significant difference was observed between the RPH samples for droplet size ($p<0.05$).

Figure 2. Comparation between droplet size and interfacial tension.

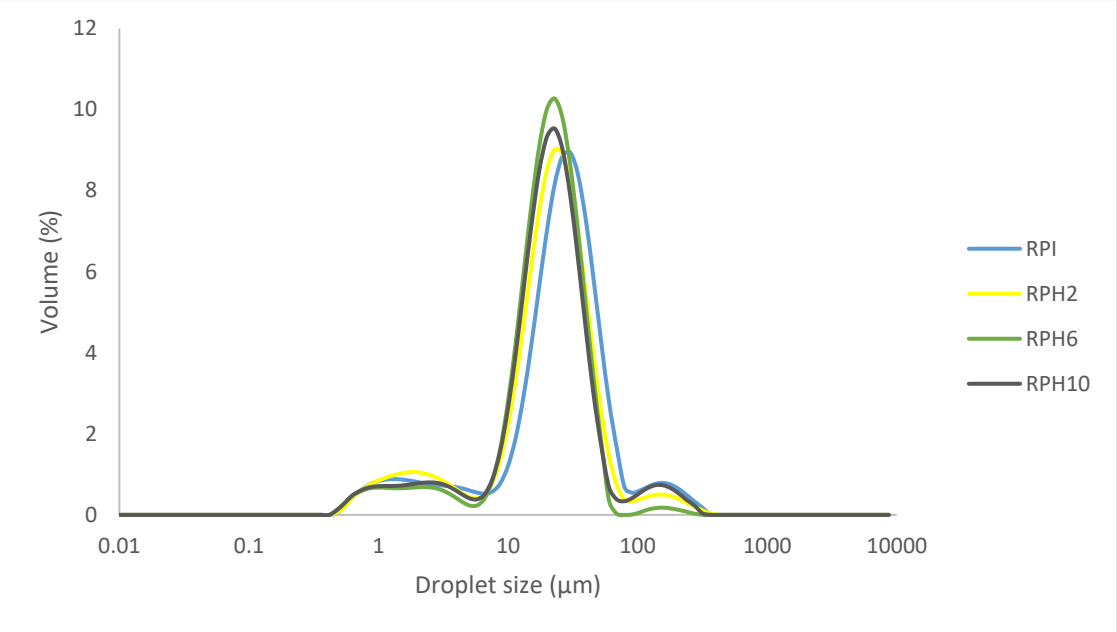


Generally, the formation of lower droplet size depends on the ability of a biopolymer (or emulsifier) rapidly absorbing on the oil-water interface, and then underwent a conformational ordering to form a viscoelastic film that surrounded the oil droplets. Therefore, a high interfacial tension means that protein generated weak adsorption on the oil droplets. The droplet size of the RPH emulsions reached a minimum of 8.62 ± 0.32 µnm, while RPI emulsion the particle size was 9.06 ± 1.21 µm. As showed in our previous study, the hydrolysates samples with specific molecular size

and good solubility indicated their capacity to form strong viscoelastic films at the oil droplets surface and decrease the droplet size (Gomes & Kurozawa, 2023).

The droplet size distributions for all emulsions are presented in Figure 3. Although the DH significantly reduced the droplet size compared to RPI, the same was not able to avoid polydisperse distributions ranging from 0.5 to 100 um. The droplet size of all samples showed a predominant peak with a narrow distribution but showed a two more peak. Probably, some of the oil droplets may not have been covered by the hydrolyzed proteins in a very short period during emulsion preparation, or part of these droplets may be incompletely covered, indicating droplet-droplet aggregation (coalescence). Furthermore, this can also be attributed to some insoluble proteins in the system. Even like that, the stronger film formed by RPH6 helped to preserve the interface integrity of the droplets during spray drying. In the fresh RPH6 emulsion, the span (1.97) of the spray dried droplet size distribution was narrower than in the RPI emulsion (2.11), which induces a better overall drying performance.

Figure 3. Oil droplet size distribution of emulsions stabilized by RPI and maltodextrin and RPH with 2%,6% and 10% of DH and maltodextrin.



6.3.2. Impact of enzymatic hydrolysis of rice protein on particle surface composition

The concentrations of the chemical elements (carbon C, nitrogen N and oxygen O) on the microparticle surface were quantified in three depth levels through X-ray photoelectron spectroscopy (XPS) (Table 1), in order to understand their effect on the oil retention and some physicochemical characteristics of the microparticles,

Table 1. Effect of degree of hydrolysis (2, 6, and 10%) on the surface composition obtained from XPS for the spray-dried orange oil powders.

Sample	Level 1*			Level 2			Level 3			% Protein
	C (%)	O (%)	N (%)	C (%)	O (%)	N (%)	C (%)	O (%)	N (%)	**
RPI	75.71	21.42	0.19	80.16	16.70	0.17	81.79	14.94	0.13	1.07
RPH2	84.25	11.87	0.16	84.62	11.62	0.16	85.52	10.69	0.18	0.56
RPH6	76.59	20.04	0.40	81.05	15.70	0.35	83.13	13.79	0.14	2.24
RPH10	75.63	21.71	0.41	80.49	16.82	0.19	82.44	14.86	0.08	2.30

RPI is rice protein isolate, and RPH is rice protein hydrolysate;
* XPS measurement depth. Level 1 corresponds to the particle surface; level 2 is an approximate 0.2 nm depth; and level 3 is a 0.3 nm depth
** Calculated using N × 5.61 (Sosulski & Imafidon, 1990) referring to level 1.

By analyzing the particle surface, we observed that there was a slight increase in nitrogen as the degree of hydrolysis also increased. The lowest nitrogen content for all matrix particle surfaces was detected for formulations containing RPI. By hydrolyzing the proteins, their kinetic speed increases and they become more capable to migrate to the surface of the atomized droplets during spray drying. It can be attributed to reducing peptide size, which resulted in higher surface activity. As the residence time of a droplet during drying process is very short, the emulsifier surface adsorption is adopted to be mainly diffusion-controlled (Landström et al., 2000). Thus, an increase in the content of small peptides results in higher nitrogen content on the powder surface.

It is worth emphasizing that the amount of nitrogen compared to the other chemical elements was lower, due to low quantity of protein/hydrolysates in the feed emulsions. Even so, we can state that even at low concentrations, % protein on particle surface had a strong and positive influence (Pearson's correlation of 0.96) on the retention of orange essential oil (Table 2). This can be attributed to the formation of a less porous and more uniform matrix due to the smaller protein with specific characteristics, as demonstrated in our previous study (Data not published).

The integrity of the protein film on the surface powder also depends on how the protein behaves during the drying of atomized droplet. Loss of water during drying causes a shrinkage of the protein film and its destabilization together with a leakage of oil onto the powder surface, which leads to local changes in surface area and thus surface protein content (Drusch et al., 2012). Thus, based on the interfacial tension results and consequently the droplet size, we can assume that RPH was able to remain in the atomized droplet during drying.

Besides that, there is a correlation between protein structures and their surface properties (Fang et al., 2013). Hydrolysis promotes the release of amino acids and

aggregates of highly soluble small active peptides with lower molecular weight than the intact protein. This release reduces the size of the protein molecules (Gomes & Kurozawa, 2020, 2021; Xu et al., 2016), which may have facilitated their displacement from the RPH to the surface of the microparticle.

6.3.3. Physicochemical Characteristics of Spray-Dried Emulsions

In this study, some of the properties of spray-dried emulsions, such as powder recovery (as an efficiency and economic factor in spray-drying process), hygroscopicity (as a physical stability index during preservation), and particle size (as a determining factor in packaging conditions) were examined. Table 2 shows the properties of powders obtained from the spray drying of essential orange oil emulsions stabilized with different RPH, including non-hydrolyzed protein RPI.

