UFRRJ

INSTITUTO DE TECNOLOGIA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

Dissertação

ENCAPSULAMENTO DO β-CAROTENO PRESENTE NO ÓLEO DE SACHA INCHI PELA COACERVAÇÃO COMPLEXA: FORMAÇÃO, CARACTERIZAÇÃO E LIBERAÇÃO

Ahmad El Ghazzaqui Barbosa

2020

UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO
INSTITUTO DE TECNOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

ENCAPSULAMENTO DO β-CAROTENO PRESENTE NO ÓLEO DE SACHA INCHI PELA COACERVAÇÃO COMPLEXA: FORMAÇÃO, CARACTERIZAÇÃO E LIBERAÇÃO

AHMAD EL GHAZZAQUI BARBOSA

Sob a Orientação do Professor

Dr. Edwin Elard Garcia Rojas

Dissertação submetida como requisito parcial para obtenção do grau de **Mestre em Ciências e Tecnologia de Alimentos**, no Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos. Área de Concentração em Ciência de Alimentos.

Seropédica, RJ

Julho de 2020

Universidade Federal Rural do Rio de Janeiro

Biblioteca Central / Seção de Processamento Técnico

Ficha catalográfica elaborada com

os dados fornecidos pelo(a) autor(a)

B238e Barbosa, Ahmad El Ghazzaqui, 1989-
ENCAPSULAMENTO DO β-CAROTENO PRESENTE NO ÓLEO DE SACHA INCHI PELA COACERVAÇÃO COMPLEXA: FORMAÇÃO, CARACTERIZAÇÃO E LIBERAÇÃO / Ahmad El Ghazzaqui Barbosa. - Volta Redonda, 2020.
80 f.

Orientador: Edwin Elard Garcia Rojas.
Dissertação(Mestrado). -- Universidade Federal Rural do Rio de Janeiro, Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, 2020.

1. Biopolímeros. 2. Isolado proteico do soro. 3. Interação eletrostática. 4. Eficiência de encapsulamento. 5. Carotenoides. I. Elard Garcia Rojas, Edwin, 1972-, orient. II Universidade Federal Rural do Rio de Janeiro. Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos III. Título.

O presente trabalho foi realizado com o apoio da Coordenação de Aperfeiçoamento de Nível Superior-Brasil (CAPES) – Código de Financiamento 001

MINISTÉRIO DA EDUCAÇÃO

UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

ATA Nº 1492/2020 - PPGCTA (12.28.01.00.00.00.00.41)

Nº do Protocolo: 23083.031439/2020-69

Seropédica-RJ, 16 de julho de 2020.

UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO
INSTITUTO DE TECNOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

Ahmad El Ghazzaqui Barbosa

Dissertação submetida como requisito parcial para a obtenção do grau de **Mestre**, no Programa de Pós Graduação em Ciência e Tecnologia de Alimentos, Área de Concentração em Ciência de Alimentos.

DISSERTAÇÃO APROVADA EM 09/07/2020

_____

EDWIN ELARD GARCIA ROJAS (Dr) EEIMVR/UFF

(orientador)

_____

ANDRESA VIANA RAMOS (Drª) UFRJ

_____

LÍVIA PINTO HECKERT BASTOS (Drª)

Conforme deliberação número 001/2020 da PROPPG, de 30/06/2020.

(Assinado digitalmente em 16/07/2020 22:35)
EDWIN ELARD GARCIA ROJAS
ASSINANTE EXTERNO
CPF: 014.548.996-54

(Assinado digitalmente em 16/07/2020 21:41)
LÍVIA PINTO HECKERT BASTOS
ASSINANTE EXTERNO
CPF: 122.476.067-09

(Assinado digitalmente em 16/07/2020 14:44)
ANDRESA VIANA RAMOS
ASSINANTE EXTERNO
CPF: 109.595.097-55

Para verificar a autenticidade deste documento entre em https://sipac.ufrrj.br/public/documentos/index.jsp informando seu número: **1492**, ano: **2020**, tipo: **ATA**, data de emissão: **16/07/2020** e o código de verificação: **def6843c89**

AGRADECIMENTOS

Ao Dr. Edwin Elard Garcia Rojas, por todo o empenho, dedicação e esforço ao trabalho, a mim e à pesquisa realizada. Espero um dia me tornar um profissional, pesquisador e ser humano como você. Registro todo carinho e gratidão pelos seus ensinamentos do que realmente é ciência e pesquisa.

A minha família em especial a Paulo Henrique, que sempre me deu apoio e foi uma luz em todo momento de escuridão, a minha mãe que sempre foi apoio em todos os caminhos que quisesse seguir e ao meu irmão pelo companheirismo.

Agradeço à UFF pela permissão do uso de seus laboratórios. Agradecer a todos os professores e do programa de ciência e tecnologia de alimentos da Universidade Federal Rural do Rio de Janeiro.

A todos os amigos que tive a imensa alegria e oportunidade de conhecer no decorrer do mestrado, aos amigos do Laboratório de Tecnologia e engenharia de agroindustrial (LETA/UFF), em especial a Lívia, uma grande amiga, que sempre esteve presente com seu conhecimento, generosidade e bondade, sempre pronta em ajudar. Augusto, um grande amigo, de discussões, troca de conhecimentos e cultura. Bárbara que sempre ofereceu auxílios e nos presenteou com Lorena. Jéssica, Clysellen, Bruno, Monique, Aline que sempre disponibilizaram seu tempo para ensinar com carinho e paciência.

A todos os amigos que auxiliaram de alguma maneira no desenvolvimento deste trabalho, muito obrigado pelo apoio, acolhimento e carinho!

RESUMO

O óleo sacha inchi (OSI) (*Plukenetia volubilis L*) é rico em ácidos graxos e carotenoides como o β-caroteno (β-C). O β-C é percussor da vitamina A e possui propriedades antioxidantes. Tais compostos apresentam sensibilidade a fatores externos (calor, oxidação e alcalinidade) e sob tais circunstâncias podem ter seu potencial biológico reduzido. A microencapsulação é uma alternativa na proteção do óleo sacha inchi e seus componentes. Dentre os métodos de microencapsulação, a coacervação complexa apresenta vantagens como baixa concentração de materiais de parede, elevada eficiência de encapsulação, e uma variedade de biopolímeros que podem ser utilizados como materiais de parede. A coacervação complexa consiste na interação eletrostáticas entre duas ou mais soluções poliméricas, que possuem cargas opostas. Consiste em três etapas básicas: emulsificação, coacervação e reticulação. Os biopolímeros como proteínas e polissacarídeos são os mais utilizados como materiais de parede na microencapsulação por coacervação complexa, estes são naturais e apresentam propriedades funcionais. O objetivo deste trabalho foi encapsular o β-C presente no OSI através da técnica de coacervação complexa utilizando como material de parede o isolado proteico do soro (IPS) e carboximetilcelulose (CMC). O sistema IPS e CMC mostrou-se eficiente como material de parede, apresentaram alta eficiência de encapsulação do β-C (96.21%). O sistema de simulação gastrointestinal indicou que a liberação de β-C ocorreu principalmente no intestino (92%) e uma parcela relativamente menor na fase gástrica (11-16%). A bioacessibilidade demonstrou que 33,14% do β-C está disponível para absorção, enquanto a estabilidade das microcapsulas foi de 82,73%. A simulação em alimentos oleosos ocorreu por difusão *Fickian* de acordo com modelo *Rigger-Peppas*. Os resultados alcançados sugerem a eficácia dos materiais parede utilizados para encapsular ingredientes ativos.

Palavras-chave: Biopolímeros, isolado proteico do soro, interação eletrostática, eficiência de encapsulamento, carotenoides

ABSTRACT

Sacha inchi oil (OSI) (*Plukenetia volubilis* L) is rich in fatty acids and carotenoids such as β-carotene (β-C). β-C is a precursor of vitamin A and has antioxidant properties. Such compounds are sensitive to external factors (heat, oxidation and alkalinity) and under such conditions may have their biological potential reduced. Microencapsulation is an alternative in protecting sacha inchi oil and its components. Among microencapsulation methods, complex coacervation has advantages such as low concentration of wall materials, high encapsulation efficiency, and a variety of biopolymers that can be used as wall materials. Complex coacervation consists of electrostatic interactions between two or more polymeric solutions, which have opposite charges. It consists of three basic steps: emulsification, coacervation and cross-linking. Biopolymers such as proteins and polysaccharides are the most used as wall materials in microencapsulation by complex coacervation, these are natural and have functional properties. The objective of this work was to encapsulate the β-C present in the OSI through the complex coacervation technique using whey protein (WPI) and carboxymethylcellulose (CMC) as wall material. The WPI and CMC system proved to be efficient as a wall material, with high β-C encapsulation efficiency (96.21%). The gastrointestinal simulation system indicated that the release of β-C occurred mainly in the intestine (92%) and a relatively smaller portion in the gastric phase (11-16%). Bioaccessibility demonstrated that 33.14% of β-C is available for absorption, while the stability of microcapsules was 82.73%. The simulation in oily foods occurred by Fickian diffusion according to the Rigger-Peppas model. The results achieved suggest the effectiveness of the wall materials used to encapsulate active ingredients.

Key words: biopolymers, whey protein isolate, electrostatic interaction, encapsulation efficiency, carotenoids

LISTA DE FIGURAS

CAPITULO I – REVISÃO DE LITERATURA

CAPITULO II - MICROENCAPSULATION OF B-CAROTENE FROM SACHA INCHI OIL BY COMPLEX COACERVATION: CHARACTERIZATION, SIMULATED GASTROINTESTINAL CONDITIONS AND MODELING RELEASE KINETICS

LISTA DE TABELAS

LISTA DE ABREVIAÇÕES E SÍMBOLOS

IPS - Isolado proteico do soro

WPI – Whey protein isolate

WPIn- Whey protein isolate heated

CMC- Carboximetilcelulose

Pdi - Índice de polidispersividade

β-C – β-caroteno

pH - Potencial de hidrogênio

OSI – Óleo sacha inchi

kDa - Kilodaltons

pI - Isoeletric Point

FT-IR- Fourier Transform Infrared Spectroscopy

IUPAC - International Union of Pure and Applied Chemistry

$CaCl_2$ – Calcium Chloride

EE- Encapsulation efficiency

TG- Transglutaminase

SSF - Simulated Salivary Fluid

SGF - Simulated Gastric Fluid

SIF - Simulated Intestinal Fluid

DLS - Dynamic Light Scattering

SEI - Strength of the Electrostatic Interaction

SUMÁRIO

INTRODUÇÃO

Nos últimos anos, existe um maior interesse na aplicação de tecnologias envolvendo a utilização de polissacarídeos e proteínas. E grandes investimentos têm sido realizados pela indústria de alimentos para fabricar produtos com alto valor agregado (GAONKAR et al., 2014). Busca-se uma melhoria nas propriedades de alimentos existentes; e tal aperfeiçoamento depende de uma melhor compreensão da complexa inter-relação entre estrutura e desempenho dos alimentos (DESAI; PARK, 2005; GIRARDI et al., 2016; McCLEMENTS, 2014; McCLEMENTS et al., 2009).

Associado a tal fato, estudos na área de formação de complexos coacervados entre proteínas e polissacarídeos vêm sendo realizados. Como o caso do IPS e CMC (KOUPANTSIS; PAVLIDOU; PARASKEVOPOULOU, 2016; ROJAS-MORENO et al., 2018), ovoalbumina e alginato de sódio (SOARES et al., 2019), lactoferrina e alginato de sódio (BASTOS; CARVALHO; GARCIA-ROJAS, 2018).

A celulose, possui papel na organização estrutural, crescimento das plantas e manutenção da resistência à tração. É um polímero linear, higroscópico, insolúvel em água (DICK- PÉREZ et al., 2011; GOPINATH et al., 2018). A carboximetilcelulose é biodegradável, também conhecida como goma de celulose, é um polímero linear aniônico, em consequência de seus consideráveis grupos carboxílicos ionizados, obtido ao se agrupar grupos de carboximetil à molécula celulose nativa (McCLEMENTS, 2014).

O soro é o subproduto fluido resultante da precipitação de proteínas do leite (KILARA; VAGHELA, 2017). Possui proteínas (α-lactalbumina, β-lactoglobulina, albumina sérica bovina, lactoferrinas e imunoglobulinas) que exibem propriedades: antioxidantes, funcionais (coagulação, gelificação e formação de espuma), ação antimicrobiana e nutricionais. São, comumente, combinadas com carboidratos (maltodextrina, amido modificado e celulose modificada) na formação de complexos. Com isso, o Isolado Proteico do Soro (IPS) é frequentemente utilizado como material de parede para obter microcápsulas (GARCÍA-MORENO et al., 2018). O tratamento térmico do IPS leva a alterações características nas conformações da proteína (JONES; DECKER; McCLEMENTS, 2010). O IPS é instável ao calor e pode desnaturar, alterando suas funcionalidades como: emulsificação e formação de espuma, e possui seu ponto de desnaturação em torno de 75-80°C (BERNAL; JELEN, 1985; HAQUE et al., 2013; SETIOWATI et al., 2017).

O óleo de sacha inchi (*Plukenetiavolubilis L.)* possui características funcionais, como a elevada percentagem de ácidos graxos insaturados essenciais cerca de 42-48% de ácido linolênico (ω-3), 32-37% de ácido linoleico (ω-6) e 9-12% de ácido oleico (ω-9) e contém cerca de 0,07-0,09 mg de β-C equivalente por 100 g de semente (FANALI et al., 2011; WANG; ZHU; KAKUDA, 2018). O β-C é um carotenoide que possui benefícios à saúde como a prevenção de doenças cardiovasculares (GAMMONE; RICCIONI; NICOLANTONIO, 2014) previne o desenvolvimento de câncer (LE GOFF et al., 2019). É percursor da vitamina A e atua como anti-inflamatório (BOIKO et al., 2017). Porém tais propriedades podem ser afetadas quando é exposto a condições do trato gastrointestinal, altas temperaturas, oxigênio e luz, também, a baixa solubilidade do composto em meio aquoso torna sua bioacessibilidade extremamente baixa (THAKUR et al., 2017).

A técnica de encapsulação envolve um ingrediente ativo em um material de parede formado por polímeros, produzindo pequenas partículas em que o principal objetivo é proteger o encapsulado de fatores ambientais tais como umidade, luz, e temperatura com o propósito de melhorar as propriedades de liberação e estender sua vida útil (SHAHIDI; HAN, 1993; GHARSALLAOUI; CHAMBIN, 2007; SANTOS et al., 2015; ZHOU et al., 2018).

Dentre as técnicas de encapsulação, pode-se destacar a coacervação complexa, que ocorre principalmente por interações eletrostáticas, em que os polímeros apresentam cargas opostas. Para que haja ideal encapsulação os tipos de material devem apresentar características funcionais, ser biodegradável, ter capacidade de formar uma barreira entre a fase interna e suas imediações, o custo e segurança do material de parede e o método devem ser acessíveis (FAVARO-TRINDADE; PINHO, 2008; McCLEMENTS, 2014).

Assim, esta dissertação foi organizada em capítulos. Onde no capítulo I trata de uma revisão de literatura na qual foram apresentados temas estudados no capítulo seguinte. Uma revisão sobre microencapsulação, coacervação complexa, matérias de parede e lipídeos bioativos (óleo sacha inchi), β-C, simulação gastro intestinal *in vitro* e liberação controlada em alimentos.

No capítulo II foi estudado o processo de formação dos complexos coacervados entre IPS aquecido e CMC em função da razão de proteína e polissacarídeo em diferentes pHs, microencapsulação do óleo sacha inchi pela técnica de coacervação complexa, caracterização morfológica da microcápsula, simulação gastro intestinal *in vitro* e liberação em alimentos de matriz lipídica

OBJETIVO GERAL

Encapsular o β-caroteno presente no óleo de sacha inchi por coacervação complexa utilizando os biopolímeros carboximetilcelulose e isolado proteico do soro desnaturado.

OBJETIVO ESPECÍFICO

- Estudar a influência dos biopolímeros na formação do complexo coacervado entre carboximetilcelulose e isolado proteico do soro desnaturado, e caracterizá-lo quanto as suas propriedades químicas.

- Encapsular o óleo de sacha inchi rico em β-caroteno por coacervação complexa empregando carboximetilcelulose e isolado proteico do soro desnaturado como materiais de parede. e caracterizar (morfologicamente e quimicamente) as capsulas.

- Estudar a estabilidade do β-caroteno presente no óleo de sacha inchi encapsulado por coacervação complexa, durante a simulação gastrointestinal *in vitro* e sua liberação em matriz alimentícia simulada

CAPÍTULO I REVISÃO BIBLIOGRÁFICA

1 Microencapsulação

A microencapsulação é definida como processo no qual um ingrediente ativo (encapsulado) é rodeado por um revestimento (material de parede), formando microcápsulas e protegendo o encapsulado contra condições ambientais adversas (GAONKAR et al., 2014; LI et al., 2019) .Tal revestimento pode ser composto por múltiplos ou um componente, e sua estrutura pode ser heterogênea ou homogênea (CHAMPAGNE e FUSTIER, 2007; GAONKAR et al., 2014; ZHOU et al., 2018).

O emprego desta tecnologia adquiriu aplicação em indústrias farmacêuticas, alimentícia, química e agrícola para fins de promover o bem-estar e a saúde humana. Recentemente vêm-se usando o encapsulamento de compostos bioativos como lipídeos, vitaminas, minerais e nutracêuticos (FATHI; MARTÍN; McCLEMENTS, 2014; GAONKAR et al., 2014).

A microencapsulação fornece uma barreira física para proteger compostos contra fatores ambientais externos (calor, oxidação, umidade, evaporação, alcalinidade), para assim melhorar a estabilidade do composto quando o mesmo for liberado (GAONKAR et al., 2014; ZHOU et al., 2018). Essas partículas protegidas, permanecem inertes em cápsulas por período específico de tempo e liberam o seu material de núcleo quando necessário para sua função específica (ARSHAD; ALI; HASNAIN, 2018).O componente do núcleo é denominado o encapsulado, núcleo e agente ativo. Já os polímeros de revestimento, que envolvem o núcleo, são chamados de revestimento, material da parede, transportador ou agente encapsulante (DESAI e PARK, 2005).

Quanto ao tamanho das cápsulas, estas podem ir desde poucos nanômetros até vários micrômetros, são classificadas como: macrocápsulas (>5,000μm), microcápsulas (0,2 a 5000 μm) e nanocapsulas (<0,2 μm) (FAVARO-TRINDADE e PINHO, 2008).

Diferentes tipos de partículas podem ser obtidos a depender das propriedades físico-químicas da composição da parede, núcleo e da técnica de microencapsulação utilizada. As microcápsulas são caracterizadas por seu núcleo ser concentrado na região central, circundado pelo material de parede. Microsferas o núcleo é uniformemente disperso em uma matriz. O que distingue uma microcápsula de uma microesfera, é que nas microesferas uma pequena porção do material encapsulado permanece exposto na superfície, o que é evitado pela “verdadeira” encapsulação. No entanto, o termo ‘encapsulação’ tem sido usado em seu sentido amplo, englobando tanto a formação de microcápsulas quanto de microesferas (RÉ, 1998; GHARSALLAOUI e CHAMBIN, 2007). A Figura 1 demonstra diferentes configurações estruturais de sistemas de microencapsulação.

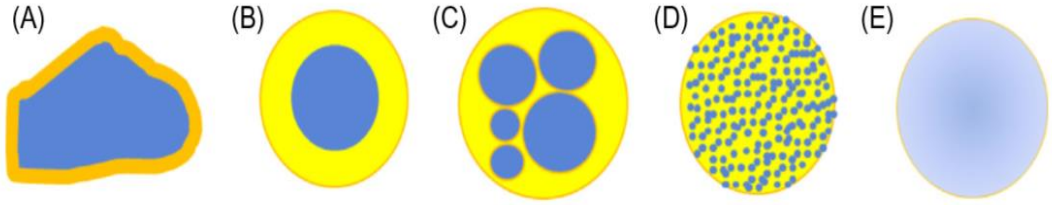

Figura 1. Comparação das Morfologias da microcápsula (A, B e C) e microesfera (D e E).
Fonte: Adaptado de GAONKAR et al., (2014).