Table 2. Physicochemical properties of orange oil microparticles formulated with maltodextrin (MD) and rice protein isolate (RPI) or rice protein hydrolysate (RPH).

Sample	Hygroscopicity (g H_2O/100g)	Moisture (% wet basis)	Water activity (a_w)	$D_{4,3}$ (µm)	Span	Powder recovery (%)	Oil retention (%)
RPI	8.97 ± 0.10^b	0.87 ± 0.26^a	0.116 ± 0.00^a	19.75 ± 2.04^a	2.11 ± 0.21^a	21.47 ± 1.47^b	23.03 ± 3.35^b
RPH2	9.93 ± 0.20^a	1.16 ± 0.71^a	0.129 ± 0.00^a	13.35 ± 1.04^b	2.02 ± 0.11^a	24.08 ± 1.52^b	24.77 ± 0.31^b
RPH6	10.09 ± 0.19^a	0.57 ±0.12^a	0.075 ± 0.00^a	14.68 ± 0.81^b	1.97 ± 0.10^a	27.19 ± 0.19^a	47.95 ± 3.35^a
RPH10	9.65 ± 0.35^a	0.75 ± 0.20^a	0.114 ± 0.02^a	13.28 ± 0.58^b	1.91 ± 0.12^a	25.89 ± 0.11^a	49.97 ± 0.75^a

Different letters represent statistically significant differences ($p < 0.05$).

Although the DH have shown positive influence on the powder recovery ($p<0.05$), the recovery was only <30%. Other studies reported the low recovery is also due to the matrix composition (Adhikari et al., 2009; Fang et al., 2013). When compared to the RPI emulsion, the performance of the hydrolysates RPH6 and RPH10 in the emulsion was better in overcoming the coalescence of droplets, as well as the sticky interactions of the droplets or particles on the wall forming a surface film on the formed particles, thus increasing the recovery. Although the nature of the films of both RPI and RPH6 were similar, the hydrolysates were able to protect the oil droplet during drying.

As reported in our previous study, in which rice protein hydrolysate was used to microencapsulate flaxseed oil, protein hydrolysates have a greater kinetic advantage as they migrate more easily to the surface of the sprayed emulsion droplet during spray drying forming a surface film on the formed particles. This observation was also corroborated by the XPS results, where more proteins were found on the surface of microparticles using RPH6 and RPH10 (Table 1).

Regardless of the numerous advantages displayed by this technology of surface modification of droplets/particles to minimize stickiness, the recovery also strongly depends on the work scale. According showed for several authors, the recovery is high in larger scale setups because the fraction lost is an increasingly smaller component of the total production volume, while in laboratory scale they are still far from optimal, the recovery being in the 20–70% range (Sosnik & Seremeta, 2015). Generally, low recovery is due to the loss of product in the walls of the drying chamber, amounts being relatively constant.

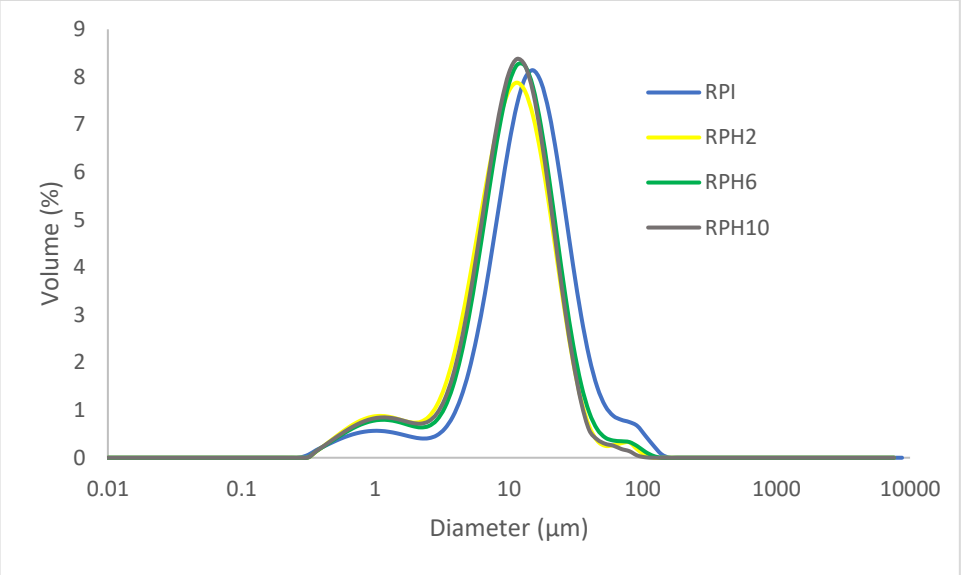
The studies of spray drying at the production plant scale are expensive. Thus, the use of the laboratory scale spray dryer is a way to simplify the system and set up new drying models to scale up to the production plant level using a minimal amount of material.

Therefore, we can affirm that hydrolysates protein can be used as ‘smart drying aids’ to minimize the stickiness during spray drying process presents new opportunities for insights in the details of microencapsulation by spray drying at different scales. Besides that, the drying process indicated be efficient to avoid microbial growth on the powders, once showed low levels of moisture content (0.75-1.16%), and aw (0.07-0.149), essential factors to storage.

Water adsorption is a critical factor in microencapsulation of oils, since the presence of water can influence the lipid oxidation, powder flowability, and caking (Juarez-Enriquez et al., 2017). Table 2 shows the hygroscopicity of all samples with different DH. DH had a significant effect ($p < 0.05$) on the water adsorption capacity of the samples. The hygroscopicity of powders containing RPH was greater than that sample with RPI. This can be due to structural changes in the enzyme-treated RPI, which lead to high accessibility, exposure of hydrophilic regions, and high solubility (Gomes & Kurozawa, 2021). The decrease in the surface oil of the final powder can also be another reason for this result, exposing a major number of active sites to water molecules adsorption, as also observed by Pereyra-Castro et al. (2018). In microparticles containing RPH6, the ratio N/C was higher than in the microparticles with RPI based in XPS results (Table 1). This could indicate that the formulation with RPI has a higher concentration of unencapsulated oily material, as also observed by Josefina Porras-Saavedra et al (2018). Besides that, this result can be also attributed to the higher surface affinity of the RPH6 than RPI, which migrate preferentially to the droplet's surface during drying. Depending on proteins nature, different results were found on lipids surface enrichment. Gaiani et al. (2010) reported that casein presents lower lipids surface enrichment than whey proteins due to its lower surface tension at air-water interface

Besides that, the particle size can also affect the powder hygroscopicity since moisture absorption takes place primarily on the particle surface. As such, smaller particle sizes have a relatively larger exchange surface for water absorption to occur, and vice versa. In the current study, powder hygroscopicity increased with decreasing particle size (Persons' correlation of -0.85). Overall, all powders had relatively small particles (13.28-19.75µm) and were affected by the presence of protein hydrolysates ($p<0.05$). The variation in the particle size may also be attributed to the agglomeration of microparticles because of the presence of surface oil. Analyzing the particle size distribution (Figure 4), it can be observed a small displacement of the RPI curve which resulted in a slight increase of the peak at the right that corresponds to agglomerates.

Figure 4. Particle size distributions for microparticles produced by RPI or different RPH (2, 6 and 10% DH).



6.3.4. Oil retention

The oil retention reflects the amount of oil recovered from powder particles, which is composed of both surface and internal oil entrapped within the wall matrix. DH had

significant effect on oil retention ($p<0.05$) (Table 2). The lowest oil retention level (23.03%) was obtained for wall system containing RPI, while the highest (49.97%) was for sample with RPH10.

An increase in the flavor retention can be related to the rapid formation of a semi-permeable membrane at the beginning of the drying process (Fernandes et al., 2013). As hydrolyzed proteins have higher kinetic velocity than intact proteins and the emulsifier surface adsorption is adopted mainly diffusion-controlled, the glassy hydrolysate protein film on the surface: prevented flavor release; minimized the permeation of both oxygen in the particle and water from the surrounding environment; and also reduced the adhesive interactions of the particles on the drying wall. This membrane acts as a barrier to most flavor compounds but remains permeable to water molecules, avoiding the loss of volatile flavors (Huynh et al., 2008). While molecular size of protein is the major factor, several authors pointed out that other factors, including surface hydrophobicity, hydrodynamic radius, the number of ionizable groups, and aggregation state of protein also likely main play a role on protection and encapsulation (Drusch et al., 2012).

The use of RPI led to a reduction in oil retention, which may be due to its compact globular structure that confers lower molecular flexibility and its poor emulsifying capability compared with protein hydrolysates (Gomes & Kurozawa, 2023). The RPH presented a good capacity of adsorbing on the oil–water interface as a consequence of their amphiphilic molecular structure, improved by enzymatic hydrolysis. Such modification affords the adsorption of RPH at the oil–water interface more efficiently, thus reducing the interfacial tension of the system (Figure 1) and favoring the kinetic stabilization of the emulsion. Therefore, there is a sufficient amount of wall material available to form a sufficiently strong structural matrix around to the droplet, improving the protection of the essential orange oil during drying and, as consequence, increasing the oil retention. These

physic-chemical characteristics of the emulsifying matrices also influenced straightly the droplet size, which affects the oil retention. Many authors reported that a smaller droplet size reflects in a greater oil retention (Peng et al., 2022; Soottitantawat et al., 2003), as observed in our results for RPH10 sample. Thus, it can be reasonably hypothesized that for the RPH10 emulsions, both the droplet size and interfacial protein load contributed to the oil retention of the essential orange oil when compared with RPI. Carmona et al. (2013) studied the effect of the emulsion droplets size on the properties of essential orange oil powders obtained by spray drying. The authors observed that flavor retention markedly decreased with the increase of droplets mean diameter, concluding that is a significant factor to be considered for flavor retention.

On the other hand, we can observe that, although the droplet size and interfacial tension were similar for the RPH2 and RPH10 emulsions, the oil retention of the RPH10 spray-dried powder was considerably higher (Table 2). This observation clearly means that there are some parameters other than the droplet size or interfacial tension that affect the oil retention. Several studies reported an accumulation of protein on the particles surface during spray-drying, resulting in the formation of a surface protective film (Elversson & Millqvist-Fureby, 2005; Fäldt & Bergenståhl, 1994; Landström et al., 2000; Munoz-Ibanez et al., 2016). In this case, the RPH2 was not able to maintain at the surface during spray drying and forming a surface film on the particles formed, providing a protective barrier. Andersen et al. (2000) demonstrated that the thickness of the encapsulation layer is one of the key factors governing rate diffusional loss of aroma compounds. This hypothesis was corroborated by XPS results toward particle surface, in which was observed a thicker layer of protein to RPH6 samples (Table 1).

6.4. CONCLUSION

The use of rice protein hydrolysates proved to be efficient as green label-natural and sustainable ingredients to be used for the microencapsulation of essential oil via spray drying. The hydrolyzed proteins were able to decrease the interfacial tension and consequently showed smaller droplet size than the non-hydrolyzed protein. Thus, emulsions stabilized by hydrolyzed protein combined with maltodextrin showed a higher stability behavior compared to intact protein emulsions. These effects directly influenced the protein's interaction with oil and maltodextrin, and the formation of microparticles.

The hygroscopicity of the microparticles containing RPH was greater than that for sample with RPI. The protein hydrolysate with DH 10% doubled the oil retention (49.97%) compared to RPI (23.03%), which can be attributed to the formation of a protein film on particles surface, as demonstrated by the XPS analysis.

It is important to determine correlations between emulsion properties, surface powder composition and final product properties once these correlations could allow us to determine optimal microparticle formulation and improve the protection and the controlled release of bioactive compounds.

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CAPÍTULO 7 - DISCUSSÃO GERAL

A microencapsulação por *spray drying* tem sido uma tecnologia importante para proteção de compostos susceptíveis a oxidação e retenção de *flavors*. As principais razões para a sua popularidade são as vantagens tanto de processo como de produto, como, por exemplo, a possibilidade de ser uma operação continua e em larga escala que resulta em um produto com baixo teor de umidade, garantindo uma elevada estabilidade microbiológica e físico-química. Muitas propriedades das micropartículas secas por *spray drying* são afetadas pela presença e composição dos materiais usados na matriz e na superfície. As características desses materiais são um fator essencial, pois qualquer dano ou alteração na superfície da micropartícula pode afetar a proteção e retenção do sistema de entrega carregado de bioativos e influenciar sua interação com o meio ambiente, seja água durante a reidratação ou ar durante o armazenamento.

Sendo assim, a primeira etapa da pesquisa (**Capítulo 4**) visou verificar o efeito da hidrólise enzimática de isolado de proteína de arroz comercial em propriedades interfaciais e funcionais e investigar a concentração mínima de hidrolisado de proteína de arroz necessária para formar e estabilizar emulsões de óleo em água (O/A) que seriam usadas em processos de microencapsulação via *spray drying*. Durante décadas, houve pouco interesse no processamento de proteínas de arroz, principalmente devido ao teor relativamente baixo de proteínas no arroz, à baixa solubilidade em água entre outros fatores. No entanto, ao longo dos anos, pesquisadores e indústrias demonstraram interesse em utilizar a proteína do arroz como substituto das proteínas de origem animal em formulações de novos produtos, principalmente como emulsificante. E para melhorar algumas propriedades tecno-funcional dessas proteínas, a hidrólise enzimática vem sendo aplicada. Tais modificações permitem reduzir consideravelmente a quantidade de proteína utilizada como emulsificante e essas pequenas quantidades podem ocupar os pequenos poros da matriz formada pela maltodextrina durante a microencapsulação por *spray drying*, resultando em

melhor proteção do princípio ativo e evitando o processo de oxidação durante o armazenamento.

Assim, as proteínas hidrolisadas RPH10 e o RPH6 apresentaram solubilidade 20% ligeiramente superior ao RPI em uma ampla faixa de pH, considerando seu potencial em diversas aplicações industriais. E mesmo em concentrações mais baixas, os hidrolisados RPH10 e o RPH6 reduziram a tensão interfacial entre o óleo de linhaça e a água, no qual diminuiu de 17,6 mN/m para 9,9 mN/m quando comparado com RPI. Além disso, os RPH produziram emulsões de óleo em água mais estáveis do que a RPI, mesmo em altas concentrações. Esses resultados confirmam a relevância de realizar medições de tensão interfacial para selecionar o DH apropriado para avaliar a estabilidade da emulsão. A propriedade anfifílica aprimorada de RPH, suportada pelos resultados de tensão interfacial e solubilidade, foi confirmada pela maior estabilidade de emulsão indicada pelo Turbiscan e índices de estabilidade de emulsão. Emulsões estabilizadas por RPH (6% e 10%) em concentrações de proteína mais baixas (1%) exibiram melhor estabilidade física do que RPI em concentrações de proteína mais altas (1,5%). Os nossos resultados também sugerem que o processo de homogeneização utilizando homogeneizador de alta pressão pode melhorar a estabilidade cinética das emulsões. A emulsão RPH-1,5% foi capaz de resistir à coalescência e outros mecanismos de desestabilização durante quase todos os períodos de armazenamento.