1.1 Técnicas de Microencapsulação

A concepção de partículas pode ser dividida em: métodos físicos, químico e físico-químicos (FAVARO-TRINDADE; PINHO, 2008; McCLEMENTS et al., 2009). A escolha do método dependerá da aplicação dada à microcápsula, mecanismo de liberação, tamanho desejado, propriedades físico-químicas do material encapsulado (RÉ, 1998)**.**

As técnicas físicas são caracterizadas pelo uso de temperatura e pressão para impulsionar a formação da parede que reveste o material do núcleo. A exemplo :*spray drying* (SANTANA et al., 2016; TAMM et al., 2016; GIORGIO; SALGADOAND; MAURI, 2018), *spray chilling* (ARSLAN-TONTUL; ERBAS; GORGULU, 2019; CHALELLA MAZZOCATO; THOMAZINI, 2019), *spray cooling* (GAVORY et al., 2014; MATOS-JR et al., 2015), extrusão (ZHANG; ZHANG; McCLEMENTS, 2016; PRADEEP; NAYAK, 2019), co-cristalização (SARABANDI; MAHOONAK; AKBARI, 2019) e liofilização (HUGUES-AYALA et al., 2020; LI et al., 2019).

Os métodos químicos são: coacervação simples (NGAMEKAUE; CHITPRASERT, 2019), coacervação complexa (WANG et al., 2015a, 2018a; TIMILSENA, 2016), envolvimento por lipossomos (CHAVES; PINHO, 2019; LINSLEY et al., 2019).

Métodos físico-químicos: emulsificação (SANTOS et al., 2015) e polimerização interfacial (FATHI; MARTÍN; McCLEMENTS, 2014).

1.1.1 Coacervação simples e coacervação complexa

De acordo com a IUPAC (União Internacional de Química Pura e Aplicada), coacervação pode ser definida como uma separação coloidal de sistemas em duas fases líquidas (IUPAC, 2014). Pode-se classificar a coacervação como: coacervação simples ou complexa. A coacervação complexa dá-se através de interações eletrostáticas entre duas ou mais soluções poliméricas, que possuem cargas opostas, o que resulta em duas fases líquidas imiscíveis, onde

uma delas é a fase que contém menos polímero, e a outra, a fase densa é abundante em polímero, enquanto a coacervação simples envolve apenas um polímero (SCHMITT e TURGEON, 2011; GAONKAR et al., 2014).

A coacervação complexa apresenta vantagens como: baixa concentração de materiais de parede (SOARES et al., 2019), elevada eficiência de encapsulação, aproximadamente 90% (RUTZ et al., 2017), condições brandas de temperatura no processamento, integridade do material de parede, boa taxa de liberação controlada do material do núcleo, variedade de biopolímeros que podem ser utilizados como materiais de parede (GAONKAR et al., 2014).

A técnica de coacervação complexa é composta de seguintes passos: emulsificação, coacervação e reticulação (ROJAS-MORENO et al., 2018; WANG et al., 2018b).

A primeira etapa consiste na formação da emulsão. Uma solução contendo óleo e polímero (usualmente um polissacarídeo ou uma proteína) a um pH acima do ponto isoelétrico é emulsificada. Para a produção de emulsão vários métodos podem ser utilizados, como: homogeneização a alta pressão, agitação mecânica ou inversão de fase (GAONKAR et al., 2014; WANG et al., 2018b).

Na segunda etapa ocorre a coacervação complexa, devido a atração eletrostática dos biopolímeros que possuem cargas opostas sendo usualmente um polissacarídeo aniônico e uma proteína. Ocorre pela redução do pH da solução abaixo do ponto isoelétrico Wang et al., (2015b). Quando o pH é reduzido abaixo do pI (ponto isoelétrico) da proteína o número de cargas positivas na proteína aumenta ($-NH_2 \rightarrow -NH^+_3$) enquanto o número de cargas negativa reduz ($-CO_2H \rightarrow -CO^-_2$). Assim os grupos aniônicos do polissacarídeo se associam com os grupos catiônicos da superfície da proteína (SCHMITT; TURGEON, 2011). Geralmente os coacervados são mais adequados para uso em *delivery systems*, porque tendem a formar partículas com propriedades com melhor definição (tamanho e carga) e têm melhor estabilidade à agregação e sedimentação (GONNET; LETHUAUT; BOURY, 2010; BARROW et al., 2013; McCLEMENTS, 2014).

Proteína e polissacarídeos, quando misturados em ambiente aquoso, para formação de complexos, se originam principalmente de interações eletrostáticas entre macromoléculas de cargas opostas, e ainda outros fatores como pH e força iônica (SCHMITT e TURGEON, 2011). Pode-se observar na Tabela 1 os biopolímeros utilizados como material de parede, o material encapsulado, o pH estabelecido e as análises e metodologias realizadas na técnica de coacervação complexa para encapsulação.

Tabela 1. Biopolímeros aplicados como material de parede através da técnica de coacervação complexa, pH, ingredientes encapsulados e análises/técnicas realizadas

Material de parede	Material Encapsulado	Emulsão	pH	Agente Crosslink	Metodologia/análises Realizadas	Fontes
Lactoferrina e Alginato de sódio	Óleo de pimenta preta	Óleo em água	4,0	Transglutaminase	EE; FT-IR, MEV, DI	(BASTOS et al., 2020a)
B-lactoglobulina e Alginato de sódio	Óleo de pimenta preta	Óleo em água	4,5	Transglutaminase	EE; FT-IR; DTP; DI; MEV	(BASTOS et al., 2020b)
IPS- Goma guar.	Óleo de atum	Óleo em água	4,75	Cloreto de Cálcio	EE; FT-IR; TG	(WANG; ADHIKARI; BARROW, 2019)
IPS e CMC	Óleo essencial de Laranja	Óleo em água	3,0	Ácido Tânico. Transglutaminase. Sodiumtripolyphosphate	EE; MM; EEO; DTP; AM.	(ROJAS-MORENO et al., 2018)
IPS e CMC	β- pineno	Óleo em Água	2,8	Ácido tânico	MEV; FTIR; EE; RE; TGA.	(KOUPANTSIS; PAVLIDOU; PARASKEVOPOULOU, 2016)
IPS e Goma Acácia	β - C	Óleo em água	4,2	Glutaraldeído	EE; DTP; FT-IR; DI	(JAIN et al., 2015)
Gelatina e CMC	Limoneno	Óleo em água	4-6	Glutaraldeído	MEV; FTIR; DTP;	(SÁNCHEZ-NAVARRO et al., 2015)
IPS e CMC	β-pineno	Óleo em água	2,8	Ácido tânico	ZP; MM; EE; RE; GC- FID.	(KOUPANTSIS; PAVLIDOU; PARASKEVOPOULOU, 2014)
Gelatina – NaCMC	Tetracloretileno	Óleo em água	5,0	Glutaraldeído	FT-IR; MEV; MM	(WU et al., 2011)

Legenda: AB= Atividade Biológica**;** AM= Análise morfológica; AMP= Análise microbiológica dos Probióticos; ANP= Avaliação nutricional da Proteína; CC= Coacervação complexa; CQM = Composição Química das microcápsulas; DI= Digestão *in vitro*; DGV= Dinâmica de Gelatinização e Viscoelasticidade; DT= Determinação da Turbidez; DTP= Distribuição do Tamanho Da Partícula;*;* EE= Eficiência de Encapsulação; EEO= Eficiência de Encapsulação do Óleo; EDL= Espalhamento Dinâmico De Luz; MEV: Microscopia Eletrônica de Varredura; MM= Morfologia das Microcápsulas; PZ= Potencial Zeta; PT= Propriedades de Textura; RC= rendimento de Coacervação; RE= Rendimento de encapsulamento; TGA = Análise Termogravimétrica.; TG= Temperatura de Transição Vítrea.

Na última etapa, acontece o endurecimento das paredes das microcápsulas devido a adição de agentes de reticulação. As paredes das microcápsulas formadas pelo processo de coacervação geralmente possuem instabilidade e baixa resistência mecânica, por conta da natureza iônica da interação entre os biopolímeros (GAONKAR et al., 2014). Alguns agentes reticulantes usualmente utilizados são *tween* 80, *tween* 20, ácido tânico e transglutaminase (ROJAS-MORENO et al., 2018).

A Transglutaminase é encontrada nos tecidos de animais, plantas e de microrganismos, porém é normalmente isolada de bactérias por conta da facilidade de separação, purificação e abundância (ZEEB; McCLEMENTS; WEISS, 2017).Estudos empregaram a transglutaminase como agente reticulante no processo de produção de microcápsulas para coacervação complexa (LV et al., 2014; IFEDUBA; AKOH, 2015; YE et al., 2017; ROJAS-MORENO et al., 2018; DA SILVA et al., 2019; RIOS-MERA et al., 2019)

1.2 Materiais de Parede

O desenvolvimento de um material de parede adequado ao encapsulado necessita de vários fatores, incluindo a seleção do processo, *design* de formatação e seleção de material. Pode ser complexo construir um material de revestimento ideal, em especial porque muitos parâmetros de interação determinam o sucesso de um determinado material de parede para a cápsula. Cada situação exige uma solução personalizada devido à grande variação de alimentos, armazenamento, necessidade de diferentes disparadores de liberação e requisitos de consumo (GAONKAR et al., 2014).

No processo de encapsulamento, a eficácia e estabilidade da cápsula são influenciadas pelo material de parede. Logo o agente encapsulante ideal deve ter capacidade de selar e manter o núcleo no interior da capsula, não ser reativo com o núcleo, ser capaz de fornecer o máximo de proteção ao núcleo contra condições externas adversas, não possuir gosto desagradável quando aplicado a alimentos e viabilidade econômica (GAONKAR et al., 2014; LUCY et al., 2014). A maioria dos materiais de parede não possui todas as propriedades desejadas, e a junção de dois ou mais materiais de parede geralmente é realizada para alcançar os atributos desejados (FAVARO-TRINDADE; PINHO, 2008).

Proteínas e polissacarídeos são largamente usados em indústrias de alimentos, farmacêutica e biomédica, tendo em vista suas propriedades funcionais (ZHANG et al., 2012; COMUNIAN et al., 2013; RUTZ et al., 2017; SHADDEL et al., 2017;). E podem ser utilizados como material de parede em microencapsulação de vitaminas e compostos bioativos (TOLSTOGUZOV, 2003; McCLEMENTS et al., 2009; TURGEON e LANEUVILLE, 2009).

1.2.1 Celulose

Derivada de plantas como: cascas de laranja, casca de aveia, casca de banana e bagaço de cana-de-açúcar, mas também pode ser encontrado em bactérias, algas e fungos (KLEMM et al., 2005; MOHAMED et al., 2017). Polímero natural com maior abundância, estima-se que a natureza é capaz de produzir anualmente 10^{11}-10^{12} toneladas/ano de celulose. Esta macromolécula tem um papel na organização estrutural, crescimento das plantas e manutenção da resistência à tração (DICK- PÉREZ et al., 2011; KUKRETY ET AL., 2017). Sendo utilizada pelo ser humano em vasta escala na indústria do papel, mineração, construção e fonte de bioenergia (KLEMM et al., 2005; COFFEY; BELL; HENDERSON, 2006). A celulose é um homopolímero linear, higroscópico, insolúvel em água, porém com capacidade de inchar em ácido diluído, água e em solventes; possui alta massa molecular, constituído de unidades repetidas de β-D-glicopiranosil (1-4), unidas por ligações glicosídicas, com a estrutura química de $(C_6H_{10}O_5)n$ (COFFEY; BELL; HENDERSON, 2006; FENNEMA; PARKIN; CLADERA-OLIVERA, 2010; GOPINATH et al., 2018).

A celulose, em seu estado natural, não é indicada para ser utilizada como componente estrutural em alimentos, pela formação de fortes ligações de hidrogênio intermoleculares, tornando-a insolúvel em água. Contudo, o biopolímero em questão pode ser modificado por diferentes formas para obter produtos que são úteis como componentes estruturais no projeto de *delivery systems* (KLEMM et al., 2005; McCLEMENTS, 2014).

1.2.1.1 Carboximetilcelulose (CMC)

Este biopolímero é um polissacarídeo biodegradável e pode ser inteiramente degradado em sistemas ambientais, mostrando-se também como não tóxico, tem aplicação em áreas *e.g* alimentos, farmacêutica, cosméticos entre outros (VAN GINKEL; GAYTON, 1996; TOGRUL; ARSLAN, 2003; XUE; NGADI, 2009; KARIMI; M.R. NAIMI-JAMAL, 2018).

O CMC constitui-se de uma longa e relativamente rígida molécula com carga negativa, devido a seus numerosos grupos carboxílicos ionizados, a repulsão eletrostática faz com que essas moléculas, quando em solução, fiquem estendidas. Da mesma forma, as cadeias adjacentes repelem umas às outras. Como consequência, as soluções de CMC tendem a ser, altamente viscosas e estáveis, estando disponível em uma ampla faixa de graus de viscosidade. A CMC estabiliza dispersões de proteínas, particularmente perto do valor do pH isoelétrico da proteína (FENNEMA; PARKIN; CLADERA-OLIVERA, 2010).

Obtém-se a carboximetilcelulose pela reação da celulose com hidróxido de sódio, originando a álcali-celulose. Da reação da álcali-celulose com monocloroacetato de sódio obtém-se a carboximetilcelulose sódico (CMC). A celulose é constituída de uma longa cadeia de unidades anidroglucose, sendo que cada unidade contém três grupos de hidroxilas disponíveis para a derivatização. Assim se todos esses grupos hidroxila fossem substituídos, o produto teria grau de substituição(DS) de 3 (MACHADO, 2000;KLEMM et al., 2005; COFFEY; BELL; HENDERSON, 2006;KARIMI; M.R. NAIMI-JAMAL, 2018).

A associação inter-cadeias dos grupos hidroxila adjacentes é importante determinador da solubilidade. O DS no CMC possui efeito nas propriedades físicas das soluções. Em DS com baixas concentrações, a eterificação ocorreu nas regiões superficiais amorfas e cristalinas da celulose, obtendo um produto com baixa solubilidade em água; com isso CMC com DS <0,3 é apenas solúvel em álcali. No DS mais alto, as associações inter-cadeias são mais rompidas, produzindo material parcialmente solúvel. À medida que o DS se aproxima de 0,7, as regiões cristalinas foram suficientemente rompidas para produzir material altamente solúvel em água (COFFEY; BELL; HENDERSON, 2006; ZHIVKOV, 2013; KUKRETY et al., 2017).Comercialmente o CMC é encontrado na forma de sais de sódio ou cálcio e está disponível em diferentes pesos moleculares e DS, variando de 0,4 -1,3. (VAN GINKEL; GAYTON, 1996; McCLEMENTS, 2014).

1.2.2 Isolado proteico do soro

O soro é o subproduto fluido resultante da precipitação de proteínas no leite. A precipitação pode ser facilitada por: adição de ácido (fabricação de caseína ácida), crescimento de microrganismos (soro de queijo), ou pela adição de enzimas (coalho fabricação de caseína). Sua composição é aproximadamente 94% de água (6% de sólidos totais), 4,5% de lactose, 0,8% de proteína e 0,7% de minerais (KILARA; VAGHELA, 2017).

As proteínas do leite, são altamente solúveis e funcionais sob condições adequadas de pH, temperatura e de interação iônica. Em sua forma nativa existem como moléculas compactas de proteína globular com um alto grau de estrutura secundária e terciária (MORR, 1989). Tais proteínas, que também exibem propriedades antioxidantes, são geralmente combinadas com carboidratos (maltodextrina, xarope de glicose, lactose, amido modificado e celulose modificada) e por suas propriedades funcionais são frequentemente usadas passa obter microcápsulas (ADJONU et al., 2014; GARCÍA-MORENO et al., 2018).

As proteínas do soro de leite possuem solubilidade em uma ampla faixa de pH, perfil nutricional favorável em relação aos aminoácidos essenciais e funcionalidade diversificada. O

IPS possui de (90-92%) de teor de proteína, (0,5-10%) de lactose (0,5-10%) de gordura e (6-7,5%) de misturas (KILARA; VAGHELA, 2017). 60% do IPS é constituído por β-lactoglobulina, que possui uma massa molecular de 18,4 kDa e um ponto isoelétrico (pI) de 5,3. A α-Lactoglobulina representa 20% do IPS, sua massa molar é de 14,2 kDa e seu pI é de 4,8. BSA representa aproximadamente 3% das proteínas do IPS e possui pI de 4,9. A lactoferrina constitui cerca de 1-2% das proteínas do IPS com massa molar de 76,1 kDa e pI de 8,9 (SANTOS;et al., 2018).

O tratamento térmico de proteínas (*e.g* IPS) leva a alterações características nas interações e conformações da proteína, e essas modificações podem ser utilizadas como base para formar micropartículas ou nanopartículas. A título de exemplo, o tratamento térmico da β-lactoglobulina acima da temperatura de desnaturação térmica e valores de pH abaixo de 6 leva à formação de nanopartículas de proteína com tamanho em torno de 50 – 150 nm em diâmetro. O tamanho das partículas dos agregados depende da taxa de aquecimento, duração do aquecimento e concentração de proteínas (JONES; DECKER; McCLEMENTS, 2010).

O IPS é muito instável ao calor e pode facilmente desnaturar, e sua desnaturação induzida pelo calor, leva à alteração de suas propriedades funcionais. Ao desnaturar, o IPS, alteram-se as suas funcionalidades como: formação de espuma e emulsificação, e possui seu ponto de desnaturação em torno de 75-80°C (BERNAL; JELEN, 1985; HAQUE et al., 2013; SETIOWATI et al., 2017).

Diferentes trabalhos utilizaram o IPS aquecida para a microencapsulação de compostos bioativos. (COMUNIAN et al., 2020), encapsularam óleo de semente de romã utilizando IPS aquecida através de emulsão simples. (BAGHERI et al., 2013) estudaram a microemulsão composta por IPS tratado termicamente, pó de extrato aquoso de palmito de dendê em óleo de girassol. (WANG et al., 2016), encapsularam através de *spray drying* óleo de peixe utilizando IPS aquecido como material de parede. (BOSNEA; MOSCHAKIS; BILIADERIS, 2014) utilizaram o ISP aquecido para encapsular *lactobacilli strains*, L. *paraplantarum* pela técnica de coacervação complexa.

1.3 Lipídios Bioativos

A categoria de lipídios alimentares contém um amplo espectro de compostos quimicamente diversos que são insolúveis em água e solúveis em solventes orgânicos (McCLEMENTS, 2014). O consumo regular de muitos desses ingredientes lipofílicos tem sido

associado a benefícios específicos para a saúde, como redução da incidência de obesidade, doença coronariana, diabetes, hipertensão, câncer, doenças cerebrais e doenças oculares. E com isso cresce o interesse em sua incorporação em alimentos e bebidas. Porém, muitos deles são altamente suscetíveis à degradação química durante o armazenamento (oxidação de lipídios poli-insaturados) (REDGWELL e FISCHER, 2005;McCLEMENTS et al., 2009; IORA et al., 2015). Compostos bioativos, como o ômega-3, são regularmente chamados de nutracêuticos e possuem componentes que normalmente ocorrem em pequenas quantidades nos alimentos, além de portarem a capacidade de modular um ou mais processos metabólicos (AJILA; JAGANMOHAN RAO; PRASADA RAO, 2010; VICENTE et al., 2017)

1.3.1 β-Caroteno

Os carotenoides são um grupo diverso de polienos lipossolúveis com coloração amarela a vermelha e estão presentes nas folhas, raízes, sementes, frutos e flores. A vitamina A é um nutriente essencial obtido de carotenoides como o β-C (FENNEMA; PARKIN; CLADERA-OLIVERA, 2010; VINHA et al., 2018). O β-C é lipofílico, solúvel em óleos e solventes orgânicos como: acetona, etanol e hexano. Para sua detecção, o comprimento de onda ultravioleta máximo, com a utilização de solventes etanol e hexano, são de 450 nm (RODRIGUEZ-AMAYA, 2001). A Figura 2 apresenta a representação da estrutura do β-C.