O desempenho melhorado dos RPH nas emulsões com as mesmas concentrações foram também analisadas nas micropartículas de óleo de linhaça obtidas por *spray drying* (**Capítulo 5**). A concentração de RPH e RPI não afetaram a solubilidade (83 – 91%), higroscopicidade (6,89 – 8,59 g H_2O/100g), tempo de umedecimento e tamanho de partícula (19,85 – 38,35 μm). No entanto, uma correlação forte e positiva foi encontrada entre DH e estabilidade oxidativa. O menor valor de peróxido (2,48 – 1,99 meq/kg de óleo)

foi encontrado no pó formulado com proteína hidrolisada RPH10-1,5% durante todo o estudo de armazenamento. Conforme apresentado em nosso estudo anterior (**Capítulo 4**), RPH10-1,5% apresentou superfície mais ativa que RPI-1,5% e através da análise XPS, foi possível observar que DH mais elevado favoreceu o acúmulo superficial de proteínas na superfície do pó. Esses resultados mostraram que o grau de hidrólise exerceu influência na distribuição dos componentes e na porcentagem de proteína na superfície do pó. Isso pode levar a um efeito protetor melhorado da proteína nas micropartículas e a menos vazamento de gordura na superfície do pó em comparação com as micropartículas RPI.

A análise AFM ajudou a entender o papel da proteína hidrolisada na formação de superfícies de micropartículas. A imagem AFM permitiu a visualização da topografia dos pós, que se caracterizou por um aspecto mais rígido que podem representar as gotículas de óleo localizadas mais próximas à superfície da micropartícula recobertas por moléculas de proteína, formando o filme superficial. Por outro lado, as micropartículas estabilizadas com RPI apresentaram nanoporos em sua superfície, indicando que a proteína intacta não foi capaz de formar um filme de proteína. No geral, observou-se que os efeitos positivos são mais evidentes para as micropartículas estabilizadas com RPH6 e RPH10, que apresentaram o maior percentual de proteína de superfície.

Acredita-se que devido a sua alta difusividade e pronunciada característica de atividade de superfície, tais efeitos foram mais pronunciados, resultando assim em um efeito mais protetor. Em alguns casos, parecia haver um estado saturado de concentração de proteína (cerca de 1,0 e 1,5%) na emulsão, pois concentrações mais altas de proteína não aumentavam significativamente a proteção das micropartículas.

Em nosso estudo anterior (**Capítulo 5**), foram relatados os efeitos de diferentes níveis de DH (2, 6 e 10%) e concentrações (0,5 – 1,5%) da proteína de arroz nas propriedades físico-químicas das micropartículas de óleo de linhaça obtidas via *spray*

drying, especialmente na oxidação lipídica durante o armazenamento. No entanto, mudanças na composição química da superfície das micropartículas de óleo essencial de laranja não foram investigadas. Neste estudo (**Capítulo 6**), examinamos a influência dos RPH na concentração de 1,5% na formação de um filme de proteína na superfície das micropartículas de óleo essencial de laranja obtidos por *spray drying*, verificando como afeta a liberação e retenção dos compostos voláteis. Em geral, os RPHs foram capazes de reduzir a tensão interfacial (3,78-4,04 mN/m) quando comparados aos RPI (4,74 mN/m). Nenhuma diferença significativa foi observada entre as amostras de RPH.

Com base em nosso estudo anterior (**Capítulo 4**), a diminuição da tensão interfacial não depende apenas do tipo de proteína. O tamanho da cadeia de ácidos graxos e dos compostos do óleo essencial, bem como a proporção de grupos polares/não polares do óleo interagindo na interface, podem ter desempenhado um papel importante. Utilizando óleo de linhaça como fase lipídica, a tensão interfacial em equilíbrio encontrada para RPH foi de 9,88±0,72 mN/m, muito superior à encontrada neste trabalho. A correlação entre a redução do valor da tensão interfacial e a diminuição do tamanho da gota também foi observada, em que as emulsões RPH apresentaram 8,29-8,66 µm enquanto RPI 9,06 µm.

Assim, com base nos resultados da tensão interfacial e consequentemente no tamanho da gota, podemos supor que o RPH conseguiu permanecer na gota atomizada durante a secagem, conforme observado nos resultados de XPS. As micropartículas RPH10 apresentou maior presença de proteína (2,30%) na superfície do que as micropartículas RPI (1.07%) e consequentemente dobrou a retenção de óleo (49,97%) e em relação ao RPI (23,03%). Em contraste, esta amostra apresentou alta higroscopicidade, atribuída ao percentual de proteína na superfície das micropartículas. É importante determinar as correlações entre as propriedades da emulsão, a composição do pó de superfície e as propriedades do produto final, uma vez que essas correlações podem nos permitir

determinar a formulação ideal de micropartículas e melhorar a proteção e a liberação controlada de compostos bioativos.

CAPÍTULO 8 – CONCLUSÃO GERAL

A utilização de protease visando melhorar as propriedades funcionais e tecnologias da proteína isolada de arroz foram verificadas. Além disso, o grande desafio deste projeto foi avaliar o papel da proteína hidrolisada na formação de um possível filme protetor nas micropartículas de óleo de linhaça e óleo essencial de laranja obtidos por *spray dryer*.

Conforme exposto nos capítulos anteriores, a utilização da enzima Flavourzyme melhorou drasticamente as propriedades físico-químicas e funcionais da proteína isolada de arroz. Dependendo do grau de hidrolise utilizado, por exemplo 10%, a solubilidade da proteína aumentou cerca de 27% quando comparada com a proteína intacta. Com isso, a gama de processos e produtos na qual essa proteína hidrolisada pode ser utilizada é ampliada. Para produtos com características de emulsão, a proteína hidrolisada também desempenha melhor eficiência do que a proteína intacta, independente da concentração da proteína utilizada dentro da faixa de 0,5 a 1,5%. A análise de tensão interfacial provou isso, tanto para o óleo de linhaça quanto para o óleo essencial de laranja, na qual a proteína hidrolisada conseguiu desempenhar um papel mais eficiente em diminuir a tensão interfacial mais rápido e consequentemente estabilizar a emulsão. Estudos relacionados à estabilização de emulsões e atividade emulsificante dos hidrolisados comprovaram mais ainda a eficiência deles para serem utilizados em processos e produtos emulsionados. Para o processo de microencapsulação via *spray drying* de ambos os ativos de características destintas, a utilização dos hidrolisados foram mais estáveis durante a secagem.

As micropartículas de óleo de linhaça estabilizadas com as proteínas hidrolisadas e em diferentes concentrações foram submetidas a diversos experimentos a fim de verificar a influência dos hidrolisados sobre o papel protetor das micropartículas e nas características tecnológicas dos pós obtidos. No geral, observaram-se que as

proteínas hidrolisadas e suas concentrações não influenciaram de forma significativa as propriedades tecnológicas dos pós (solubilidade, higroscopicidade, molhabilidade e tamanho de partícula). No entanto, as propriedades de barreira das micropartículas contra o oxigênio foram melhoradas para as micropartículas obtidas com proteína hidrolisadas DH10% e com 1,5% de concentração, como observado através da análise de estabilidade oxidativa. Já com relação às micropartículas de óleo de laranja, a retenção do óleo foi melhorada com o aumento do grau de hidrólise. Verificou-se para ambos os ativos, através da análise XPS, que micropartículas com alto grau de hidrólise proporcionaram o acúmulo superficial de proteínas na interface ar-água, levando a um filme rico em proteínas nas partículas superficiais, sendo assim impedindo a passagem do oxigênio e liberação dos compostos voláteis. Vale reforçar que para algumas partículas microencapsuladas com o óleo de linhaça não houve diferença entre si, por exemplo 1% e 1,5%, o que pode indicar uma saturação da interface já na concentração mais baixa.

Algumas microscopias, por exemplo, a AFM, mostraram pequenos poros para as partículas de óleo de linhaça utilizando a proteína intacta, enquanto para partículas formuladas com hidrolisados observou-se um filme proteico protegendo o óleo. De modo geral, essas diferenças podem ser devidas a vários fatores, incluindo o tamanho dos peptídeos e consequentemente suas difusividades, cargas relativas de cada um deles, distribuição dos aminoácidos na cadeia polipeptídica entre outros fatores. Assim, a implementação de técnicas conjuntas como um protocolo de análise de superfície das partículas é sugerida.

A aplicação de uma nova abordagem para microencapsular diferentes ativos com diferentes susceptibilidades a oxidação e liberação utilizando proteína hidrolisada de arroz em pequenas, se mostrou eficiente com relação às propriedades de barreiras. Além

disso, confirmou-se que os hidrolisados têm um papel crucial na retenção dos ativos durante a formação e estabilização da emulsão, podendo assim ser utilizados em diversos processos de formulação.

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APÊNDICES

APÊNDICE A – Material suplementar referente ao capítulo 4

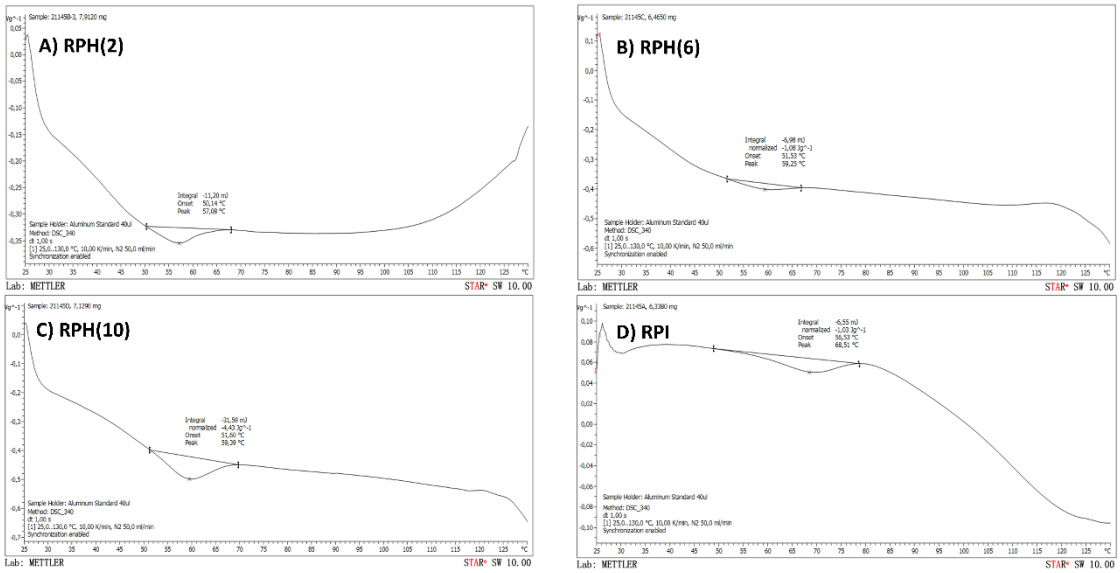


Figure S1. DSC thermogram rice protein isolate (RPI) and rice protein hydrolysates (RPH) at different degrees of hydrolysis (2, 6 and 10%).

APÊNDICE B – Relatório científico do projeto de bolsa SWE 200408/2022-4

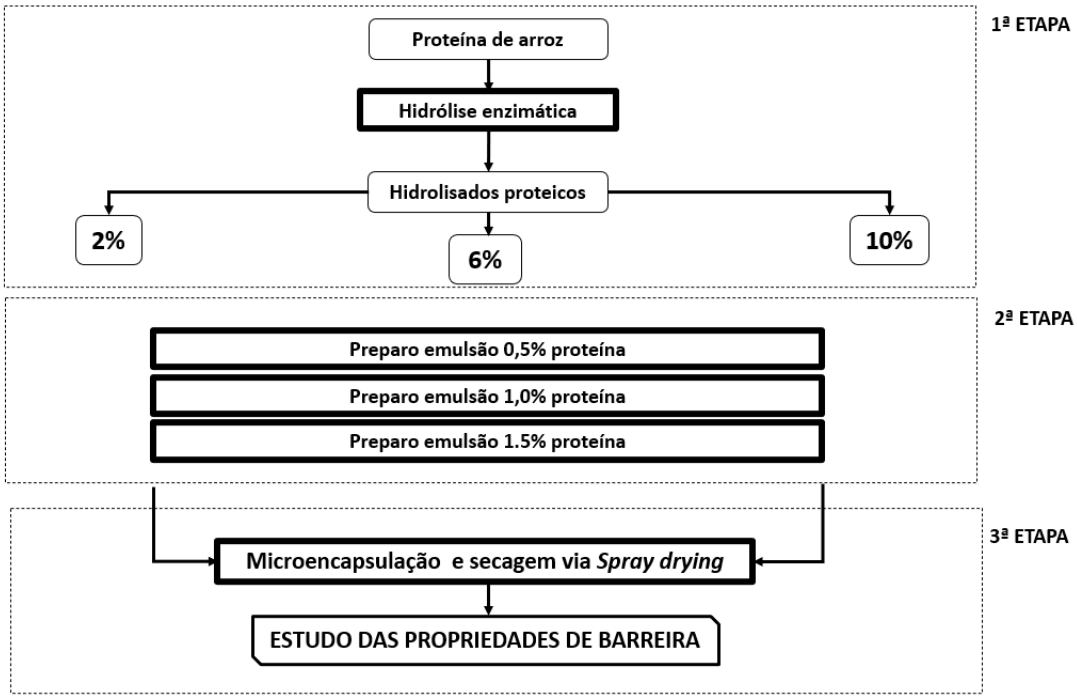
RESUMO

O presente projeto visou a colaboração entre os grupos de pesquisas coordenados pela proponente e pelo Prof. Dr. Gary Reineccius da Universidade de Minnesota - EUA. O Prof. Dr. Gary Reineccius é um dos principais investigadores de referência mundial na área de encapsulação de aromas e emulsões por spray dryer, como observado pela sua elevada publicação (>250 trabalhos) com mais de 4000 citações, resultado em índice h de 37 (Web of Science). A colaboração deste investigador no nosso projeto de pesquisa foi fundamental na contribuição no andamento científico das linhas de pesquisa da proponente e para a criação de uma rede de pesquisa entre as instituições. O grande desafio deste projeto foi a quantificação da taxa de transferência de massa de oxigênio e de compostos voláteis através de micropartículas de óleo de linhaça e de óleo essencial de laranja obtidas por spray dryer. Para isso, algumas análises foram realizadas visando entender o comportamento e o mecanismo de deposição das proteínas e hidrolisados proteicos sobre a superfície das micropartículas formadas, bem como as propriedades de barreira são afetadas. Devido à facilidade de acesso a algumas técnicas e à infraestrutura, realizou-se análises de liberação/oxidação e tamanho de poros das partículas utilizando PALS. A oxidação lipídica das micropartículas contendo óleo de linhaça durante o *shelf-life* foi monitorada através da análise do teor de propanal e hexanal no *headspace* por meio da cromatografia gasosa. As quantidades de compostos de aroma provenientes do óleo essencial de laranja microencapsulado pelo processo de spray drying também foram determinadas por análise cromatográfica gasosa (CG) assim como os produtos de oxidação. Com base nessas técnicas, podemos ter informações necessárias para avaliar a migração do oxigênio que entra na partícula e como afeta a oxidação do óleo de linhaça encapsulado, além de verificar a saída de compostos voláteis de óleo essencial de laranja microencapsulado. Além destas análises/técnicas disponíveis na Universidade de Minnesota, a contribuição científica do Prof. Dr. Reineccius no desenvolvimento do projeto foi fundamental, uma vez que o pesquisador possui amplo conhecimento na área de encapsulação de aromas.