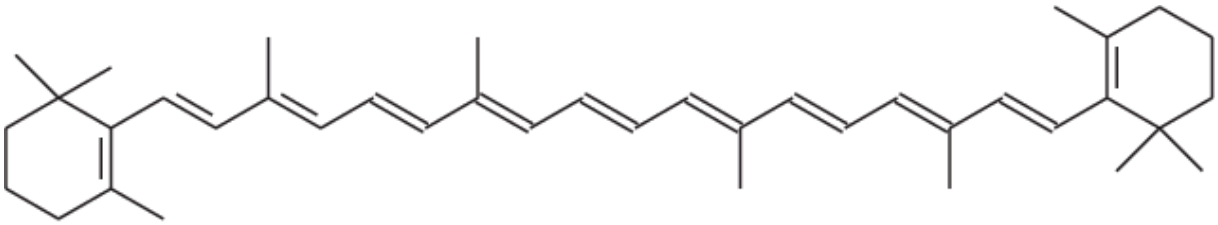

Figura 2: Representação estrutural do β-caroteno
Fonte: Adaptado de (ALVES et al., 2010)

Existe larga referência quanto a pesquisas com β-C em fontes naturais e suas respectivas quantidades: polpa da bocaiúva (49,0 μg/g), Acerola (26,23 μg / g), polpa de mamão (20,24 μg / g), pitanga (*Eugenia uniflora*; 15,64 μg / g), pasta de oliva (0,019 μg / g); óleo vegetal de batuá (*Oenocarpusbataua* 0,0024 μg /g), óleo de soja (0,003 μg/g), óleo de oliva (0,007 μg/g), óleo desemente de linhaça (0,007 μg/g) (RAMOS et al., 2008; ANNIVA; TSIMIDOU, 2009;

MONTÚFAR et al., 2010; SILVA et al., 2014). A Tabela 2 demonstra a técnica de microencapsulação aplicada a ingredientes lipofílicos e lipídeos bioativos, os materiais de parede utilizados no processo de encapsulamento, material coacervado, pH de coacervação e análises realizadas.

Tabela 2. Coacervação complexa aplicada a ingredientes lipofílicos e lipídeos bioativos.

Material de Parede	Material Encapsulado	pH	Análises/ Metodologias	Referência
Caseinato de Sódio e Alginato	β-C	4,5	ZP; DBE; DV; DE;	(LIU et al., 2018)
Quitosana e Goma de caju	Óleo de Pequi	4,5	ZP; RC; DT; EE;	(CARVALHO et al., 2018)
Caseína e goma Guar	β-C	5,0	ZP; AM; FTIR; EE; DV; DAA;	(THAKUR et al., 2017)
Quitosana e Goma Xantana	β-C contido no óleo de Palma	6,2	EE; AM; DSC; DV; AMA	(RUTZ et al., 2017)
IPS e Goma Acácia	β-C	3,75	HPLC; EE; AM; FTIR; DAA; AA	(MIHALCEA et al., 2017)
Gelatina e Goma Arábica	Óleo de Peixe	4,0	MM; CG-MS; DTP; MC.	(HABIBI et al., 2016)
IPS e Goma acácia	β-C	4,2	RC; EE; ZP; MM; FTIR; DV; DE.	(JAIN et al., 2015)
Quitosana e CMC	β-C contido no óleo de Palma	6,2	EE; AM; DSC; DV; AMA	(RUTZ et al., 2016)
Gelatina e Hexametafosfato de sódio	Óleo de Atum e vitaminas A, D3, E, K2	4,7	FTIR; AM; EE; TOS;	(WANG et al., 2015a)
Caseinato de Sódio e Pectina	Óleo de milho	5,0	ZP; DTP; DIC; DTP; DE; MM	(MATALANIS; McCLEMENTS, 2013)
Gelatina e Acácia	Capsaicina		DV;DTP; DSC; TGA;	(JINCHENG; XIAOYU; SIHAO, 2010)

Legenda: AA= Atividade Antifúngica; AMA= Adição das Micropartículas em Alimentos; AM= Análise morfológica; DAA= Determinação de Atividade Antioxidante; DE= Determinação da Estabilidade; DBE= Degradação do e β-carotene em Estoque DGV/DV= Dinâmica de Gelatinização e ou Viscoelasticidade; DTP= Distribuição do Tamanho da Partícula; DV= Digestão *in Vitro;* EE= Eficiência de Encapsulação; MC= Medição da Cor MM= Morfologia das Microcápsulas; PZ= Potencial Zeta; RC= rendimento de Coacervação;

1.4 Sacha Inchi

Sacha inchi (*Plukenetia volubilis L.*), também chamada de Inca inchi, e conhecida como "amendoim inca", "amendoim selvagem" ou "amendoim de montanha", é uma planta oleaginosa selvagem, trepadeira, perene e semi-lenhosa da família Euphorbiaceae que cresce nas florestas tropicais da América Latina, em altitudes entre 200 e 1500 m. Essa leguminosa tem sementes de formato lenticular, que são ricas em óleo (35-60%) e proteínas (27%) e proteínas e contêm substâncias lábeis ao calor com um sabor amargo. Contém cerca de 0,07-0,09 mg de β-C equivalente por 100 g de semente. Sacha Inchi tem sido e ainda é usado como um nutriente tradicional e pela população amazônica, em refeições, cosméticos e como medicamento (GUILLÉN et al., 2003; FOLLEGATTI-ROMERO et al., 2009; HANSSEN; FANALI et al., 2011; WANG; ZHU; KAKUDA, 2018). O OSI, possui elevadas quantidades de ácidos graxos insaturados essenciais, cerca de 42-48% de ácido linolênico (ω-3), 32-37% de ácido linoleico (ω-6) e 9-12% de ácido oleico (ω-9), contêm substâncias termolábeis, são ricos em cisteína, tirosina, treonina e triptofano (GUILLÉN et al., 2003; WANG; ZHU; KAKUDA, 2018).

Diferentes estudos realizaram o encapsulamento do OSI através de diferentes técnicas: (SOARES et al., 2019) encapsulou OSI por coacervação complexa utilizando os biopolímeros ovoalbumina e alginato de sódio em um pH 3,8. (SILVA et al., 2019) utilizou emulsões para encapsular OSI usando alginato de sódio e Tween 20 e 80. (VICENTE et al., 2017) encapsulou OSI através de emulsão utilizando diferentes biopolímeros (ovoalbumina, pectina e goma xantana).

1.5 Simulação Gastrointestinal e Liberação Controlada de compostos Bioativos

1.5.1 Simulação Gastrointestinal

Devido ao aumento de doenças relacionadas à dietas, ensaios de digestão veem sendo realizados para correlacionar a dieta com a saúde de diferentes grupos de pessoas. Porém para entender melhor a resposta fisiológica de alimentos específicos, é necessário seguir, de forma mais detalhada, os complexos processos digestivos no sistema gastrointestinal humano. Pode-se realizar isso com procedimentos invasivos (aspiração do estômago ou intestino) ou técnicas menos invasivas (ressonância magnética) (MINEKUS et al., 2014). Modelos em animais também são utilizados, muito embora seu uso passe por embates éticos (e.g. morte de animais e cirurgias). Por esses motivos, os modelos *in vitro* são utilizados há muitas décadas para simular a digestão dos alimentos (BRODKORB et al., 2019).

A simulação gastrointestinal *in vitro* inclui três etapas: oral, gástrica e a fase intestinal. (MINEKUS et al., 2014). A digestão gastrointestinal simulada *in vitro* é amplamente empregada em muitos campos das ciências nutricionais e alimentares (LUCINDA-SILVA et al., 2010; ZHANG et al., 2012, TIMILSENA et al., 2017; WANG et al., 2017).

Bioacessibilidade refere-se à fração do composto bioativo (*e.g.* carotenoide) ingerido que torna-se disponível para utilização em funções fisiológicas normais ou para armazenamento no corpo humano (YUWEI et al., 2012). A absorção de carotenoides envolve a decomposição da matriz alimentar, liberação de carotenoides, dispersão em partículas de emulsão lipídica, solubilização em micelas mistas de sal biliar, movimento através da camada de água sem agitação adjacente aos microfilos, captação de carotenoides pelas células da mucosa intestinal e incorporação em lipoproteínas linfáticas (RODRIGUEZ-AMAYA, 2010).

Estudos realizaram testes de digestão *in vitro* e bioacessibilidade de microcápsulas contendo β-C (YUWEI et al., 2012; SOUKOULIS et al., 2016; ZHANG; ZHANG; McCLEMENTS, 2016; FAN et al., 2017; MUN; McCLEMENTS, 2017; LIU et al., 2019).

1.5.2 Liberação controlada

A libertação controlada pode ser definida como um método pelo qual um ou mais agentes ativos são disponibilizados num local e tempo desejado e a uma taxa específica (MADENE; JACQUOT, 2006). Os dados da liberação podem ser usados para prever a liberação do composto *in vivo*, o que contribui para entender as características da formulação e fornece uma base para a preparação de uma melhor formulação de liberação sustentada (JAIN; JAIN, 2016).

Para estudar ainda mais os comportamentos de liberação de drogas in vitro, os pesquisadores vêm trabalhando no ajuste dos dados de liberação de drogas com alguns modelos cinéticos para julgar os modelos e mecanismos de liberação de drogas (WANG et al., 2019). Os modelos cinéticos de liberação comum incluem modelo cinético de ordem zero, modelo cinético de primeira ordem, modelo Weibull, modelo Higuchi, modelo Ritger-Peppas e modelo cinético de duas fases (JAIN; JAIN, 2016).

A liberação controlada vem sendo estudada por diversos autores (LUCINDA-SILVA et al., 2010; ZHANG et al., 2012; LUO; TENG; WANG, 2012; ATAY et al., 2018; SOLANKI; PATEL, 2018). Para simular diferentes tipos de alimentos que possuam características distintas tais quais: alimentos hidrofílicos, lipofílicos, diferentes faixas pH, emulsões de óleo em água, diferentes conteúdos de álcool, utiliza-se de simuladores de alimentos: etanol 10, 20 e 50%, ácido acético 3%, óleo vegetal e água (COMMISSION REGULATION 10/ 2011 EU, 2011).

2. REFERÊNCIAS

ADJONU, R. et al. Whey protein peptides as components of nanoemulsions: A review of emulsifying and biological functionalities. **Journal of Food Engineering**, v. 122, n. 1, p. 15–27, 2014.

AJILA, C. M.; JAGANMOHAN RAO, L.; PRASADA RAO, U. J. S. Characterization of bioactive compounds from raw and ripe Mangifera indica L. peel extracts. **Food and Chemical Toxicology**, v. 48, n. 12, p. 3406–3411, 2010.

ANNIVA, C.; TSIMIDOU, M. Z. On the quality control of "olive paste", a specialty based on olives and olive oil. **European Journal of Lipid Science and Technology**, v. 111, n. 4, p. 328–336, 2009.

ARSHAD, H.; ALI, T. M.; HASNAIN, A. Native and modified Sorghum starches as wall materials in microencapsulation of nutmeg oleoresin. **International Journal of Biological Macromolecules**, v. 114, p. 700–709, 2018.

ARSLAN-TONTUL, S.; ERBAS, M.; GORGULU, A. The Use of Probiotic-Loaded Single- and Double-Layered Microcapsules in Cake Production. **Probiotics and Antimicrobial Proteins**, v. 11, n. 3, p. 840–849, 2019.

ATAY, E. et al. Development and characterization of chitosan/gelatin electrosprayed microparticles as food grade delivery vehicles for anthocyanin extracts. **Food Hydrocolloids**, v. 77, p. 699–710, 2018.

Bagheri, L., Madadlou, A., Yarmand, M., & Mousavi, M. E. Nanoencapsulation of date palm pit extract in whey protein particles generated via desolvation method. **Food Research International**, 51(2), 866–871, 2013.

BARROW, C. J. et al. Spray drying and encapsulation of omega-3 oils. In: **Food enrichment with omega-3 fatty acids**. [s.l.] Woodhead Publishing Limited, 2013. p. 194–225.

BASTOS, L. P.; CARVALHO, C. W. P.; GARCIA-ROJAS, E. E. Formation and characterization of the complex coacervates obtained between lactoferrin and sodium alginate. **International Journal of Biological Macromolecules**, 2018.

BASTOS, L. P.; Vicente, J., Santos, C. dos C. H., Carvalho, M. G. de, & Garcia-Rojas, E. E.. Encapsulation of black pepper (Piper nigrum L.) essential oil with gelatin and sodium alginate by complex coacervation. **Food Hydrocolloids.** (2020)

BERNAL, V.; JELEN, P. Thermal Stability of Whey Proteins - A Calorimetric Study Several factors affecting thermal sta- bility of the proteins present in whey were studied over 2 . 5 to 6 . 5 pH range using differential scanning calorimetry and heat-induced precipitation . Heat. **Journal of Dairy Science**, v. 68, p. 2847e2852, 1985.

BOIKO, Y. A. et al. Extraction, identification and anti-inflammatory activity of carotenoids out of Capsicum Anuum L. **Journal of HerbMed Pharmacology**, v. 6, n. 1, p. 10–15,

2017.

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., … Recio, I.. INFOGEST static in vitro simulation of gastrointestinal food digestion. **Nature Protocols**, *14*(4), 991–1014, 2019.

CARVALHO, L. et al. Optimization of cashew gum and chitosan for microencapsulation of pequi oil by complex coacervation. **Journal of Food Processing and Preservation**, v. 42, n. 3, p. 1–8, 2018.

CHALELLA MAZZOCATO, M.; THOMAZINI, M.; FAVARO-TRINDADE, C. S. Improving stability of vitamin B12 (Cyanocobalamin) using microencapsulation by spray chilling technique. **Food Research International**, v. 126, n. September, p. 108663, 2019.

CHAMPAGNE, C. P.; FUSTIER, P. Microencapsulation for the improved delivery of bioactive compounds into foods. **Current Opinion in Biotechnology**, p. 184–190, 2007.

CHAVES, M. A.; PINHO, S. C. Curcumin-loaded proliposomes produced by the coating of micronized sucrose: Influence of the type of phospholipid on the physicochemical characteristics of powders and on the liposomes obtained by hydration. **Food Chemistry**, v. 291, n. November 2018, p. 7–15, 2019.

COFFEY, D. G.; BELL, D. A.; HENDERSON, A. **Food Polysaccharides and Their Applications**. Boca Raton, Florida: 2006.

COMMISSION REGULATION 10/ 2011 EU, (10/2011/EC). **COMMISSION REGULATION (EU) No 10/2011 of 14 January 2011**, 2011. Disponível em: <https://www.fsai.ie/uploadedFiles/Reg10_2011.pdf>

COMUNIAN, T. A. et al. Microencapsulation of ascorbic acid by complex coacervation : Protection and controlled release. **Food Research International journal**, v. 52, n. 1, p. 373–379, 2013.

DA SILVA, T. M. et al. The effect of enzymatic crosslinking on the viability of probiotic bacteria (Lactobacillus acidophilus) encapsulated by complex coacervation. **Food Research International**, v. 125, n. April, p. 108577, 2019.

DAI, R. et al. Gelatin/carboxymethylcellulose/dioctyl sulfosuccinate sodium microcapsule by complex coacervation and its application for electrophoretic display. **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, v. 362, n. 1–3, p. 84–89, 2010.

DESAI, K. G. H.; PARK, H. J. Drying Technology : An Recent Developments in Microencapsulation of Food Ingredients. **Drying Technology: An International Journal**, p. 37–41, 2005.

DICK- PÉREZ, M. et al. Structure and Interactions of Plant Cell-Wall Polysaccharides by Two- and Nmr, Three-dimensional Magic-angle-spinning Solid-state. **Biochemistry**, v. 50, p. 989–1000, 2011.

FAN, Y. et al. Physicochemical stability and in vitro bioaccessibility of β-carotene nanoemulsions stabilized with whey protein-dextran conjugates. **Food Hydrocolloids**, v. 63, n. 2017, p. 256–264, 2017.

FANALI, C. et al. Chemical characterization of Sacha inchi (Plukenetia volubilis L.) oil. **Journal of Agricultural and Food Chemistry**, v. 59, n. 24, p. 13043–13049, 2011.

FATHI, M.; MARTÍN, Á.; MCCLEMENTS, D. J. Nanoencapsulation of Food Ingredients using Carbohydrate Based Delivery Systems. **Trends in Food Science & Technology**, 2014.

FAVARO-TRINDADE, C. S.; PINHO, S. C. DE. Revisão : Microencapsulação de ingredientes alimentícios. **Brazilian Journal Food Technology**, p. 103–112, 2008.

FENNEMA, O. R.; PARKIN, K. L.; CLADERA-OLIVERA, F. **Química de Alimentos de Fennema**. 4. ed. Porto Alegre: [s.n.].

FOLLEGATTI-ROMERO, L. A. et al. Supercritical CO2 extraction of omega-3 rich oil from Sacha inchi (Plukenetia volubilis L.) seeds. **Journal of Supercritical Fluids**, v. 49, n. 3, p. 323–329, 2009.

GAMMONE, M.; RICCIONI, G.; NICOLANTONIO, D. Carotenoids: potential allies of cardiovascular health? **Food and Nutrition Research**, 2014.

GAONKAR, A. et al. **Microencapsulation in the food Industry: A Pratical Implementation Guide**. 1° ed. San Diego: Academic Press: Elsevier, 2014.

GARCÍA-MORENO, P. J. et al. Physicochemical characterization and oxidative stability of fish oil-loaded electrosprayed capsules: Combined use of whey protein and carbohydrates as wall materials. **Journal of Food Engineering**, v. 231, p. 42–53, 2018.

GAVORY, C. et al. Encapsulation of a pressure sensitive adhesive by spray-cooling: Optimum formulation and processing conditions. **Advanced Powder Technology**, v. 25, n. 1, p. 292–300, 2014.

GHARSALLAOUI, A.; CHAMBIN, O. Applications of spray-drying in microencapsulation of food ingredients : An overview. v. 40, p. 1107–1121, 2007.

GIORGIO, L. DI; SALGADOAND, P. R.; MAURI, A. N. Encapsulation of fish oil in soybean protein particles by emulsification and spray drying. **Food Hydrocolloids**, 2018.

GIRARDI, N. S. et al. Microencapsulation of Peumus boldus oil by complex coacervation to provide peanut seeds protection against fungal pathogens. **Industrial Crops & Products**, v. 92, p. 93–101, 2016.

GONNET, M.; LETHUAUT, L.; BOURY, F. New trends in encapsulation of liposoluble vitamins. **Journal of Controlled Release**, v. 146, n. 3, p. 276–290, 2010.

GOPINATH, V. et al. A review of natural polysaccharides for drug delivery applications : Special focus on cellulose , starch and glycogen. **Biomedicine & Pharmacotherapy**, v. 107, n. July, p. 96–108, 2018.

GUILLÉN, M. D. et al. Characterization of Sacha Inchi (Plukenetia volubilis L.) Oil. **Journal of the American Oil Chemists' Society**, v. 80, n. 8, p. 755–762, 2003.

HABIBI, A. et al. Preparation of fish oil microcapsules by complex coacervation of gelatin – gum arabic and their utilization for fortification of pomegranate juice. **Journal of Food Process Engineering**, v. 4, p. 1–11, 2016.

HANSSEN, H. P.; SCHMITZ-HÜBSCH, M. Sacha Inchi (Plukenetia volubilis L.) Nut Oil and Its Therapeutic and Nutritional Uses. **Nuts and Seeds in Health and Disease Prevention**, p. 991–994, 2011.

HAQUE, M. A. et al. Comparative study of denaturation of whey protein isolate (WPI) in convective air drying and isothermal heat treatment processes. **Food Chemistry**, v. 141, n. 2, p. 702–711, 2013.

HUGUES-AYALA, A. M. et al. Airbrush encapsulation of Lactobacillus rhamnosus GG in dry microbeads of alginate coated with regular buttermilk proteins. **Lwt**, v. 117, n. September 2019, p. 108639, 2020.