1. REALIZAÇÕES DO PROJETO

Para um melhor entendimento da realização das análises experimentais que foram conduzidas na Universidade de Minnesota com a colaboração do Prof. Dr. Gary Reineccius, nesse item estão descritas brevemente as etapas anteriores que foram realizados na FEA/UNICAMP. Desta forma, o projeto de doutorado do aluno foi dividido em três etapas conforme o diagrama de fluxo abaixo (Figura 1).

Figura 1. Diagrama de fluxo das etapas do projeto.



A primeira etapa consistiu na caracterização dos agentes ativos: composição de compostos voláteis por Cromatografia Gasosa do óleo essencial de laranja; e composição lipídica em ácidos graxos por análise de cromatografia gasosa de ésteres metílicos de ácidos graxos no óleo de linhaça. A hidrólise enzimática da proteína de arroz utilizando a protease Flavourzyme foi conduzida para obtenção de hidrolisados proteicos com diferentes graus de hidrólise (2%, 6% e 10%). Estes hidrolisados foram escolhidos por apresentarem melhores propriedades funcionais, resultados obtidos no nosso trabalho anterior.

Na segunda etapa, realizada na FEA/UNICAMP, foram preparadas emulsões contendo o ativo óleo essencial de laranja e óleo de linhaça. Cada emulsão foi estabilizada pela proteína de arroz intacta ou hidrolisada com diferentes graus de

hidrólise (2%, 6% e 10%) e em três concentrações (0,5, 1,0 e 1,5%). O preparo das emulsões foi feito pela solubilização dos materiais de parede (maltodextrina + proteína ou hidrolisado proteico) em água destilada, com agitação constante, seguida da homogeneização em um homogeneizador Ultra-Turax. A concentração de sólidos foi fixada em 45% (33.5-35% maltodextrina, 0.5-1,5% proteína, 10% óleo de laranja ou óleo de linhaça). Todas as emulsões obtidas foram caracterizadas.

Na terceira etapa, executadas nos EUA, as micropartículas de óleo de linhaça e as micropartículas de óleo essencial de laranja foram obtidas da secagem das emulsões contendo 1,5% de proteína. Essas emulsões foram secas utilizando um Mini Spray Dryer Büchi B-290 (Flawil, Suíça) com um bico atomizador duplo fluido de 0,7 mm de diâmetro. A secagem das emulsões foi realizada conforme as condições de processos utilizadas nos nossos estudos anteriores.

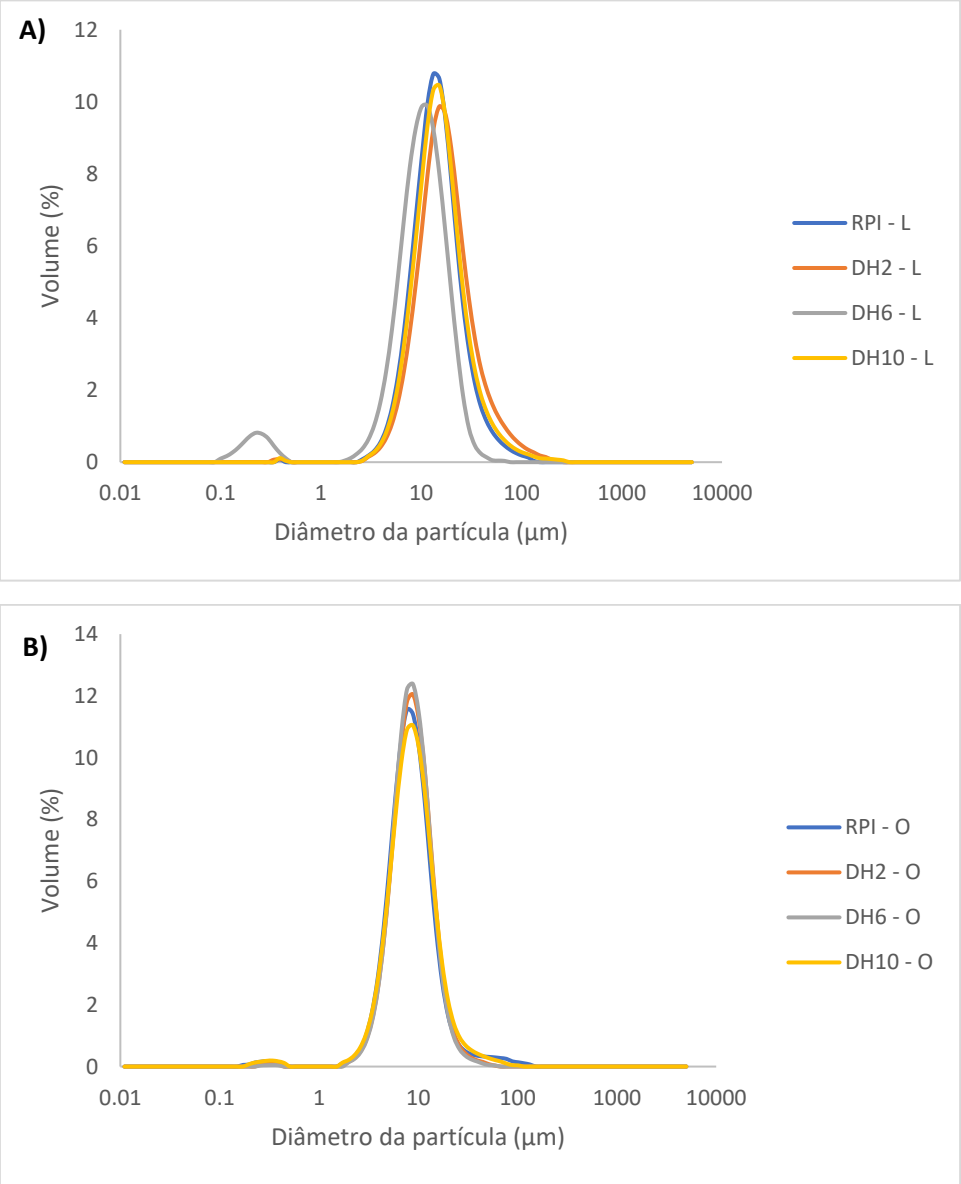
Com o objetivo de avaliar a influência dos hidrolisados proteicos de arroz em proteger os diferentes ativos encapsulados, foram avaliados: diâmetro médio das partículas $D_{4,3}$, distribuição das partículas e atividade de água (Tabela 1).

Tabela 1. Diâmetro médio das partículas $D_{4,3}$ e atividade de água (Aw) das micropartículas de óleo essencial de laranja e óleo de linhaça obtidas por spray drying.

Amostra	$D_{4,3}$ (µm)	Atividade de água (Aw)
RPI-L	15,96 ± 1,37	0,093 ± 0,013
DH2-L	20,42 ± 2,03	0,088 ± 0,004
DH6-L	10,43 ± 1,21	0,109 ± 0,001
DH10-L	17,89 ±0,65	0,091 ± 0,003
RPI-O	9,75 ± 1,22	0,088 ± 0,007
DH2-O	8,98 ± 0,20	0,075 ± 0,007
DH6-O	8,83 ± 0,16	0,072 ± 0,002
DH10-O	9,58 ± 0,31	0,097 ± 0,001

L- óleo de linhaça; O – óleo essencial de laranja

Figura 2. Distribuição do tamanho de partícula de pós secos obtidos por spray drying contendo isolado de proteína de arroz ou hidrolisados com diferentes graus de hidrólise (2, 6 e 10%); A) óleo essencial de laranja e B) óleo de linhaça.



As micropartículas contendo óleo de linhaça e óleo essencial de laranja foram avaliadas quanto a estabilidade oxidativa a 45°C por cromatografia gasosa durante 30 dias de estocagem. Previamente, as amostras foram estocadas durante 30 dias em uma umidade relativa controlada até atingir o equilíbrio.