IFEDUBA, E. A.; AKOH, C. C. Microencapsulation of stearidonic acid soybean oil in complex coacervates modified for enhanced stability. **Food Hydrocolloids**, v. 51, p. 136–145, 2015.

IORA, S. R. F. et al. Evaluation of the bioactive compounds and the antioxidant capacity of grape pomace. **International Journal of Food Science & Technology**, v. 50, n. 1, p. 62–69, 2015.

IUPAC. **Compendium of Chemical Terminology Gold Book**. [s.l: s.n.].

JAIN, A. et al. Microencapsulation by Complex Coacervation Using Whey Protein Isolates and Gum Acacia : An Approach to Preserve the Functionality and Controlled Release of β -Carotene. **Food and Bioprocess Technology**, p. 1–10, 2015.

JAIN, A.; JAIN, S. K. In vitro release kinetics model fitting of liposomes: An insight. **Chemistry and Physics of Lipids**, v. 201, p. 28–40, 2016.

JINCHENG, W.; XIAOYU, Z.; SIHAO, C. Preparation and properties of nanocapsulated capsaicin by complex coacervation method preparation and properties of nanocapsulated capsaicin by complex coacervation. **Chemical Engineering Communications**, n. September 2014, p. 37–41, 2010.

JONES, O. G.; DECKER, E. A.; MCCLEMENTS, D. J. Comparison of protein-polysaccharide nanoparticle fabrication methods: Impact of biopolymer complexation before or after particle formation. **Journal of Colloid and Interface Science**, v. 344, n. 1, p. 21–29, 2010.

KARIMI, M.; M.R. NAIMI-JAMAL. Carboxymethyl cellulose as a green and biodegradable catalyst for the solvent-free synthesis of benzimidazoloquinazolinone derivatives. **Journal of Saudi Chemical Society**, p. 1–6, 2018.

KILARA, A.; VAGHELA, M. N. Whey proteins. In: **Proteins in Food Processing: Second Edition**. Second Edi ed. [s.l.] Elsevier Ltd., 2017. p. 93–126.

KLEMM, D. et al. Cellulose : Fascinating Biopolymer and Sustainable Raw Material Angewandte. **Angewandte Chemie 3358**, v. 44, p. 3358–3393, 2005.

KOUPANTSIS, T.; PAVLIDOU, E.; PARASKEVOPOULOU, A. Food Hydrocolloids

Flavour encapsulation in milk proteins e CMC coacervate-type complexes. **Food hydrocolloids**, v. 37, p. 134–142, 2014.

KOUPANTSIS, T.; PAVLIDOU, E.; PARASKEVOPOULOU, A. Glycerol and tannic acid as applied in the preparation of milk proteins e CMC complex coavervates for flavour encapsulation. **Food hydrocolloids**, v. 57, p. 62–71, 2016.

KUKRETY, A. et al. Comprehension on the Synthesis of Carboxymethylcellulose (CMC) Utilizing Various Cellulose Rich Waste Biomass Resources. **Waste and Biomass Valorization**, v. 9, n. 9, p. 1587–1595, 2017.

LE GOFF, M. et al. Microalgal carotenoids and phytosterols regulate biochemical mechanisms involved in human health and disease prevention. **Biochimie**, v. 167, p. 106–118, 2019.

LI, K. et al. Microencapsulation of Lactobacillus casei BNCC 134415 under lyophilization enhances cell viability during cold storage and pasteurization, and in simulated gastrointestinal fluids. **Lwt**, v. 116, n. February, p. 108521, 2019.

LINSLEY, C. S. et al. Preparation of photothermal palmitic acid/cholesterol liposomes. **Journal of Biomedical Materials Research - Part B Applied Biomaterials**, v. 107, n. 5, p. 1384–1392, 2019.

LIU, W. et al. Encapsulation of β -carotene-loaded oil droplets in caseinate / alginate microparticles : Enhancement of carotenoid stability and bioaccessibility. **Journal of Functional Foods**, v. 40, n. September 2017, p. 527–535, 2018.

LIU, W. et al. Stability, rheology, and β-carotene bioaccessibility of high internal phase emulsion gels. **Food Hydrocolloids**, v. 88, n. September 2018, p. 210–217, 2019.

LUCINDA-SILVA, R. M. et al. Alginate – chitosan systems : In vitro controlled release of triamcinolone and in vivo gastrointestinal transit. **Carbohydrate Polymers**, v. 81, p. 260–268, 2010.

LUCY, L. et al. Microencapsulation : concepts , mechanisms , methods and some applications in food technology. **Ciência Rural. Santa Maria**, p. 1304–1311, 2014.

LUO, Y.; TENG, Z.; WANG, Q. Development of zein nanoparticles coated with carboxymethyl chitosan for encapsulation and controlled release of vitamin D3. **Journal of Agricultural and Food Chemistry**, v. 60, n. 3, p. 836–843, 2012.

LV, Y. et al. Formation of heat-resistant nanocapsules of jasmine essential oil via gelatin/gum arabic based complex coacervation. **Food Hydrocolloids**, v. 35, p. 305–314, 2014.

MACHADO, G. DE O. **Universidade de são paulo**. Universidade de São Paulo, 2000.

MADENE, A.; JACQUOT, M. Review Flavour encapsulation and controlled release – a review. **International Journal of Food Science and Technology**, v. 41, p. 1–21, 2006.

MATALANIS, A.; MCCLEMENTS, D. J. Hydrogel microspheres for encapsulation of lipophilic components : Optimization of fabrication & performance. **Food hydrocolloids**, v. 31, n. 1, p. 15–25, 2013.

MATOS-JR, F. E. et al. Development and characterization of solid lipid microparticles loaded with ascorbic acid and produced by spray congealing. **Food Research International**, v. 67, p. 52–59, 2015.

MCCLEMENTS, D. J. et al. Structural Design Principles for Delivery of Bioactive Components in Nutraceuticals and Functional Foods Structural Design Principles for Delivery of Bioactive Components in Nutraceuticals and Functional Foods. **Critical Reviews in Food Science and Nutrition**, n. May 2013, p. 37–41, 2009.

MCCLEMENTS, D. J. **Nanoparticle and microparticle based delivery systems**. Boca Raton, Florida: CRC PRESS, 2014.

MIHALCEA, L. et al. Encapsulation of carotenoids from sea buckthorn extracted by CO2 supercritical fluids method within whey proteins isolates matrices. **Innovative Food Science and Emerging Technologies**, v. 42, p. 120–129, 2017.

MINEKUS, M. et al. A standardised static in vitro digestion method suitable for food – an international consensus. **Food & Function**, v. 5, p. 1113–1124, 2014.

MOHAMED, M. A. et al. An overview on cellulose-based material in tailoring bio-hybrid nanostructured photocatalysts for water treatment and renewable energy applications. **International Journal of Biological Macromolecules**, p. 1232–1256, 2017.

MONTÚFAR, R. et al. Oenocarpus bataua Mart. (Arecaceae): Rediscovering a Source of High Oleic Vegetable Oil from Amazonia. **JAOCS, Journal of the American Oil Chemists' Society**, v. 87, n. 2, p. 167–172, 2010.

MORR, C. V. Beneficial and Adverse Effects of Water-Protein Interactions in Selected Dairy Products. **Journal of Dairy Science**, v. 72, n. 2, p. 575–580, 1989.

MUN, S.; MCCLEMENTS, D. J. Influence of simulated in-mouth processing (size reduction and alpha-amylase addition) on lipid digestion and β-carotene bioaccessibility in starch-based filled hydrogels. **LWT - Food Science and Technology**, v. 80, p. 113–120, 2017.

NGAMEKAUE, N.; CHITPRASERT, P. Effects of beeswax-carboxymethyl cellulose composite coating on shelf-life stability and intestinal delivery of holy basil essential oil-loaded gelatin microcapsules. **International Journal of Biological Macromolecules**, v. 135, p. 1088–1097, 2019.

PRADEEP, H. N.; NAYAK, C. A. Enhanced stability of C-phycocyanin colorant by extrusion encapsulation. **Journal of Food Science and Technology**, v. 56, n. 10, p. 4526–4534, 2019.

RAMOS, M. I. L. et al. Nutritional quality of the pulp of bocaiuva Acrocomia aculeata (Jacq.) Lodd. **Ciência e Tecnologia de Alimentos**, v. 28, p. 1–8, 2008.

RÉ, M. I. MICROENCAPSULATION BY SPRAY DRYING. **Drying Technology : An International Journal**, p. 37–41, 1998.

REDGWELL, R. J.; FISCHER, M. Dietary fiber as a versatile food component: An industrial perspective. **Molecular Nutrition and Food Research**, v. 49, n. 6, p. 521–535, 2005.

RIOS-MERA, J. D. et al. Encapsulation optimization and pH- and temperature-stability of the complex coacervation between soy protein isolate and inulin entrapping fish oil. **Lwt**, v. 116, n. August, p. 108555, 2019.

RODRIGUEZ-AMAYA, D. B. **A GUIDE TO CAROTENOID ANALYSIS IN FOODS**. 2001.

RODRIGUEZ-AMAYA, D. B. Quantitative analysis, in vitro assessment of bioavailability and antioxidant activity of food carotenoids-A review. **Journal of Food Composition and Analysis**, v. 23, n. 7, p. 726–740, 2010.

ROJAS-MORENO, S. et al. Effect of the cross-linking agent and drying method on encapsulation efficiency of orange essential oil by complex coacervation using whey protein isolate with different polysaccharides. **Journal of Microencapsulation**, p. 165–180, 2018.

RUTZ, J. K. et al. Elaboration of microparticles of carotenoids from natural and synthetic sources for applications in food. **FOOD CHEMISTRY**, v. 202, p. 324–333, 2016.

RUTZ, J. K. et al. Microencapsulation of palm oil by complex coacervation for application in food systems. **Food Chemistry**, v. 220, p. 59–66, 2017.

SÁNCHEZ-NAVARRO, M. M. et al. Scent properties by natural fragrance microencapsulation for footwear applications. **Polymer International**, v. 64, n. 10, p. 1458–1464, 2015.

SANTANA, A. A. et al. Influence of different combinations of wall materials on the microencapsulation of jussara pulp *(Euterpe edulis)* by spray drying. **Food Chemistry**, v. 212, p. 1–9, 2016.

SANTOS, M. B.; DA COSTA, N. R.; GARCIA-ROJAS, E. E. Interpolymeric Complexes Formed Between Whey Proteins and Biopolymers: Delivery Systems of Bioactive Ingredients. **Comprehensive Reviews in Food Science and Food Safety**, v. 17, n. 3, p. 792–805, 2018.

SANTOS, M. G. et al. Microencapsulation of xylitol by double emulsion followed by complex coacervation. **FOOD CHEMISTRY**, v. 171, p. 32–39, 2015.

SARABANDI, K.; MAHOONAK, A. S.; AKBARI, M. Physicochemical properties and antioxidant stability of microencapsulated marjoram extract prepared by co-crystallization method. **Journal of Food Process Engineering**, v. 42, n. 1, p. 1–11, 2019.

SCHMITT, C.; TURGEON, S. L. Protein / polysaccharide complexes and coacervates in food systems. **Advances in Colloid and Interface Science**, v. 167, n. 1–2, p. 63–70, 2011.

SETIOWATI, A. D. et al. Improved heat stability of whey protein isolate stabilized emulsions via dry heat treatment of WPI and low methoxyl pectin: Effect of pectin concentration, pH, and ionic strength. **Food Hydrocolloids**, v. 63, p. 716–726, 2017.

SHADDEL, R. et al. Use of gelatin and gum Arabic for encapsulation of black raspberry anthocyanins by complex coacervation. **International Journal of Biological Macromolecules**, v. 107, p. 1800–1810, 2018.

SHAHIDI, F.; HAN, X. Q. Encapsulation of Food Ingredients. **Critical Reviews in Food**

Science and Nutrition, v. 33, n. 6, p. 501–547, 1993.

SILVA, L. M. R. et al. Quantification of bioactive compounds in pulps and by-products of tropical fruits from Brazil. **Food Chemistry**, v. 143, p. 398–404, 2014.

SOARES, DA S. B. et al. Microencapsulation of sacha inchi oil (Plukenetia volubilis L.) using complex coacervation: Formation and structural characterization. **Food Chemistry**, v. 298, n. February, p. 125045, 2019.

SOLANKI, P.; PATEL, A. In vitro release of l-arginine and cysteine from MCM-48: a study on effect of size of active biomolecules on release rate. **Journal of Porous Materials**, v. 25, n. 5, p. 1489–1498, 2018.

SOUKOULIS, C. et al. Chemical stability and bioaccessibility of β-carotene encapsulated in sodium alginate o/w emulsions: Impact of Ca2+ mediated gelation. **Food Hydrocolloids**, v. 57, p. 301–310, 2016.

TAMM, F. et al. Functional properties of pea protein hydrolysates in emulsions and spray-dried microcapsules. **Food Hydrocolloids**, v. 58, p. 204–214, 2016.

THAKUR, D. et al. Microencapsulation of β -Carotene Based on Casein / Guar Gum Blend Using Zeta Potential-Yield Stress Phenomenon : an Approach to Enhance Photo-stability and Retention of Functionality. **AAPS PharmSciTech**, v. 18, n. 5, p. 1447–1459, 2017.

TIMILSENA, Y. P. Microencapsulation of chia seed oil in chia seed protein-gum complex coacervates. 2016.

TIMILSENA, Y. P. et al. Digestion behaviour of chia seed oil encapsulated in chia seed protein-gum complex coacervates. **Food hydrocolloids**, v. 66, p. 71–81, 2017.

TOGRUL, H.; ARSLAN, N. Production of carboxymethyl cellulose from sugar beet pulp cellulose and rheological behaviour of carboxymethyl cellulose. **Carbohydrate Polymers**, v. 54, p. 73–82, 2003.

TOLSTOGUZOV, V. Some thermodynamic considerations in food formulation. **Food Hydrocolloids**, v. 17, 2003.

TURGEON, S. L.; LANEUVILLE, S. I. Protein + Polysaccharide Coacervates and complexes: From Scientific Background to their Application as functional Ingredients in Food Products. In: **Modern Biopolymer Science**. 1 ed ed. Burlington. p. 327–363.

VAN GINKEL, C. G.; GAYTON, S. THE BIODEGRADABILITY AND NONTOXICITY OF CARBOXYMETHYL CELLULOSE (DS 0 . 7) AND INTERMEDIATES. **Environmental Toxicology and Chemistry**, v. 15, n. 3, p. 270–274, 1996.

VICENTE, J. et al. Microencapsulation of sacha inchi oil using emulsion-based delivery systems. **Food Research International**, v. 99, n. June, p. 612–622, 2017.

VINHA, A. F. et al. **Natural pigments and colorants in foods and beverages**. [s.l.] Elsevier Inc., 2018.

WANG, B. et al. Microencapsulation of tuna oil fortified with the multiple lipophilic ingredients vitamins A , D 3 , E , K 2 , curcumin and coenzyme Q 10. **Journal of Functional Foods**, p. 1–9, 2015a.

WANG, B. et al. Preparation and study of digestion behavior of lactoferrin-sodium alginate complex coacervates. **Journal of Functional Foods**, v. 37, p. 97–106, 2017.

WANG, B. et al. Anchovy oil microcapsule powders prepared using two-step complex coacervation between gelatin and sodium hexametaphosphate followed by spray drying. **Powder Technology**, v. 358, p. 68–78, 2018a.

WANG, B. et al. Encapsulation and Delivery Tool for Hydrophobic Biofunctional Compounds. In: **Role of Materials Science in Food Bioengineering**.. Elsevier Inc., 2018b. p. 236–261.

WANG, B.; ADHIKARI, B.; BARROW, C. J. Highly stable spray dried tuna oil powders encapsulated in double shells of whey protein isolate-agar gum and gellan gum complex coacervates. **Powder Technology**, v. 358, p. 79–86, 2019.

WANG, L. et al. Hydrogen bonding enhances the electrostatic complex coacervation between κ-carrageenan and gelatin. **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, v. 482, p. 604–610, 2015b.

WANG, S. S. S. et al. Study of release kinetics and degradation thermodynamics of ferric citrate liposomes. **Chemistry and Physics of Lipids**, v. 225, n. June, 2019.

WANG, S.; ZHU, F.; KAKUDA, Y. Sacha inchi (Plukenetia volubilis L.): Nutritional composition, biological activity, and uses. **Food Chemistry**, v. 265, n. April, p. 316–328, 2018.

WU, G. et al. Preparation of stable gelatin/sodium carboxymethylcellulose/sodium lauryl sulfonate microcapsules with ultra-thin capsule wall for electrophoretic displays. **Current Applied Physics**, v. 11, n. 3, p. 321–326, 2011.

XUE, J.; NGADI, M. ARTICLE IN PRESS Effects of methylcellulose , xanthan gum and carboxymethylcellulose on thermal properties of batter systems formulated with different flour combinations. **ARTICLE IN PRESS Food Hydrocolloids**, v. 23, p. 286–295, 2009.

YE, S. et al. Preparation of safflower oil microcapsules with gelatin and a novel exopolysaccharide as wall matrix. **Journal of Food Process Engineering**, v. 40, n. 6, p. 1–9, 2017.

YUWEI, L. et al. Investigation into the bioaccessibility and microstructure changes of β-carotene emulsions during in vitro digestion. **Innovative Food Science and Emerging Technologies**, v. 15, p. 86–95, 2012.

ZEEB, B.; MCCLEMENTS, D. J.; WEISS, J. Enzyme-Based Strategies for Structuring Foods for Improved Functionality. **Annual Review of Food Science and Technology**, v. 8, n. 1, p. 21–34, 2017.

ZHANG, K. et al. Synthesis and release studies of microalgal oil-containing microcapsules prepared by complex coacervation. **Colloids and Surfaces B: Biointerfaces**, v. 89, n. 1, p. 61–66, 2012.

ZHANG, Z.; ZHANG, R.; MCCLEMENTS, D. J. Encapsulation of β-carotene in alginate-based hydrogel beads: Impact on physicochemical stability and bioaccessibility. **Food Hydrocolloids**, v. 61, p. 1–10, 2016.

ZHIVKOV, A. M. Electric Properties of Carboxymethyl Cellulose. In: **Cellulose – Fundamental Aspects**. [s.l: s.n.]. p. 197–226.

ZHOU, Q. et al. Evaluation of the physicochemical stability and digestibility of microencapsulated esterified astaxanthins using in vitro and in vivo models. **Food Chemistry**, v. 260, n. 5, p. 73–81, 2018.

CAPÍTULO II

MICROENCAPSULATION OF B-CAROTENE FROM SACHA INCHI OIL BY COMPLEX COACERVATION: CHARACTERIZATION, SIMULATED GASTROINTESTINAL CONDITIONS AND MODELING RELEASE KINETICS

Abstract

This study aimed to evaluated the influence of the pH and protein ratio on the formation of the complex between carboxymethylcellulose (CMC) and whey protein isolated after heat treatment (WPIn). Further, we also encapsulated sacha inchi oil (SIO) containing β-carotene (β-C) using these biopolymers and transglutaminase as a cross-linking agent. The β-C stability under *in vitro* digestion and release in the food model were studied. The ratio 1:6 (CMC/WPIn) at pH 3.5 was the ideal condition for the formation of the complex. The encapsulation efficiency and chemical and morphological characteristics suggested that SIO was microencapsulated. The β-carotene from SIO microcapsules (β-SIO microcapsules) was preserved in vegetable oil when studying the kinetic release in food stimulant, and Rigger-Peppas model indicated that the Fickian diffusion mechanism occurred. *In vitro* digestion simulation the release of the β-C from β-SIO microcapsules was preserved under oral and gastric conditions and the higher release occurred in intestinal digestion. After *in vitro* digestion, the β-C from β-SIO microcapsules presented higher stability (83.37%) and acceptable bioaccessiblility (31.16%). The results suggest that β-SIO microcapsules can be used as delivery to β-C in food systems.

.

Keywords: Carboxymethylcellulose, whey protein isolated, bioaccessibility, particle size, carotenoid.

1 Introduction

Sacha inchi oil (*Plukenetia volubilis* L) is extracted from the seed of the plant belonging to the Euphorbiaceae family also known as “Inca peanut”. This plant is native to the Amazon region that includes the northern part of Brazil, Peru, and Colombia (Li et al., 2018)and its production has promising growth in Asian countries (Guillén, Ruiz, Cabo, Chirinos, & Pascual, 2003). SIO is composed of linolenic acids (omega-3), approximately 50% of its composition, and β-C (0.8 mg / 100g of seed)(HAMAKER et al., 1992; WANG; ZHU; KAKUDA, 2018). The β-C is a carotenoid associated with health benefits such as precursor of vitamin A and antioxidants, prevents non-alcoholic fatty liver disease (CLUGSTON, 2019), acting as an anti-inflammatory, (BOIKO et al., 2017), cardiovascular diseases (GAMMONE; RICCIONI; NICOLANTONIO, 2014), and the risk of developing cancer (Le Goff et al., (2019). Such properties can be reduced when the β-C is exposed to high temperatures, oxygen, and light (da Silva et al., 2017), also, the low solubility of the compound in aqueous media makes its bioaccessibility extremely low (THAKUR et al., 2017).

Encapsulation can be used as an alternative to protect the β-C present in the SIO and consequently reduce its functional loss (FAN et al., 2017; THAKUR et al., 2017). Encapsulation creates a barrier between encapsulated material and wall materials, protecting compounds that are sensitive to external conditions. Encapsulation is defined as a process in which an active ingredient (encapsulated) is surrounded by a coating (wall material), forming microcapsules and protecting the encapsulated against adverse environmental conditions (GAONKAR et al., 2014). Studies have shown that encapsulation improved carotenoid stability and increased bioaccessiblility by controlled release during *in vitro* digestion (FAN et al., 2017).

The complex coacervation technique has been used to encapsulate bioactive ingredients due to its advantages such as high encapsulation efficiency, low concentration of wall materials, low temperature employed, and good controlled release rate (Shaddel et al., 2017). Complex coacervation is the liquid-liquid phase separation event that occurs when biopolymers that have opposite electrostatic charges are mixed under certain specific conditions. The complex coacervation process used for encapsulation runs in three stages: emulsification, coacervation, and cross-linking (ERATTE et al., 2014). Usually proteins and polysaccharides are used as wall materials to encapsulate SIO and carotenoids. Soares, Siqueira, de Carvalho, Vicente, & Garcia-Rojas, (2019) encapsulated SIO to preserve omega 3 using eggalbumin and sodium alginate as wall material; Rutz et al., (2016) encapsulated β-C contained in palm oil using

carboxymethylcellulose and chitosan as an encapsulant. However, there are still no studies relating to the encapsulation by complex coacervation of the β-C present in the SIO.

In this study, CMC and WPIn were used as wall materials in the encapsulation process. WPI is used in the food industry due to its physicochemical properties (e.g. gel formation and stabilization of emulsions), exhibits a positive charge below its Isoelectric Point (pI) (pH 4.1-5.2).When WPI is heated, aggregates are formed, improving its stability when it is part of an electrostatic complex (JONES; DECKER; McCLEMENTS, 2010). CMC is biodegradable, it is an anionic linear polymer, as a result of its considerable ionized carboxylic groups, obtained by grouping carboxymethyl groups to the native cellulose molecule (BARBUCCI; MAGNANI; CONSUMI, 2000). Studies demonstrate the use of CMC and WPI to microencapsulate flavor, vegetable oils, and β-C through complex coacervation (KOUPANTSIS; PAVLIDOU; PARASKEVOPOULOU, 2016; ROJAS-MORENO et al., 2018; RUTZ et al., 2016).

This study aimed to encapsulate of β-carotene from sacha inchi oil by complex coacervation using CMC and WPIn as wall materials, characterize the capsules and evaluate the stability of the β-C under digestion *in vitro* and release kinetics in food models.

2 Materials and methods

2.1 Materials

Sacha inchi oil (*Plukenetia volubilis L.*) was acquired from the local market in Lima, Peru. Whey Protein Isolate (purity of 90%) was purchased from Glanbia Nutritionals, carboxymethylcellulose (419303), α-amylase (A3403), porcine pepsin (P6887), pancreatin from porcine pancreas (P7545), porcine bile extract (B3883) and β-C synthetic, ≥93% (UV), powder (C9750) were obtained from Sigma- Aldrich® Company (St. Louis, USA)(419303), Transglutaminase from *Streptomyces mobaraensis* (85-121U/g) were kindly provided by Ajinomoto® (São Paulo, Brazil).Reagents utilized were of grade P.A. and bought from VETEC® Ltd. (Rio de Janeiro, Brazil). For the preparation of the solutions and experiments was used ultrapure water with a conductivity of 0.05 µS / cm (Gehaka, Master-P&D, Brazil).

2.2 Methods

2.2.1 Preparation of the CMC and WPIn solutions

The biopolymers were weighed on an analytical balance (B-TEC-210, Tecnal, Brazil) for the preparation of solutions containing a fixed concentration of 0.1% (w/w). The CMC was dissolved for 24 h at 25 ºC in in ultrapure water using a magnetic stirred (NT101, Novatecnica, Brazil). For preparation of the WPIn, the solutions of WPI were spilled in a 40 mL glass tube fitted with plastic screw-caps. These solutions were then immersed in a water bath (85 °C) for 15 min. Consecutively were cooled at room temperature for 15 min. They were immersed in a water bath in a room temperature for 2h. Subsequently the samples were placed in an overnight refrigerated storage, as reported by Jones et al., (2010).

2.2.2 Effect of the pH on the formation of CMC/WPIn complex

2.2.2.1 ζ-Potential

The ζ-potentials as a function of the pH (2.0 – 4.5) of the biopolymers were determined using a Zetasizer Nano ZS90 (Malvern Instruments, UK) coupled with an MPT-2 autotitrator (Malvern Instruments, UK). The pH was titrated using 0.5M NaOH, 0.25M HCl, and 0.025M HCl solutions. The pH varied by 0.5unit increments with a confidence interval of ± 0.1 unit.

The Strength of the Electrostatic Interaction (SEI) between oppositely charged polyelectrolytes can be estimated as reported by (YUAN et al., 2017), given by equation (1):

$$\text{SEI } (mV^2) = ZP_1 \text{ x } ZP_2 \quad (1)$$

where ZP_1 and ZP_2 are the measured □-potential of both polymers at each pHs.

2.2.3 Effect of the protein ratio on the formation of the CMC/WPIn complex

To determine the ideal ratio to the formation of the CMC/WPIn complex, CMC and WPIn were mixed at different ratios: 10:1, 08:1, 06:1, 02:1, 1:1 1:2, 1:6, 1:8, 1:1 (polysaccharide/protein) (v/v). After mixing the biopolymer solutions at varying ratios, the pH of the mixture was adjusted to 3.5 (determined in topic 2.2.2.1) using NaOH and HCl solutions, with a pHmeter (mPA210, Tecnopon, Brazil). Individual biopolymers and the ratios were measured byturbidity. Turbidity was measured at a wavelength of 400nm on spectrophotometer (Biomate 3S, Thermo Fisher Scientific, USA) calibrated with ultrapure water to 100% transmittance (%T). Turbidity was defined as 100-%T. The □-potentials of the ratios were measured using a Zetasizer Nano ZS90 (Malvern Instruments, UK) as described in the topic (2.2.2.1).Samples were read in triplicate at 25 °C, the methodology as reported by (DIARRASSOUBA et al., 2015).

2.2.4 Preparation of the β-SIO microcapsules

The β-SIO microcapsules were produced as reported by (RUTZ et al., 2016),with a few modifications. A ratio of 1:6 (CMC: WPIn) at pH 3.5 was used to prepare the capsules, these conditions were determined in topics (2.2.2 and 2.2.3). The CMC: WPIn microcapsules were tested using a solution with total concentrations of 0.5, 1.0 and 2.0% (w/w), and different core/wall concentrations were tested (2:1, 1:1 and 1:2). The primary oil-in-the water (O/W) emulsion consisting of SIO (0.0875-1.4g) and WPIn was formed at 13.600 rpm for 5 min using an ultraturrax (IKA, T25D Ultraturrax, Germany). The CMC was added, and the mixture was homogenized for 3 min using a magnetic stirrer (Novatecnica, model NT101, Brazil).The pH of the solution was adjusted to 3.5 with acetic acid 20% (v/v) using a pH meter (mPA-210, Tecnopon, Brazil). The temperature of the solution was be reduced to 10ºC in an ice-water bath for 1h (PENG et al., 2014) for complete precipitation of the formed microcapsules. Posteriorly, the supernatant was removed and 5.0 mL of the transglutaminase solution (2.5% w/w) was added, the microcapsules were shaking (Tecnal, model TE-424, Brazil) at 100rpm for 3h at 25ºC to induce a cross-linking (LV et al., 2014).Subsequently the samples were frozen with liquid nitrogen and freeze-dried (Terroni, Enterprise I, Brazil) for 48 h.

2.2.5 **Characterization of the β-SIO microcapsules**

2.2.5.1 **Determination of standard curve and encapsulation efficiency (EE)**

To determine de standard curve, the β-C synthetic powder was dissolved in hexane and formulated into 0.0 to 3.2 mg/mL standards solutions, and then there were measured by spectrophotometer (Biomate 3S, Thermo Fisher Scientific, USA) at 450 nm. The standard curve was drawn and its equation is y=0.0137x (R^2 = 0.98).

The encapsulation efficiency was adapted from (JAIN et al., 2015) with slight modifications. 100 mg of the β-OSI microcapsules were weighed (B-TEC-210, Tecnal, Brazil) and was added 10 mL of hexane. The sample was placed into a water bath 20°C for 10 min by ultrasonic bath (2510, Soni-tech, Brazil) and the samples were incubated in the shaker (TE-424, Tecnal,Brazil) for 2 h at 25ºC. The sample was homogenized on a Vortex (AP 56, Phoenix, Brazil) and centrifuged (Digicen 21R, OrtoAlresa, Spain) at 8000 rpm for 15 min. The supernatant was collected and measured in spectrophotometer (Biomate 3S, Thermo Fisher Scientific, USA). The theoretical β-C content and the final amount of β-C was used to calculate the EE given by equation (2).

$$EE(\%) = \frac{CT\ (\%)}{CI(\%)} x100 \qquad (2)$$

where the *CT* is the final amount of β-C in microcapsules and *CI* is the theoretical β-C in the initial process of microencapsulation.

2.2.5.2 **Particle size of biopolymers and the β-SIO microcapsules**

The hydrodynamic diameter (d.nm) of the WPI, WPIn, CMC and fresh β-SIO microcapsules at pH 3.5 were monitored by Dynamic Light Scattering (DLS) technique using a Zetasizer (Malvern Instruments, Nano-ZS, UK) equipped with a He– Ne laser at a wavelength of 632.8 nm and fixed detection angle of 90°.The proteins solutions were filtered through a 0.45µm low binding syringe to remove proteins aggregation and other impurities. Samples were placed in glass cuvettes and read at 25ºC.

2.2.5.3 **Fourier transform infrared spectroscopy (FT-IR)**

The FT-IR spectra of the dried samples (Microcapsules,CMC, WPIn, and SIO) were obtained. The analyses were performed with an FT-IR spectrometer (Ver-tex 70, Bruker, Germany) read in the range of 4000–400 cm^{-1}.

2.2.5.4 **Photolytic degradation of the microcapsules**

The UV photo-stability of the β-C in β-SIO microcapsules under radiation was measured as adapted by Thakur et al., (2017). The lyophilized microcapsules and SIO were placed at a distance at 15 cm under a UV-visible light (BLB, 15 W, T8) with a wavelength of 368nm.At the determined period (0,1,2,3,4, and 5 days) the samples were withdrawn. 0.1 mg of the sample was extracted in the similar way they are extracted in EE (section 2.2.5.1) and read in the spectrophotometer in a 450 nm. The degradationof β-C was expressed as C/C_0, where C_0 and C is the β-C initial concentration and the concentration at any time during storage, respectively.

2.2.6 **Release kinetic of the β-C in SIO microcapsule**

Kinetic release was performed according to (REZAEINIA et al., 2019), 200 mg of the samples were weight in analytical balance (B-TEC-210, Tecnal, Brazil), added to 10 mL of the food simulant and mixed for 95 rpm at 25ºC using the shaking (TE-424, Tecnal, Brazil). Soybean oil was chosen as the simulant medium of fatty foods according to the EU commission regulation (COMMISSION REGULATION 10/ 2011 EU, 2011). Aliquots of the 200µL were collected and were analyzed by spectrophotometer (Biomate 3S, Thermo Fisher Scientific, USA) at 450 nm. To explain the β-C release profile, the release kinetics in food models were fitted to Higuchi, First-Order, and Rigger-Peppas empirical models (REZAEINIA et al., 2019).

2.2.6.1 **Optical microscopy**

An aliquot of freshβ-SIO microcapsules was placed between the lamina and cover-slip and taken to the optical microscope (K220, Kasvi, Brazil) coupled to an Moticam camera (5MP,Kasvi, Brazil) that was viewed at 40x magnification with immersion oil.

2.2.7 *In vitro* digestion of the β-C in β-SIO microcapsules

The *In vitro* digestion of the β-SIO microcapsules was measured using a model that imitates the gastro-intestinal tract (GIT)in three stages: oral, gastric, and intestinal. This study used the INFOGEST *in vitro* digestion protocol (BRODKORB et al., 2019).

Firstly 0.7g of the lyophilized microcapsule was weighed, added to 0.56 mL of the prewarmed simulated salivary fluid (SSF) containing 0.23 µL of salivary amylase (75 U/mL in final oral solution) solution, and added to 0,067 mL of water to achieve 1/1 (w/w). For a pasty-like consistency 2 mL of ultra-pure water was added in the process. The pH of the mixture was adjusted to 7.0 with NaOH and HCl 6M using pHmeter and stirred at 95 rpm for 2 min at 37ºC using the shaking.

The gastric phase has been programmed with 1.4 mL of the prewarmed simulated gastric fluid (SGF) containing 0.07 mL of pepsin solution (2.000U/mL in final gastric solution) and mixed with the oral digested mixture. The pH of the mixture was adjusted to 3.0 with NaOH and HCl 6M using pHmeter and stirred at 95 rpm for 2 h at 37ºC using the incubate shaker.

In the final gastric digestion, 2.8 mL of prewarmed simulated intestinal fluid (SIF) was added into the mixture, with 0.7 mL of the pancreatin solution (100U/mL in final intestinal volume),0.5mL of the bile extract solution (10mM), 5,6µLof 0.3 M $CaCl_2$.The pH of the mixture was adjusted to 7.0 with NaOH and HCl 6M pHmeter (mPA210, Tecnopon, Brazil) and stirred at 95 rpm for 2 h at 37ºC using the incubate shaker. Aliquots (120µL) of gastric and intestinal juice were taken at 0, 15, 30, 60, and 120 minutes. After the withdrawal of each aliquot, 120 µL of gastric or intestinal juice was added to continue the digestion process. The quantity of β-C released was calculated as described in the topic (2.2.5.1).

2.2.8 Bioaccessiblility and stability of β-C in β-SIO microcapsules

After the simulation of the samples through the GIT, 5mL of digesta from the small intestine was collected and centrifuged at 18000 rpm for 30 min at 4 °C. Aliquots of the 200 µL were collected and were denominated micelle. The β-C in the digesta and micelle phase was read by spectrophotometer as described 2.2.5.1.

The stability and bioaccessibility of the β-SIO microcapsules after digestion were measured according to a method described by (LIU et al., 2018). The stability (S*) of β-C was defined as the fraction that remained in a non-transformed state in the small intestine after *in vitro* digestion model, given by equation (3).

$$S * = \frac{C_{Digesta}}{C_{Initial}} x100 \quad (3)$$

where $C_{Digesta}$ and $C_{Initial}$ are the β-C concentrations in the total digesta collected after the small intestine phase and in the initial samples, respectively.

The bioaccessiblility(B*) of the β-C was defined as the fraction that was released from the food matrix and solubilized in the mixed micelle phase after digestion, given by equation (4).

$$B * = \frac{C_{Micelle}}{C_{Digesta}} x100 \quad (4)$$

where $C_{Micelle}$ is the β-C concentration in the micelle fraction.

2.2.9 Statistical analysis

All experiments were conducted in triplicate, and the results were displayed in ± standard deviation. The statistical analysis was performed using Origin® Pro 9.0 (OriginLab, Northampton, USA). The Kolmogorov-Smirnov normality test was performed for the populations. After confirming normality for all populations, one-way analysis of variance (ANOVA) was applied to determine the existence of significant differences between populations. Significant differences at a level of significance of α = 0.05 were identified by Tukey´s test.

3 Resultsand discussion

3.1 Formation of the WPIn/CMC complex

3.1.1 Effect of the heat treatment the WPI

The particle size of the WPI and WPIn were 336.07 nm and 157.2nm, respectively. The size of WPI (heated protein and unheated) vary with the pH and temperature. In this study the WPI used has a pI 4.7, and the particle size was measured in pH 4.0. The formation of large particles in the proteins without heating can be attributed to the pH being close to the pI of the protein, a weak electrostatic repulsion between the protein molecules, forming larger aggregates (HARNSILAWAT; PONGSAWATMANIT; McCLEMENTS, 2006). The thermal treatment of

WPI results in changes in the interactions and conformations. The protein molecules unfold and expose hydrophobic group, which promote intermolecular non-covalent interactions (hydrogen bonding and hydrophobic interactions), this can be used to formed micro or nanoparticules, and may induce the protein denaturation and then result on aggregation (JONES; DECKER; McCLEMENTS, 2010; LV et al., 2019). A similar result was found by (JONES; DECKER; McCLEMENTS, 2010) when heated β-lactoglobulin (85°C/15 min), the protein has 177.3 nm. In another study, Jones, Decker, & McClements, (2009) observed that the particle size of the β-lactoglobulin before and after heat treatment (85° C / 15 min) was 400 nm and 177 nm, respectively.

The polydispersity index (PdI) is an important indicator, is regularly used to express the size distribution pattern of the particles. The PdI range is from 0 to 1. Lower PdI values represent the more homogeneous size distribution (MCCLEMENTS, 2014). The PdI of the WPI and WPIn were 0.5 and 0.27, respectively. This demonstrated the importance of preheating in obtaining more homogenous particles from WPI (BAGHERI et al., 2013) study the WPI before and after thermal treatment (60°C/ 30 min), PdI of the WPI and WPIn (after thermal treatment) were 0.4 and 0.36, respectively.

3.1.2 Strength of the electrostatic interaction and effect of the protein ratio on the formation of the CMC/WPIn complex

The ζ-potential and strength of the electrostatic interaction (SEI) between oppositely charged of the biopolymers was shown in Fig 1.The CMC showed a negative charge at all pH studied, due to the presence of carboxylic groups in its structure (Javanbakht &Shaabani, 2019a). In the WPIn, a positive charge is observed at pHs below the pI and slowly become negative when it comes close to pH 4.7 close to the pI of the protein verified in ζ-potential, which agrees with the reported value in the literature (WEINBRECK et al., 2003). The highest SEI value was observed at pH 3.5 where the values of zeta potential for biopolymers were WPIn (14.21 mV) and CMC (-19.07 mV). At this pH there was a greater interaction between biopolymers, with a high capacity aggregation between CMC/WPIn.

The effect of the protein ratio on the formation of the CMC/WPIn complex was demonstrated in Fig. 2. The turbidity of the ratios was observed in Fig. 2 (A), and theζ-potentials in Fig. 2 (B), both at pH 3.5.

In Fig. 2B, the charge of WPIn was 20.93mV and CMC -22.57mV. The complexes showed negative charge density in the concentrations (10: 1; 8: 1; 6: 1; 2: 1). The electrical

charge of the CMC was negative, suggesting that saturation of the polysaccharide occurred to the colloidal particles formed.