Figura 2. Oxidação de limoneno durante o armazenamento de pós secos por spray com diferentes graus de hidrólise. A) Óxido de limoneno e B) Carvone.

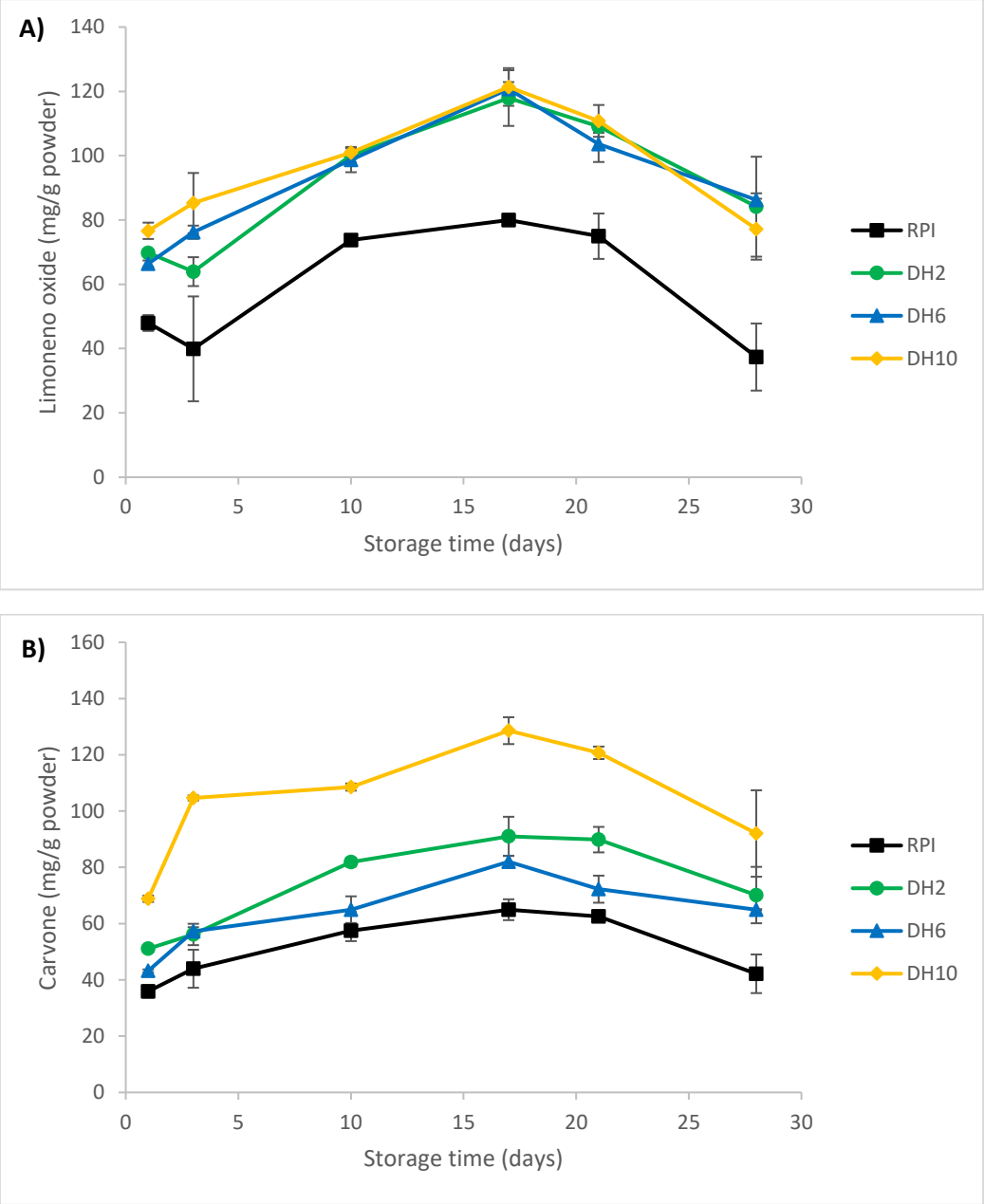
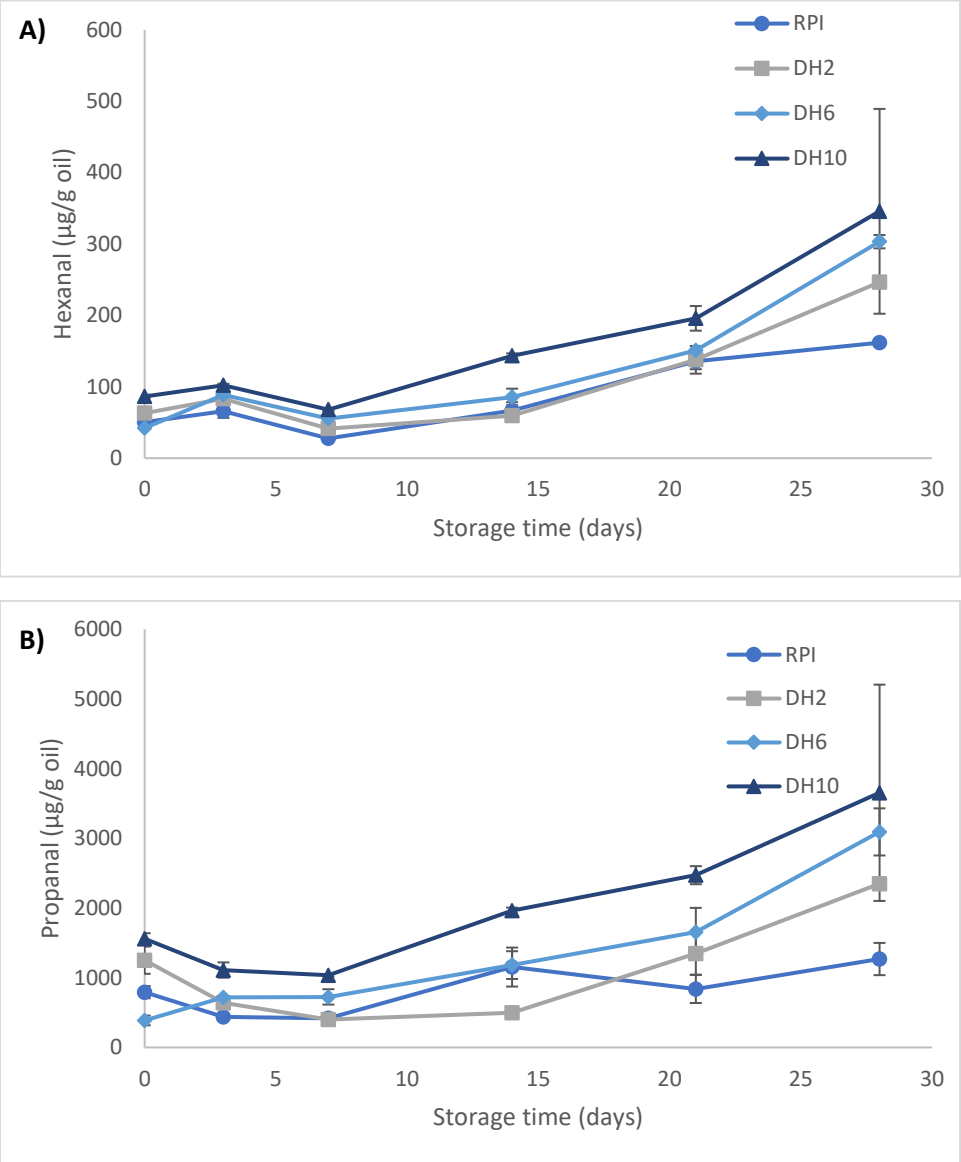


Figura 3. Produção de A) hexanal e B) propanal durante o armazenamento dos pós produzidos a partir de diferentes graus de hidrólise (2, 6 e 10%) da proteína de isolada de arroz.