At the ratios of (1:1 and 1:2) (CMC/WPIn), the zeta potential was negative, due to the fact that polysaccharide has a higher charge density than that related to the molar weight of the protein. In the ratio 1:6 (CMC/WPIn) the zeta potential became close to neutrality (0.81mV). This occurs because the WPIn is positively charged and by adding more protein to the system the zeta potential became positive. The ratios 1:8 and 1:10 demonstrated positively charged values showing that there was an excess of WPIn molecules in the complex, and the protein had a higher molecular weight in comparison with the CMC.

As WPIn concentrations increased, the potential zeta changed from negative to positive. With low concentrations of proteins in the CMC/WPIn mixtures, the negatively charged polysaccharide dominated the zeta potential value. The mixture of the biopolymers had a positive charge when there was an excess of WPIn. A similar result was described by Tavares & Zapata Noreña(2019) in complexation between WPI and chitosan.

These results obtained for the ζ-potentials and turbidity in the function of the CMC/WPIn ratios demonstrated that ratio 1:6 CMC/WPIn was appropriate for the formation of the complex between CMC and WPIn. The charge approached the neutrality and the higher turbidity, indicating a better balance between the charges of the biopolymers.

3.1.3 Effect of the pH on the formation of the CMC/WPIn complex

To confirm the optimal pH of the ratio 1:6 (CMC/WPIn), the zeta and turbidity in the function of the pH (2.0-6.0) were measured, and the results were shown in Fig.3. The ζ-potentials and turbidity were demonstrated in Fig.3A and 3B.

Fig. 3A shows the effect of pH variation on the turbidity of CMC/WPIn complexes. In the pH value range (4.5, 5.0, 5.5 and 6.0) the turbidity values were 28.1, 18.3, 10.5 and 6.0 respectively, at this pH, above the pI, the protein is negatively charged, and for this reason it did not occur complexation of biopolymers which justifies the low turbidity. The highest turbidity observed was at pH 3.5 and values were 73.0, this indicates the point occurred higher electrostatic interaction.

The ideal pH for the formation of the complex between CMC/WPIn was defined through the zeta potential at the pH evaluated Fig 3 (B). At pH 4.0-6.0 the electrical charge of the complex (1:6) CMC/WPIn was negative, not favoring the formation of coacervate complexes. This occurs because in this pH range the biopolymers have a negative charge density. At pH

2.5, 3.0 and 3.5 the complex showed positive charges of 4.56, 6.56 and 1.13 mV respectively. In this pH range, occurred the formation of the complexes due to the higher positive charges density of WPIn, because it was below the protein's isoelectric point. Electrostatic forces are predominant in complex coacervation, and pH has a fundamental factor in this interaction, as it affects the degree of ionization of the amino and carboxylic functional side groups transported by biopolymers. (LV et al., 2014). The electrostatic interaction occurred at pH 3.5, where the sum of charges approached neutrality, which shows a balance of charges between biopolymers. In a similar study Rojas-moreno et al.,(2018) studied the interaction between WPI-CMC at pH 3.0 to form complexes between biopolymers.

3.1.4 **Encapsulation efficiency (EE)**

The composition of the β-SIO microcapsules and your EE produced with different proportions of core and wall material was shown in Table 1.The EE values range from 68.92% (S4) to 96.21% (S8), treatments S8, S2 and S3 showed the highest EE% and did not show significant differences ($p < 0.05$) and their values are respectively 96.21%, 93.54% and 91.69%. The samples had different values of core material/wall S8 and S2 (1: 1) and S3 (1:2).

In sample S3, the wall material is superior to the core material, therefore, there is a possibility that this treatment has encapsulated all the core material, resulting in a high EE%. Samples S8 and S2 have the possibility that the core material is not fully encapsulated and present on the surface of the microcapsules during the lyophilization process. High EE% values were found by studies produced by Rutz et al., (2016) with 95% EE% for encapsulation of β-C in palm oil by complex coacervation.

Samples S4, S5, S6, and S7 have the lowest EE% values (Table 1). These were prepared with different core ratios/wall S4 and S7 (2: 1), S5 (1: 1), and S6 (1: 2) and intermediate biopolymer concentrations between 1% (S4, S5, S6) and 2% (S7). It is assumed that an insufficient amount of wall material was free to encapsulate the core material, succeeding in a large amount of free core and, consequently, lost in the complex coacervation process (SOARES et al., 2019).

With this, the treatment chosen was the S3 experiment shown in Table 1, with EE% of 91.69% and core: wall ratio of 1: 2. These results are in accordance with the literature, where Rojas-moreno et al., (2018) found an EE% of 83, 94% when encapsulating orange essential oil using CMC/WPI biopolymers as a complex coacervation wall material.

3.2 Characterization of β-C present on the β-SIO microcapsules

3.2.1 Morphology and particle size of microcapsules

Fig. 4 demonstrates the microscopic image of β-SIO microcapsules using the CMC: WPIn biopolymers as the wall material and the transglutaminase cross-linking agent. The microscopic structure of β-C microcapsules is formed by the ratio 1: 2 core/ wall material. The results show that the morphology of the coacervate capsules are spherical and intact, this result is in agreement with what was reported by Shaddel et al., (2017) who found continuous and regular microspheres in the process of microencapsulation of black raspberry anthocyanins by complex coacervation.

The particle size of β-SIO microcapsules, CMC and WPIn showed 2746nm, 918 nm and 157.2 nm respectively. Usually, microcapsules are particles that have a size between 1 to 1000 µm (YE; GEORGES; SELOMULYA, 2018). The PdI microcapsule was 0.16 this indicated the particle size is closer to homogeneity. The variation in particle size and its characteristics may be due to changes in pH, ionic strength, concentration, and proportion of the type of core ingredient, charge level, and type of matrix. Also, the rate of agitation speed, cooling, and drying process can change the particle size Jones et al., (2009). Thakur et al., (2017) found similar sizes in β-C capsules using casein and guar gum using complex coacervation, and found particle size of 160 – 180µm.

3.2.2 Chemical characterization of the β-SIO microcapsules

The FT-IR spectra of the biopolymers and the β-SIO microcapsules (sample S3) at pH 3.5 were demonstrated in Fig.5. In the WPI_n spectrum was identified bands corresponds to amide I (1625-1750cm^{-1}), amide II (1475-1575cm^{-1}) and amide III (1225-1425cm^{-1}). The band at 1625cm^{-1} witch refers to the stretching of the C=O(Dai et al., 2016), the band at 1540cm^{-1} refers to the stretching the NH bond or by angular deformation of the NH bond(Barth, 2007), the band 1423 cm^{-1} is attributed to the stretching of the C-N and N-H groups (Bastos, Vicente, Santos, Carvalho, & Garcia-Rojas, 2020). Similar result was observed by(K. Li et al., 2019) in WPI spectrum by FT-IR.

In CMC spectrum the bands at 2930cm^{-1} refers to anaxial strain C-H. The bands at 1610 cm^{-1} and at 1421 cm^{-1}, refers to asymmetrical and asymmetric vibrations of the carboxylic

groups present in the polysaccharide. The band at 1048cm^{-1} correspond to C-O stretching. Studies observed similar results in CMC FT-IR spectrum (Barbucci et al., 2000; Koupantsis et al., 2016; S. S. Li et al., 2020).

In SIO spectrum the bands at 3010 cm^{-1}, 2927 cm^{-1}, 2854 cm^{-1} 1745 cm^{-1}, 1165 cm^{-1}, and 798 cm^{-1} were observed. The band at 3010 cm^{-1} correspond to vibrational stretching of the cis-olefinic double bands (=CH), the band at 2927 cm^{-1} refers to asymmetric vibrations and band at 2854 cm^{-1} to symmetrical methylene vibrations (CH of CH_2). The band at 1745 cm^{-1} correspond to the ester absorption vibrations for triacylglycerols (C = O of ester) present in oils with a high degree of unsaturation such as SIO that is rich in polyunsaturated fatty acids (Soares et al., 2019; Vicente et al., 2017). The band at 1165cm^{-1} refers to the stretching vibration of the (C-O group and the vibration of the CH_2). The band at 798 cm^{-1} is assigned to (=C-H) when folding the plane of the alkene (Soares et al., 2019; Vicente et al., 2017).

The spectrum of the microcapsule showed the WPI_n characteristic bands (1625 and 1540 cm^{-1}), CMC carboxyl group at 1048cm^{-1} (COO^-). The presence of amino and carboxyl groups in the spectrum suggests that there was an interaction between CMC and WPIn pointing to the formation of coacervate complexes (Soares et al., 2019). The bands at 2927 cm^{-1}, 2854 cm^{-1} and 798cm^{-1} were bands characteristics to the SIO spectra and suggesting that SIO was encapsulated. Similar results were found in the literature when demonstrated SIO unencapsulated and after encapsulation using emulsion and complex coacervation techniques (Guillén et al., 2003; Soares et al., 2019; Vicente et al., 2017)

3.3 Stability of the β-C present in microcapsules under UV radiation

The stability of the β-C present in SIO before and after encapsulation under radiation was observed in Fig. 6. The samples were exposed to a light at 5º C for 5 days. The results show that β-C in unencapsulated SIO was completely degraded, while the β-C in encapsulated SIO was preserved in 70%, both after exposed for 4 days. This suggesting that microcapsules are effective in protecting β-C from oxidation through light. Chen & Zhong, (2015) investigated the stability of the microemulsions prepared with Tween® 20, lecithin, and β-C dissolved in peppermint oil obtained 71% of this carotenoid was protect. A similar result was observed by Thakur et al.,(2017), the dried microcapsules prepared by complex coacervation using casein and guar gum demonstrated the rate of degradation of free β-C was higher up to 3 days as compared with microcapsules.

3.4 **Release of the β-C in food simulant**

The kinetic release of the β-C in β-SIO microcapsules was shown in Fig. 7. The analysis was made in sample S3 (higher EE), and the sample was simulated in soybean oil. The maximum SIO release was observed at 9 min with 51.1% of the release, at 51 min the rate stops growing and remains constant until the end of the test. The results of the present study demonstrated that microcapsule was preserved in vegetal oil as a food simulant.

To determine the mechanism of the release of the β-C in β-SIO microcapsules, the release profile within the soybean oil system was fitted with different kinetic equations. Higuchi, First-Order, Zero-Order, and Rigger-Peppas as a mathematical model were used to evaluate the release behavior of the β-C in β-SIO microcapsules. The constants and the coefficient of determination (R^2) were shown in Table 2. Concerning the R^2 values, the Rigger-Peppas was an appropriate model for explaining the release kinetics of β-C in SIO an R^2 over 0.99.

In the Rigger-Peppas mathematical model, the values of n point to the following release mechanisms: if $n \leq 0.43$, the mechanism that controls the release will be Fickian diffusion (case I transport); If the value of n is between$0.43 \leq n < 0.85$ (non-Fickian or anomalous transport), this indicates that the release mechanism will be diffusion and swelling, and $n<0.85$ indicates that the release mechanism is zero order (transport case II)(RITGER; PEPPAS, 1987; SIEPMANN; PEPPAS, 2012). As noted in Table 2, the n value found for the Rigger-Peppas mathematical model was $n \leq 0.43$,which is confirmed in literature for spherical molecules, and mainly follow the Fickian release pattern (RITGER; PEPPAS, 1987). In the diffusion process, the material to be released can diffuse between the particle matrix and the surrounding environment. The particle matrix may remain intact throughout this process, or its properties may change, resulting in erosion, fragmentation or dissolution. The rate of release of an active ingredient from the particles depends on its relative solubility in the particle matrix and the surrounding liquid and its diffusion coefficient through the matrix (SIEPMANN; PEPPAS, 2012). Studies demonstrate the controlled release of active ingredients by Fickian diffusion, (REZAEINIA et al., 2019) observed Fickian diffusion using the Rigger-Peppas mathematical model in *Mentha longifolia L.* essential oil.

3.5 *In vitro* digestion

The study of the release of β-C in oil from microcapsules(S3) at different time intervals is shown in Fig. 8. The capsule was submitted to oral (2 min), gastric (2h) and intestinal (2h) conditions.

The pH of the SSF was 7.0, and as shown in section 3.3, at pH 6.0, the charges of the biopolymers are negative and do not form coacervate complexes, there is a repulsive electrostatic force between the protein and the polysaccharide(Santos et al., 2018). Therefore, given the exposure of the capsules to the enzyme and pH (7.0), the hypothesis is that a small release of β-C.

During the gastric phase (2h), the highest percentage of release of β-C present was 16.83% in 120 min, which demonstrates that the capsule protected 83.17% of the amount of β-C present in β-SIO microcapsules. At the beginning of the gastric phase, there was a release of around 9.35% in the first 15 minutes and there was a progressive increase up to 120 minutes. Pepsin acts in the hydrolysis of proteins during the gastric phase (DONHOWE; KONG, 2014), however, with the results presented in Fig.6, it is possible to observe that the microcapsules did not completely rupture. Such release may have occurred through the enzymatic hydrolysis of some proteins present in the WPI. WPI being made up of β-lactoglobulin (50–55%) and α-lactalbumin (20–25%)(SANTOS; DA COSTA; GARCIA-ROJAS, 2018). In the literature, it was observed that a complete hydrolysis of α-lactalbumin was found during the gastric phase, however,β-lactoglobulin remained present even after 60 min of incubation (MALAKI NIK; WRIGHT; CORREDIG, 2011). Also, it has been reported that β-lactoglobulin can interact with β-C with strong non-covalent interactions, this suggests that WPI can act as transported hydrophobic compounds during digestion (Iddir et al., 2019). Changes in the conformation of WPIn (heat-induced aggregation) can influence protein digestibility, as this alters the enzymatic cleavage sites of protein molecules (SAH; MCAINCH; VASILJEVIC, 2016). Regarding CMC, at low pH (SGF pH 3) this polysaccharide is stable and resistant to proteases (JAVANBAKHT; SHAABANI, 2019b). In addition, there is a shrinkage of this polysaccharide in acidic pH, which may delay the release of the oil. (BARKHORDARI; YADOLLAHI; NAMAZI, 2014). Studies related to the resistance of CMC at low pH and gastric juice, as well as its use for the release of pharmaceutical products (BARKHORDARI; YADOLLAHI; NAMAZI, 2014). Resistance to acidic pH may explain why the low release rate of β-C and the conservation of capsules in the gastric phase.

The release of β-C in the intestinal phase (2h) occurred more quickly when compared to the gastric phase. As shown in Fig. 8, the release increased throughout its test, reaching a maximum of 92.28% in 120 minutes, preserving only 7.72% of the core material. The SIF has a pH of 7.0 and enzymes to simulate the intestinal environment. At this pH, the wall material breaks, and the capsules are destroyed, thus resulting in the release of the core material. Regarding WPIn digestion, it starts in the stomach by the action of the enzyme pepsin, and in the intestine, it is continued by pancreatic and intestinal proteases, such as trypsin, chymotrypsin, and membrane peptidases (SAH; MCAINCH; VASILJEVIC, 2016). In a basic medium, ionization of groups of carboxylic acids (carboxylic groups in the CMC chains) occurs, this factor increases the interaction of the polysaccharide with the aqueous medium, and thus, its release rate. Besides, the action of the pancreatin enzyme acts on the wall material (CMC), facilitating the release of β-C (LIM; NYAM, 2016).Similar results were found by (Sathasivam, Muniyandy, Chuah, &Janarthanan, 2018), which encapsulated red palm oil using sago carboxymethylcellulose, where the maximum oil retention found in the intestine was 20%.

3.6 Stability and bioaccessibility of the β-C from the β-SIO microcapsules

The results for stability (S *) and bioaccessibility (B *) of β-C after *in vitro* digestion were 83.37% and 33.14% respectively. The bioavailability of β-C is caused by physiological conditions of the gastrointestinal tract (e.g. enzymes, pH, and intestinal mucus barrier) and environmental conditions (e.g. pH, light, heat, oxygen)(Bao et al., 2019). Before being absorbed, β-C must be released from its food matrix and dissolved in lipid droplets and under the influence of digestive enzymes, pH, bile and gastric peristalsis, this carotenoid is incorporated into mixed micelles, thus allowing its absorption in the intestine (IDDIR et al., 2019). Similar result was found by Ilyasoglu & El, (2014) who found a value of 36.25% bioaccessibility in fish oil nanocapsules. Other studies report similar values of up to 50% for bioaccessibility in food emulsions of canola oil with sodium alginate (Soukoulis, Cambier, Hoffmann, & Bohn, 2016). Fan et al., (2017), found in different emulsions formed by WPI, bioaccessibility values of 61.34%for the study of alginate and sodium caseinate microcapsules for microencapsulate β-C.

4 Conclusion

In this study, the best ratio and pH for complex formation were 1:6 (WPIn/CMC) and 3.5, respectively. The SIO capsules demonstrated good encapsulation efficiency and were obtained on micro-scale. The encapsulated of the SIO was suggested by the chemical and morphological characteristics. The β-C in β-SIO microcapsules was preserved in food simulant and can be added to in oily foods food. The Rigger-Peppas model demonstrated that Fickian diffusion mechanism occurred during release in food simulant. The β-SIO microcapsules showed resistance under exposure to oral and gastric conditions *in vitro*, and release in the intestine contributing to β-C absorption. After digestion, β-C in SIO presented higher stability, and acceptable bioaccessibility. The WPI after heat treatment and CMC showed potential for used as wall materials in SIO microencapsulation by complex coacervation, the β-C was preserved, and the microcapsules showed potential use as delivery systems for active ingredients.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgment

The authors thank the CNPq, Brazil (312973/2018-7); CAPES, Brazil (Código 001) and FAPERJ, Brazil (E-26/202.975/2017) for the financial support.

5 **References**

Bagheri, L., Madadlou, A., Yarmand, M., & Mousavi, M. E. (2013). Nanoencapsulation of date palm pit extract in whey protein particles generated via desolvation method. *Food Research International*, *51*(2), 866–871. https://doi.org/10.1016/j.foodres.2013.01.058

Bao, C., Jiang, P., Chai, J., Jiang, Y., Li, D., Bao, W., … Li, Y. (2019). The delivery of sensitive food bioactive ingredients: Absorption mechanisms, influencing factors, encapsulation techniques and evaluation models. *Food Research International*, *120*(February), 130–140. https://doi.org/10.1016/j.foodres.2019.02.024

Barbucci, R., Magnani, A., & Consumi, M. (2000). Swelling behavior of carboxymethylcellulose hydrogels in relation to cross-linking, pH, and charge density. *Macromolecules*, *33*(20), 7475–7480. https://doi.org/10.1021/ma0007029

Barkhordari, S., Yadollahi, M., & Namazi, H. (2014). PH sensitive nanocomposite hydrogel beads based on carboxymethyl cellulose/layered double hydroxide as drug delivery systems. *Journal of Polymer Research*, *21*(6). https://doi.org/10.1007/s10965-014-0454-z

Barth, A. (2007). Infrared spectroscopy of proteins. *Biochimica et Biophysica Acta - Bioenergetics*, *1767*(9), 1073–1101. https://doi.org/10.1016/j.bbabio.2007.06.004

Bastos, P. H. L., Vicente, J., Santos, C. dos C. H., Carvalho, M. G. de, & Garcia-Rojas, E. E. (2020). Encapsulation of black pepper (Piper nigrum L.) essential oil with gelatin and sodium alginate by complex coacervation. *Food Hydrocolloids*, *102*(December 2019). https://doi.org/10.1016/j.foodhyd.2019.105605

Boiko, Y. A., Kravchenko, I. A., Shandra, A. A., & Boiko, I. A. (2017). Extraction, identification and anti-inflammatory activity of carotenoids out of Capsicum Anuum L. *Journal of HerbMed Pharmacology*, *6*(1), 10–15.

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., … Recio, I. (2019). INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nature*

Protocols, *14*(4), 991–1014. https://doi.org/10.1038/s41596-018-0119-1

Chen, H., & Zhong, Q. (2015). Thermal and UV stability of β-carotene dissolved in peppermint oil microemulsified by sunflower lecithin and Tween 20 blend. *Food Chemistry*, *174*, 630–636. https://doi.org/10.1016/j.foodchem.2014.11.116

Clugston, R. D. (2019). BBA - Molecular and Cell Biology of Lipids Carotenoids and fatty liver disease : Current knowledge and research gaps ☆. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids*, (November).

Commission Regulation 10/ 2011 EU, (10/2011/EC). (2011). *COMMISSION REGULATION (EU) No 10/2011 of 14 January 2011*. Retrieved from https://www.fsai.ie/uploadedFiles/Reg10_2011.pdf

da Silva, M. M., Paese, K., Guterres, S. S., Pohlmann, A. R., Rutz, J. K., Flores Cantillano, R. F., … Rios, A. de O. (2017). Thermal and ultraviolet–visible light stability kinetics of co-nanoencapsulated carotenoids. *Food and Bioproducts Processing*, *105*, 86–94. https://doi.org/10.1016/j.fbp.2017.05.004

Dai, Q., Zhu, X., Yu, J., Karangwa, E., Xia, S., Zhang, X., & Jia, C. (2016). Mechanism of Formation and Stabilization of Nanoparticles Produced by Heating Electrostatic Complexes of WPI-Dextran Conjugate and Chondroitin Sulfate. *Journal of Agricultural and Food Chemistry*, *64*(27), 5539–5548. https://doi.org/10.1021/acs.jafc.6b01213

Diarrassouba, F., Remondetto, G., Garrait, G., Alvarez, P., Beyssac, E., & Subirade, M. (2015). Self-assembly of β-lactoglobulin and egg white lysozyme as a potential carrier for nutraceuticals. *Food Chemistry*, *173*, 203–209. https://doi.org/10.1016/j.foodchem.2014.10.009

Donhowe, E. G., & Kong, F. (2014). Beta-carotene: Digestion, Microencapsulation, and In Vitro Bioavailability. *Food and Bioprocess Technology*, *7*(2), 338–354. https://doi.org/10.1007/s11947-013-1244-z

Eratte, D., Wang, B., Dowling, K., Barrow, C. J., & Adhikari, B. P. (2014). Complex coacervation with whey protein isolate and gum arabic for the microencapsulation of omega-3 rich tuna oil. *Food and Function*, *5*(11), 2743–2750. https://doi.org/10.1039/c4fo00296b

Fan, Y., Yi, J., Zhang, Y., Wen, Z., & Zhao, L. (2017). Physicochemical stability and in vitro bioaccessibility of β-carotene nanoemulsions stabilized with whey protein-dextran conjugates. *Food Hydrocolloids*, *63*(2017), 256–264. https://doi.org/10.1016/j.foodhyd.2016.09.008

Gammone, M., Riccioni, G., & Nicolantonio, D. (2014). Carotenoids: potential allies of cardiovascular health? *Food and Nutrition Research*. Retrieved from http://www.world-heart-federation.org/cardiovascular-health/cardiovascular-disease-risk-factors/%5Cnhttp://www.fpcardiologia.pt/%5Cnhttp://www.heart.org/HEARTORG/Conditions/More/ToolsForYourHeartHealth/Numbers-That-Count-for-a-Healthy-Heart_UCM_305427_Art

Gaonkar, A., Vasisht, N., Khare, A. R., & Sobel, R. (2014). *Microencapsulation in the food Industry: A Pratical Implementation Guide* (1°). San Diego: Academic Press: Elsevier.

Guillén, M. D., Ruiz, A., Cabo, N., Chirinos, R., & Pascual, G. (2003). Characterization of sacha inchi (Plukenetia volubilis L.) Oil by FTIR spectroscopy and 1H NMR. Comparison with linseed oil. *JAOCS, Journal of the American Oil Chemists' Society*, *80*(8), 755–762. https://doi.org/10.1007/s11746-003-0768-z

Hamaker, B., Valles, C., Gilman, R., Hardmeier, R. M., Clark, D., Garcia, H. H., … Lescano, M. (1992). Amino acid and fatty acid profiles ofthe Inca peanut (Plukenetia volubilis L.). *Cereal Chem*, *6*, 461–463.

Harnsilawat, T., Pongsawatmanit, R., & McClements, D. J. (2006). Characterization of β-lactoglobulin-sodium alginate interactions in aqueous solutions: A calorimetry, light

scattering, electrophoretic mobility and solubility study. *Food Hydrocolloids*, *20*(5), 577–585. https://doi.org/10.1016/j.foodhyd.2005.05.005

Iddir, M., Degerli, C., Dingeo, G., Desmarchelier, C., Schleeh, T., Borel, P., … Bohn, T. (2019). Whey protein isolate modulates beta-carotene bioaccessibility depending on gastro-intestinal digestion conditions. *Food Chemistry*, *291*(April), 157–166. https://doi.org/10.1016/j.foodchem.2019.04.003

Ilyasoglu, H., & El, S. N. (2014). Nanoencapsulation of EPA/DHA with sodium caseinate-gum arabic complex and its usage in the enrichment of fruit juice. *LWT - Food Science and Technology*, *56*(2), 461–468. https://doi.org/10.1016/j.lwt.2013.12.002

Jain, A., Thakur, D., Ghoshal, G., Katare, O. P., & Shivhare, U. S. (2015). Microencapsulation by Complex Coacervation Using Whey Protein Isolates and Gum Acacia : An Approach to Preserve the Functionality and Controlled Release of β -Carotene. *Food and Bioprocess Technology*, 1–10. https://doi.org/10.1007/s11947-015-1521-0

Javanbakht, S., & Shaabani, A. (2019a). Carboxymethyl cellulose-based oral delivery systems. *International Journal of Biological Macromolecules*, *133*, 21–29. https://doi.org/10.1016/j.ijbiomac.2019.04.079

Javanbakht, S., & Shaabani, A. (2019b). Encapsulation of graphene quantum dot-crosslinked chitosan by carboxymethylcellulose hydrogel beads as a pH-responsive bio-nanocomposite for the oral delivery agent. *International Journal of Biological Macromolecules*, *123*, 389–397. https://doi.org/10.1016/j.ijbiomac.2018.11.118

Jones, O. G., Decker, E. A., & Mcclements, D. J. (2010). Comparison of protein-polysaccharide nanoparticle fabrication methods: Impact of biopolymer complexation before or after particle formation. *Journal of Colloid and Interface Science*, *344*(1), 21–29. https://doi.org/10.1016/j.jcis.2009.12.017

Jones, O. G., Decker, E. A., & McClements, D. J. (2009). Formation of biopolymer particles

by thermal treatment of β-lactoglobulin-pectin complexes. *Food Hydrocolloids*, *23*(5), 1312–1321. https://doi.org/10.1016/j.foodhyd.2008.11.013

Koupantsis, T., Pavlidou, E., & Paraskevopoulou, A. (2016). Glycerol and tannic acid as applied in the preparation of milk proteins e CMC complex coavervates for flavour encapsulation. *Food Hydrocolloids*, *57*, 62–71. https://doi.org/10.1016/j.foodhyd.2016.01.007

Le Goff, M., Le Ferrec, E., Mayer, C., Mimouni, V., Lagadic-Gossmann, D., Schoefs, B., & Ulmann, L. (2019). Microalgal carotenoids and phytosterols regulate biochemical mechanisms involved in human health and disease prevention. *Biochimie*, *167*, 106–118. https://doi.org/10.1016/j.biochi.2019.09.012

Li, K., Wang, B., Wang, W., Liu, G., Ge, W., Zhang, M., … Kong, M. (2019). Microencapsulation of Lactobacillus casei BNCC 134415 under lyophilization enhances cell viability during cold storage and pasteurization, and in simulated gastrointestinal fluids. *Lwt*, *116*(February), 108521. https://doi.org/10.1016/j.lwt.2019.108521

Li, P., Wen, J., Ma, X., Lin, F., Jiang, Z., & Du, B. (2018). Structural, functional properties and immunomodulatory activity of isolated Inca peanut (Plukenetia volubilis L.) seed albumin fraction. *International Journal of Biological Macromolecules*, *118*, 1931–1941. https://doi.org/10.1016/j.ijbiomac.2018.07.046

Li, S. S., Wang, X. L., An, Q. Da, Xiao, Z. Y., Zhai, S. R., Cui, L., & Li, Z. C. (2020). Upon designing carboxyl methylcellulose and chitosan-derived nanostructured sorbents for efficient removal of Cd(II) and Cr(VI) from water. *International Journal of Biological Macromolecules*, *143*, 640–650. https://doi.org/10.1016/j.ijbiomac.2019.12.053

Lim, W. T., & Nyam, K. L. (2016). Characteristics and controlled release behaviour of microencapsulated kenaf seed oil during in-vitro digestion. *Journal of Food Engineering*, *182*, 26–32. https://doi.org/10.1016/j.jfoodeng.2016.02.022

Liu, W., Wang, J., Julian, D., & Zou, L. (2018). Encapsulation of β -carotene-loaded oil droplets in caseinate / alginate microparticles : Enhancement of carotenoid stability and bioaccessibility. *Journal of Functional Foods*, *40*(September 2017), 527–535. https://doi.org/10.1016/j.jff.2017.11.046

Lv, L., Fu, C., Zhang, F., & Wang, S. (2019). Thermally-induced whey protein isolate-daidzein co-assemblies: Protein-based nanocomplexes as an inhibitor of precipitation/crystallization for hydrophobic drug. *Food Chemistry*, *275*(July 2018), 273–281. https://doi.org/10.1016/j.foodchem.2018.09.057

Lv, Y., Yang, F., Li, X., Zhang, X., & Abbas, S. (2014). Formation of heat-resistant nanocapsules of jasmine essential oil via gelatin/gum arabic based complex coacervation. *Food Hydrocolloids*, *35*, 305–314. https://doi.org/10.1016/j.foodhyd.2013.06.003

Malaki Nik, A., Wright, A. J., & Corredig, M. (2011). Impact of interfacial composition on emulsion digestion and rate of lipid hydrolysis using different in vitro digestion models. *Colloids and Surfaces B: Biointerfaces*, *83*(2), 321–330. https://doi.org/10.1016/j.colsurfb.2010.12.001

McClements, D. J. (2014). *Nanoparticle and microparticle based delivery systems*. https://doi.org/10.1201/b17280

Peng, C., Zhao, S. Q., Zhang, J., Huang, G. Y., Chen, L. Y., & Zhao, F. Y. (2014). Chemical composition, antimicrobial property and microencapsulation of Mustard (Sinapis alba) seed essential oil by complex coacervation. *Food Chemistry*, *165*, 560–568. https://doi.org/10.1016/j.foodchem.2014.05.126

Rezaeinia, H., Ghorani, B., Emadzadeh, B., & Tucker, N. (2019). Electrohydrodynamic atomization of Balangu (Lallemantia royleana) seed gum for the fast-release of Mentha longifolia L. essential oil: Characterization of nano-capsules and modeling the kinetics of release. *Food Hydrocolloids*, *93*(February), 374–385.

https://doi.org/10.1016/j.foodhyd.2019.02.018

Ritger, P. L., & Peppas, N. A. (1987). A simple equation for description of solute release II. Fickian and anomalous release from swellable devices. *Journal of Controlled Release*, *5*(1), 37–42. https://doi.org/10.1016/0168-3659(87)90035-6

Rojas-moreno, S., Osorio-revilla, G., Gallardo-velázquez, T., Cárdenas-bailón, F., & Meza-márquez, G. (2018). Effect of the cross-linking agent and drying method on encapsulation efficiency of orange essential oil by complex coacervation using whey protein isolate with different polysaccharides. *Journal of Microencapsulation*, 165–180. https://doi.org/10.1080/02652048.2018.1449910

Rutz, J. K., Borges, C. D., Zambiazi, R. C., Da Rosa, C. G., Da Silva, M. M., Cleonice, G., & Médelin, M. (2016). Elaboration of microparticles of carotenoids from natural and synthetic sources for applications in food. *Food Chemistry*, *202*, 324–333. https://doi.org/10.1016/j.foodchem.2016.01.140

Sah, B. N. P., McAinch, A. J., & Vasiljevic, T. (2016). Modulation of bovine whey protein digestion in gastrointestinal tract: A comprehensive review. *International Dairy Journal*, *62*, 10–18. https://doi.org/10.1016/j.idairyj.2016.07.003

Santos, M. B., da Costa, N. R., & Garcia-Rojas, E. E. (2018). Interpolymeric Complexes Formed Between Whey Proteins and Biopolymers: Delivery Systems of Bioactive Ingredients. *Comprehensive Reviews in Food Science and Food Safety*, *17*(3), 792–805. https://doi.org/10.1111/1541-4337.12350

Sathasivam, T., Muniyandy, S., Chuah, L. H., & Janarthanan, P. (2018). Encapsulation of red palm oil in carboxymethyl sago cellulose beads by emulsification and vibration technology: Physicochemical characterization and in vitro digestion. *Journal of Food Engineering*, *231*, 10–21. https://doi.org/10.1016/j.jfoodeng.2018.03.008

Shaddel, R., Hesari, J., Azadmard-Damirchi, S., Hamishehkar, H., Fathi-Achachlouei, B.,

& Huang, Q. (2017). Use of gelatin and gum Arabic for encapsulation of black raspberry anthocyanins by complex coacervation. *International Journal of Biological Macromolecules*, *107*, 1800–1810. https://doi.org/10.1016/j.ijbiomac.2017.10.044

Siepmann, J., & Peppas, N. A. (2012). Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). *Advanced Drug Delivery Reviews*, *64*(SUPPL.), 163–174. https://doi.org/10.1016/j.addr.2012.09.028

Soares, da S. B., Siqueira, R. P., de Carvalho, M. G., Vicente, J., & Garcia-Rojas, E. E. (2019). Microencapsulation of sacha inchi oil (Plukenetia volubilis L.) using complex coacervation: Formation and structural characterization. *Food Chemistry*, *298*(February), 125045. https://doi.org/10.1016/j.foodchem.2019.125045

Soukoulis, C., Cambier, S., Hoffmann, L., & Bohn, T. (2016). Chemical stability and bioaccessibility of β-carotene encapsulated in sodium alginate o/w emulsions: Impact of Ca2+ mediated gelation. *Food Hydrocolloids*, *57*, 301–310. https://doi.org/10.1016/j.foodhyd.2016.02.001

Tavares, L., & Zapata Noreña, C. P. (2019). Encapsulation of garlic extract using complex coacervation with whey protein isolate and chitosan as wall materials followed by spray drying. *Food Hydrocolloids*, *89*(October 2018), 360–369. https://doi.org/10.1016/j.foodhyd.2018.10.052

Thakur, D., Jain, A., Ghoshal, G., Shivhare, U. S., & Katare, O. P. (2017). Microencapsulation of β -Carotene Based on Casein / Guar Gum Blend Using Zeta Potential-Yield Stress Phenomenon : an Approach to Enhance Photo-stability and Retention of Functionality. *AAPS PharmSciTech*, *18*(5), 1447–1459. https://doi.org/10.1208/s12249-017-0806-1

Vicente, J., Cezarino, T. D. S., José, L., Pereira, B., Pinto, E., Raymundo, G., … Garcia-rojas, E. E. (2017). Microencapsulation of sacha inchi oil using emulsion-based delivery

systems. *Food Research International*, *99*(June), 612–622.

Wang, S., Zhu, F., & Kakuda, Y. (2018). Sacha inchi (Plukenetia volubilis L.): Nutritional composition, biological activity, and uses. *Food Chemistry*, *265*(April), 316–328. https://doi.org/10.1016/j.foodchem.2018.05.055

Weinbreck, F., Nieuwenhuijse, H., Robijn, G. W., & De Kruif, C. G. (2003). Complex Formation of Whey Proteins: Exocellular Polysaccharide EPS B40. *Langmuir*, *19*(22), 9404–9410. https://doi.org/10.1021/la0348214

Ye, Q., Georges, N., & Selomulya, C. (2018). Microencapsulation of active ingredients in functional foods: From research stage to commercial food products. *Trends in Food Science and Technology*, *78*(January), 167–179. https://doi.org/10.1016/j.tifs.2018.05.025

Yuan, Y., Kong, Z. Y., Sun, Y. E., Zeng, Q. Z., & Yang, X. Q. (2017). Complex coacervation of soy protein with chitosan: Constructing antioxidant microcapsule for algal oil delivery. *Lwt*, *75*, 171–179. https://doi.org/10.1016/j.lwt.2016.08.045

Figure Caption

Fig.1: Zeta potentialand SEI of WPIn and CMC solutions in the pH range of 2.0 - 6.0.

Fig.2: (A) Turbidity (100 -% T) observed between the CMC: WPIn complex at different ratios at a wavelength of 450 nm. (B) Zeta potential of CMC / WPIn in different proportions at pH 3.5.

Fig.3: Effect of pH on the formation of the CMC / WPIn complex. Fig. 3 (A) turbidity at 450 nm. Fig. 3 (B) zeta potential of concentration of the mixture was fixed at 1: 6 and the pH values were 2.0 – 6.0.

Fig.4: Confocal microscopy image of β-SIO microcapsules with 1: 2 core: wall ratio on a 100µm scale.

Fig.5: FT-IR spectrum from (WPIn), and CMC, Sacha Inchi Oil (SIO) and β-SIO microcapsules

Fig.6: Degradation profile of β-C and β-SIO microcapsulesduring UV-visible treatment

Fig.7: Release profile of β-C from β-SIO microcapsules in soybean oil as a food simulator

Fig.8: Release of β-carotene present in β-SIO microcapsules obtained through complex coacervation using CMC / WPIn as wall material and produced by lyophilization during in vitro digestion.