As micropartículas obtidas para o óleo essencial de laranja e o óleo de linhaça foram analisas com relação ao tamanho de poros utilizando a técnica PALS. Resumidamente, o PALS mede o tempo de vida dos pósitrons ou positrônio (Ps, o estado ligado de um pósitron e um elétron) nas amostras. O pósitron se difunde no material, perdendo energia. Em algum ponto, o pósitron irá aniquilar-se no volume, localizar-se em um poro e aniquilar-se, ou formará positrônio que se localiza em um vazio que então aniquila. Cada processo tem tempos de vida correspondentes (se presentes) que são característicos de onde ocorre a aniquilação. Assim, de acordo com

os tempos de vida do pósitron consegue-se medir o tamanho dos poros presente em cada amostra (Tabela 2-3).

Tabela 2. Análise de PALS em ar para as micropartículas obtidas por *spray drying.*

Amostra	**τ_1 (ns)**	**I_1 (ns)**	**τ_2 (ns)**	**I_2 (ns)**	**τ_3 (ns)**	**I_3 (ns)**
RPI/O	0.264 (28)	89.8 (4)	1.220 (10)	9.62 (33)	3.027 (129)	0.55 (6)
DH2/O	0.266 (10)	89.7 (1)	1.221 (6)	9. 55 (3)	3.300 (58)	0.72 (3)
DH6/O						
DH10/O	0.266 (5)	90.5 (1)	1.244 (4)	9.12 (4)	3.565 (64)	0.38 (1)
RPI/L	0.285 (5)	89.6 (1)	1.400 (10)	8.39 (6)	3.215 (31)	2.06 (6)
DH2/L	0.267 (1)	87.8 (1)	1.235 (9)	8.62 (4)	2.962 (21)	2.70 (6)
DH6/L						
DH10/L	0.291 (1)	90.2 (1)	1.326 (4)	8.04 (2)	3.273 (26)	1.05 (2)

L- óleo de linhaça; O – óleo essencial de laranja

Tabela 3. Análise de PALS em vácuo para as micropartículas obtidas por *spray drying.*

Amostra	**τ_1 (ns)**	**I_1 (ns)**	**τ_2 (ns)**	**I_2 (ns)**	**τ_3 (ns)**	**I_3 (ns)**
RPI/O	0.266 (1)	88.9 (1)	1.264 (7)	10.07 (5)	2.645 (54)	1.03 (7)
DH2/O	0.265 (1)	89.0 (1)	1.259 (5)	9.89 (3)	2.834 (31)	1.16 (4)
DH6/O						
DH10/O	0.264 (2)	89.7 (1)	1.289 (7)	9.56 (4)	2.843 (62)	0.80 (6)
RPI/L	0.285 (19)	89.0 (2)	1.454 (12)	8.49 (16)	3.078 (33)	2.47 (9)
DH2/L	0.266 (1)	87.1 (1)	1.281 (8)	8.77 (5)	2.879 (19)	3.16 (7)
DH6/L						
DH10/L	0.301 (1)	90.6 (1)	1.449 (7)	8.45 (3)	3.227 (47)	0.92 (4)

L- óleo de linhaça; O – óleo essencial de laranja

Micropartículas contendo óleo essencial de laranja

No ar, as amostras laranja têm um tempo de vida Ps (t2) em torno de 1,22 ns, correspondendo a um diâmetro de poro em torno de 0,394 nm. O tempo de vida de t2

parece aumentar ligeiramente em DH10/O para 0,400 nm de diâmetro à medida que a hidrólise aumenta. Há também uma ligeira queda na intensidade, I2. Vemos mudanças maiores na vida útil dos poros maiores, t3. Na proteína de arroz intacta (RPI/O) o tempo de vida é de cerca de 3 ns e corresponde a um diâmetro de poro de 0,723 nm. O tamanho dos poros aumenta com a hidrólise, com poros DH10/O com 0,792 nm de diâmetro.

Foi observado tendências semelhantes quando as amostras são analisadas no vácuo. Para t2, os tempos de vida são ligeiramente maiores (1,26 ns) correspondendo a diâmetros de poros de 0,405 nm. A intensidade também aumenta ligeiramente. No entanto, vemos mudanças significativas em t3. Os tempos de vida caem significativamente para 2,65 – 2,84 ns (0,67 – 0,70 nm de diâmetro). Se o bombeamento removesse o material, esperaríamos que a vida útil aumentasse, correspondendo a poros maiores, mas a tendência é na direção oposta. Vemos uma tendência semelhante em I3 com um ligeiro aumento no vácuo em oposição ao ar.

Micropartículas contendo óleo de linhaça

No ar, a amostra intacta de proteína de arroz em linhaça (RPI/L) parece substancialmente maior, com um diâmetro t2 de 0,440 nm, do que a amostra de laranja (RPI/O), 0,394 nm. Uma tendência semelhante ocorre com t3, embora com uma diferença menor; o diâmetro nas micropartículas de óleo de laranja é 0,723 e é 0,748 nm nas micropartículas formuladas com óleo de linhaça. A intensidade total de Ps em ambas as amostras RPI é semelhante 10,2 – 10,5%, porém tem menor intensidade em t2 e maior intensidade em t3. Os tamanhos dos poros nas amostras DH2 são muito semelhantes, mas a distribuição das intensidades é diferente entre óleo essencial de larajan e óleo de linhaça, como visto nas amostras PRI. Observamos que para a linhaça, isso significa que o tamanho dos poros cai substancialmente para DH2 em relação à amostra RPI/L, enquanto na amostra laranja o menor, t2, não mudou e o maior, t3, ficou maior. Nas micropartículas de óleo de linhaça ficou evidente um aumento de tamanho de poros com o aumento do grau de hidrólise.

Em resumo:

- Todas as amostras são adequadas para 3 vidas, as 2 mais longas são devidas à aniquilação de Ps nos poros e são de interesse primário;

- Observou-se diferenças entre as amostras de laranja e linhaça tanto no tamanho dos poros (relacionado ao tempo de vida do Ps) quanto nas distribuições relativas (relacionadas à intensidade do Ps) das populações de poros;
- O grau de hidrólise parece afetar o tamanho dos poros, com maior DH10 apresentando poros maiores do que DH2;
- As tendências são semelhantes independente se as amostras são analisadas no ar ou no vácuo. No entanto, os poros menores no vácuo parecem ficar ligeiramente maiores, enquanto os poros maiores tendem a ser (amplamente) menores;
- A tendência de oxidação está totalmente relacionada com o tamanho dos poros, sendo as amostras DH10 que sofreram maior oxidação.

APÊNDICE C – Carta de aceite do artigo referente ao capítulo 4

Fw: Your Submission

Louise Emy Kurozawa <louisek98@yahoo.com.br>
Sex, 09/06/2023 12:18
Para: Matheus Gouveia <matheus_hgg@hotmail.com>

----- Mensagem encaminhada -----
De: Food Research International <em@editorialmanager.com>
Para: Louise Emy Kurozawa <louisek98@yahoo.com.br>
Enviado: sexta-feira, 9 de junho de 2023 10:57:06 BRT
Assunto: Your Submission

Ms. Ref. No.: FOODRES-D-23-00874R1
Title: Performance of rice protein hydrolysates as a stabilizing agent on oil-in-water emulsions
Food Research International

Dear louisek98,

A final disposition of "Accept" has been registered for the above-mentioned manuscript.

Kind regards,

Food Research International

Anderson de Souza Sant'Ana, Ph.D.
Editor-in-Chief

Comments from the Editors and Reviewers:

%COMMENTS_TO_AUTHOR%

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