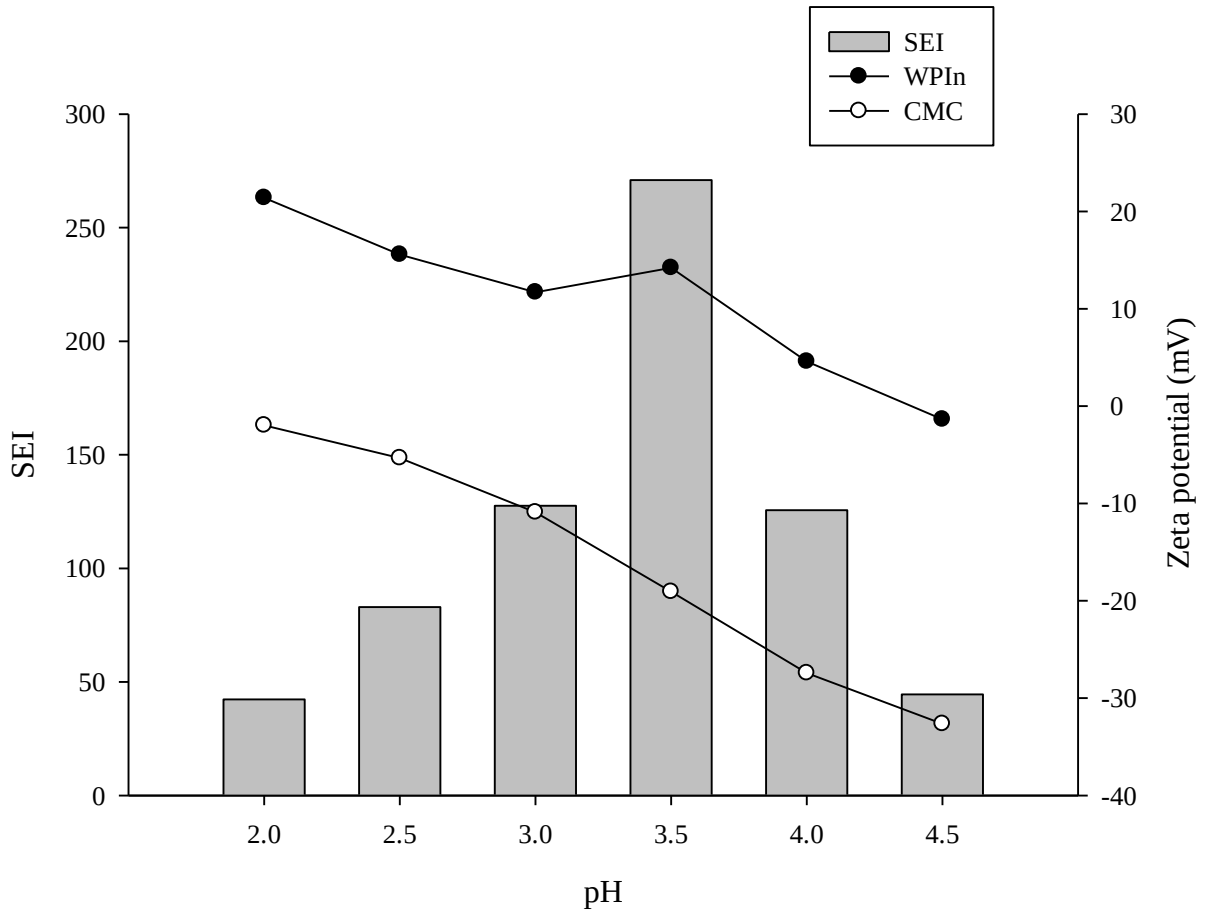
SEI
WPIn
CMC
300
250
200
150
100
50
0
SEI
30
20
10
0
-10
-20
-30
-40
Zeta potential (mV)
2.0
2.5
3.0
3.5
4.0
4.5
pH

Fig.1

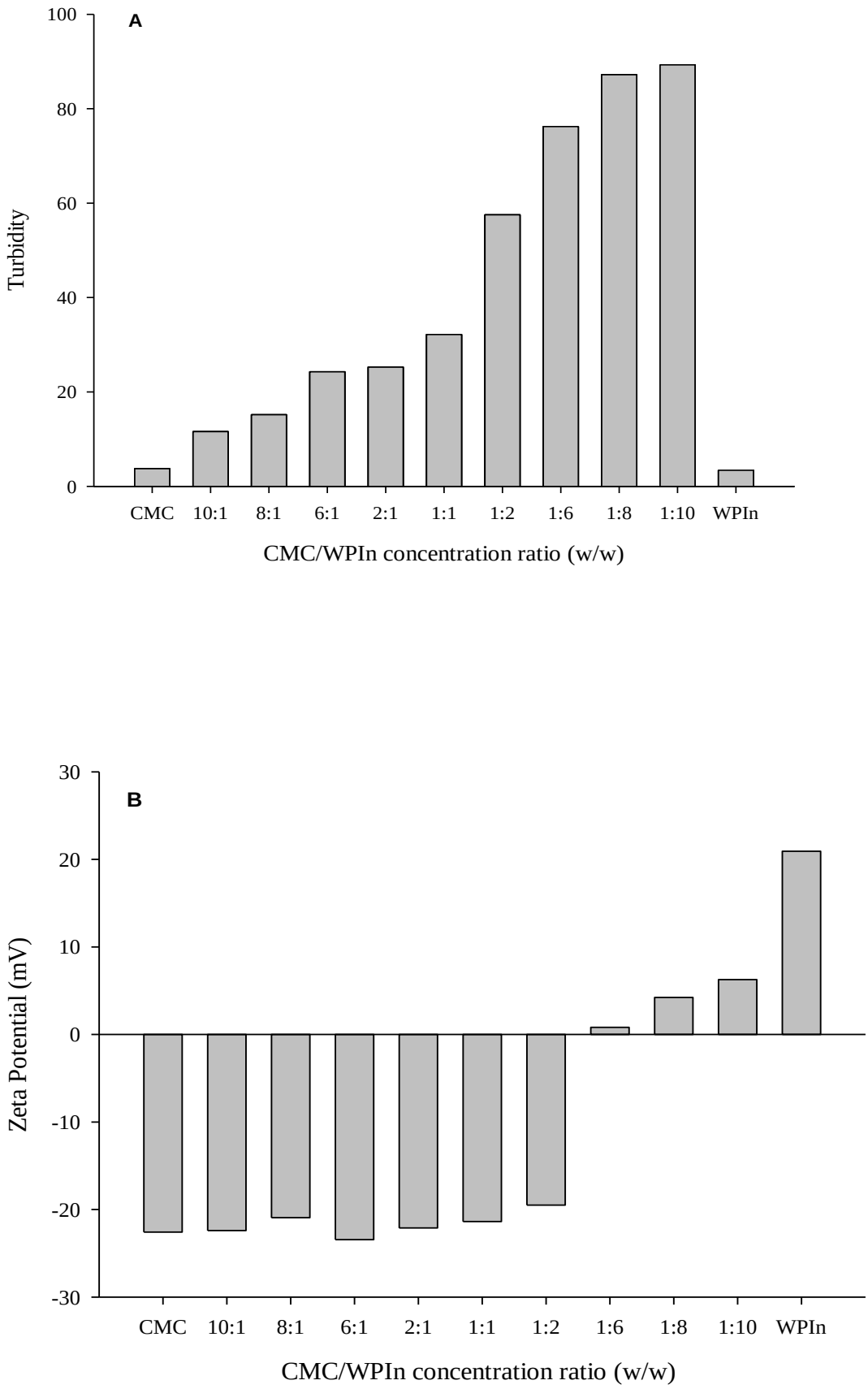
A
Turbidity
100
80
60
40
20
0
CMC
10:1
8:1
6:1
2:1
1:1
1:2
1:6
1:8
1:10
WPIn
CMC/WPIn concentration ratio (w/w)
B
Zeta Potential (mV)
30
20
10
0
-10
-20
-30
CMC
10:1
8:1
6:1
2:1
1:1
1:2
1:6
1:8
1:10
WPIn
CMC/WPIn concentration ratio (w/w)

Fig.2

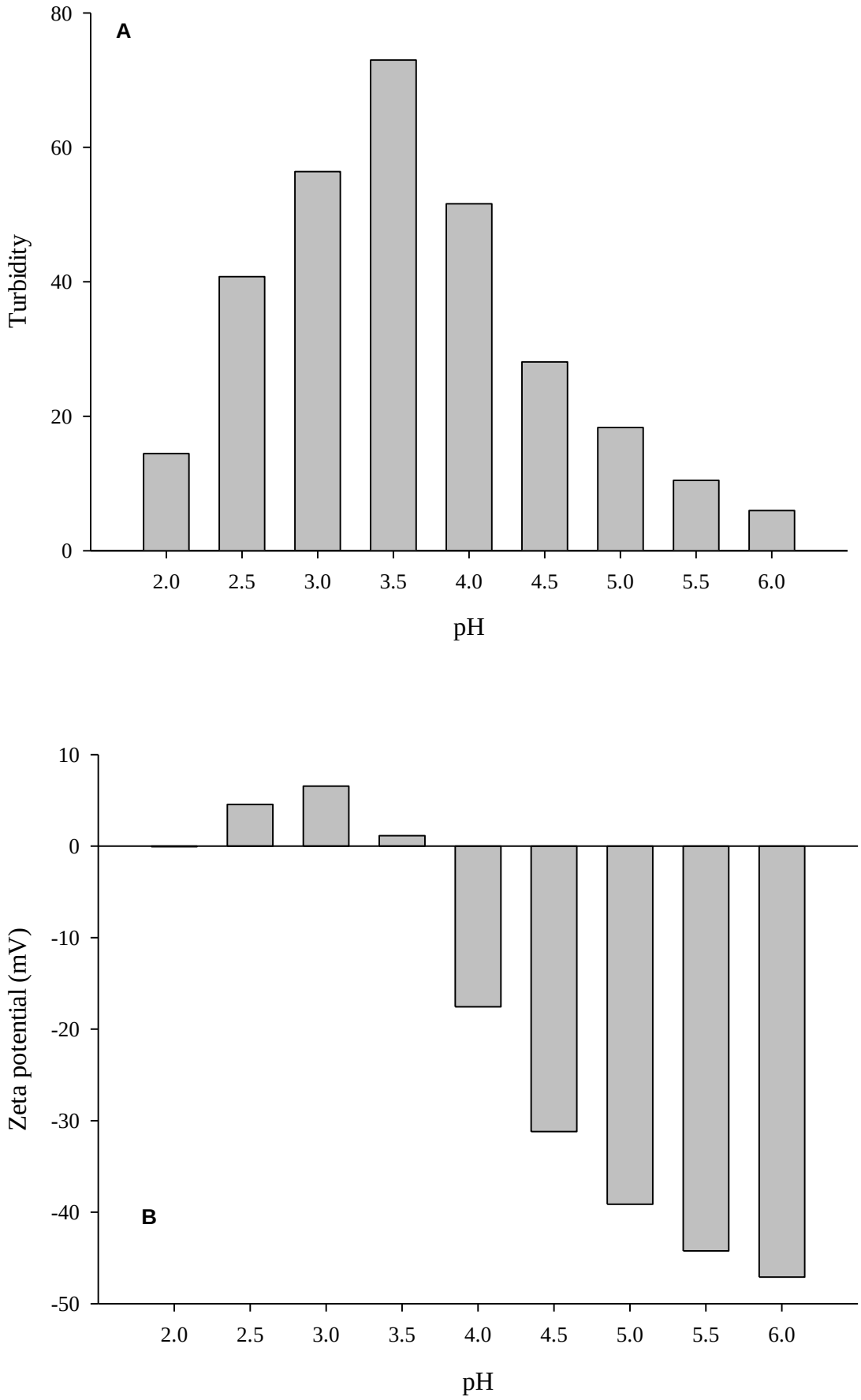
A
Turbidity
80
60
40
20
0
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
pH
B
Zeta potential (mV)
10
0
-10
-20
-30
-40
-50
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
pH

Fig.3

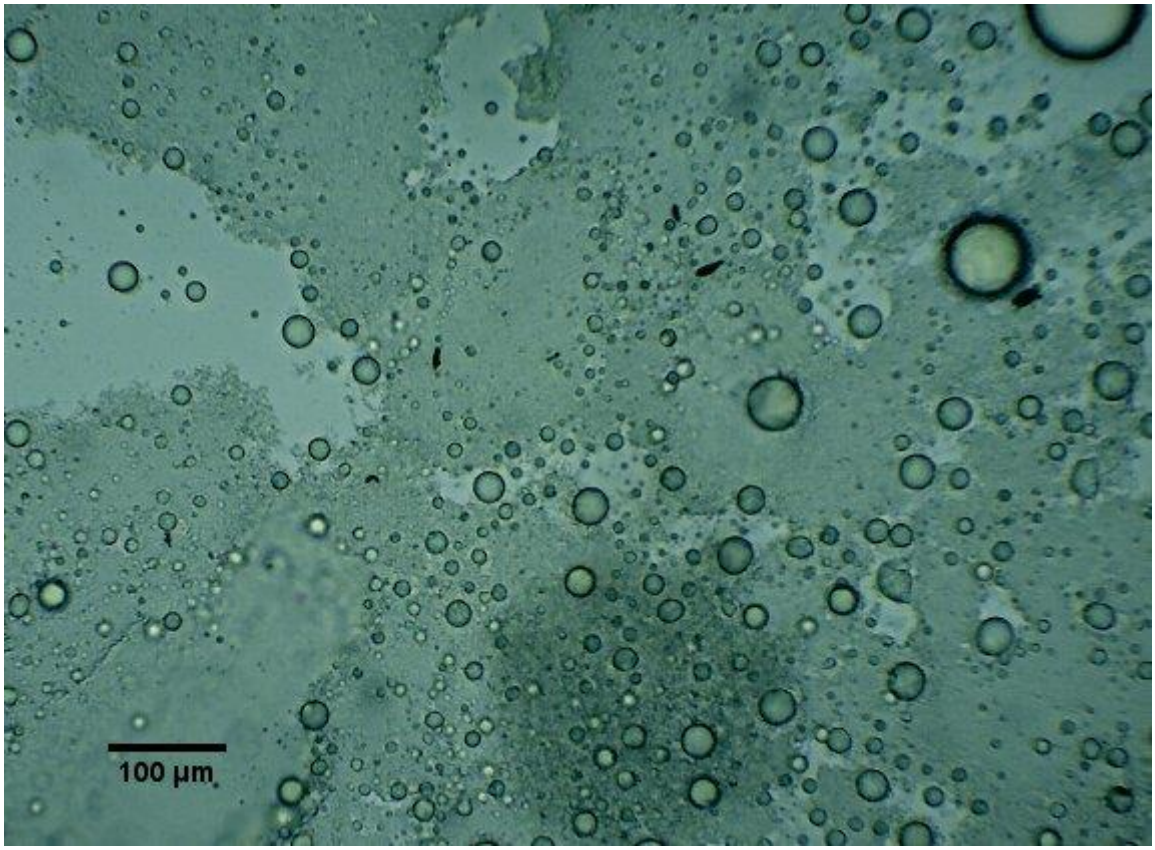

Fig.4

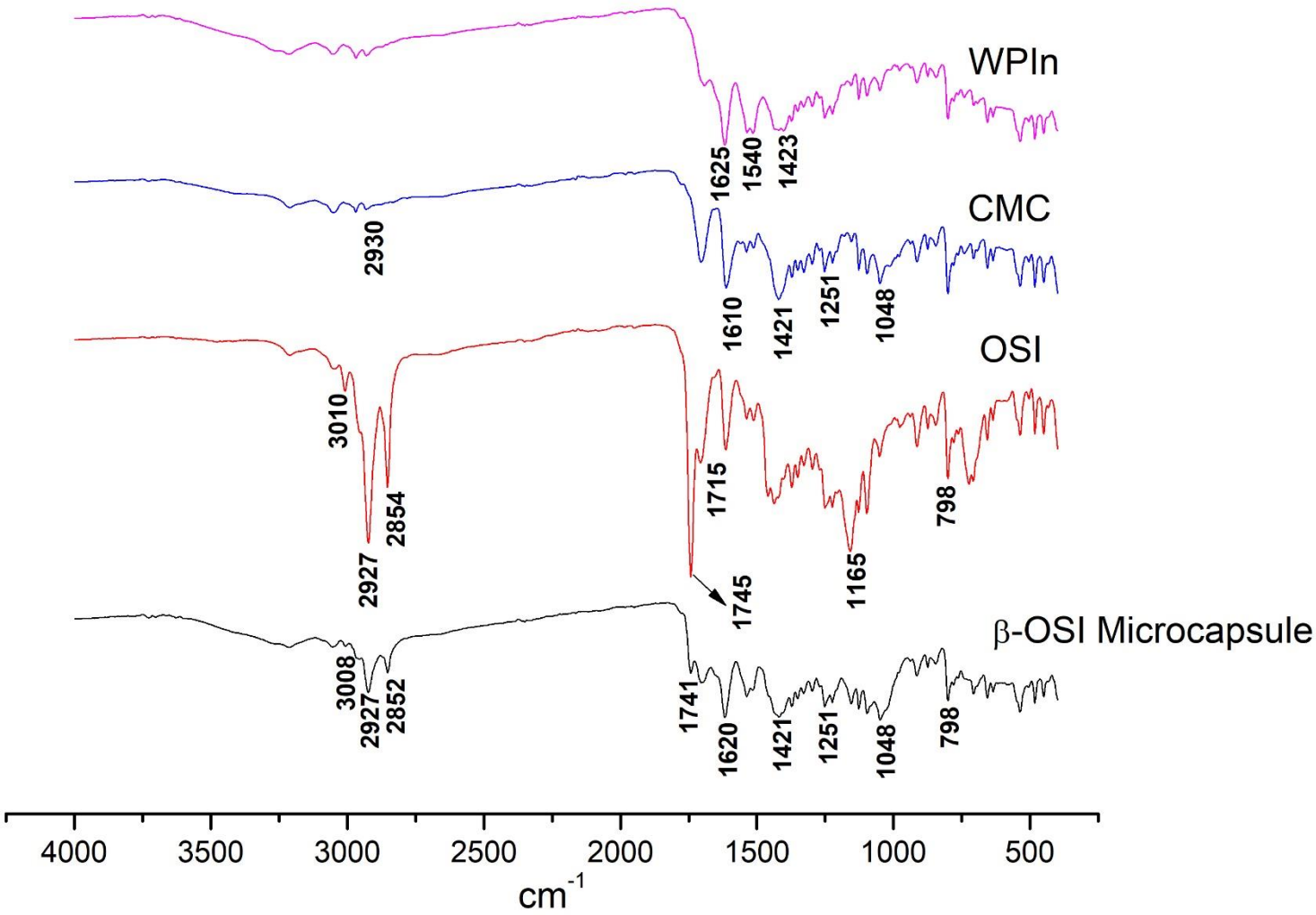
WPIn
1625
1540
1423
CMC
2930
1610
1421
1251
1048
OSI
3010
2927
2854
1715
1745
1165
798
β-OSI Microcapsule
3008
2927
2852
1741
1620
1421
1251
1048
798
4000
3500
3000
2500
2000
1500
1000
500
cm-1

Fig.5

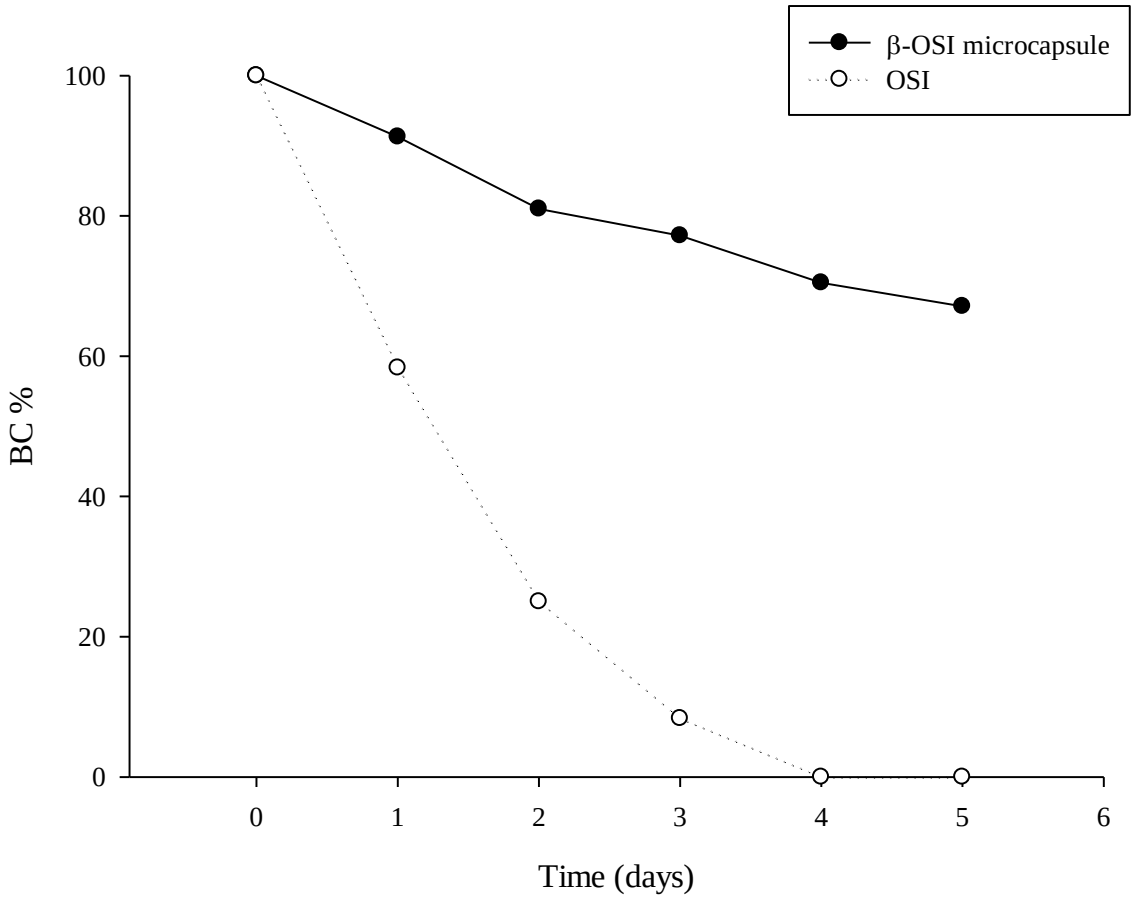

Fig.6

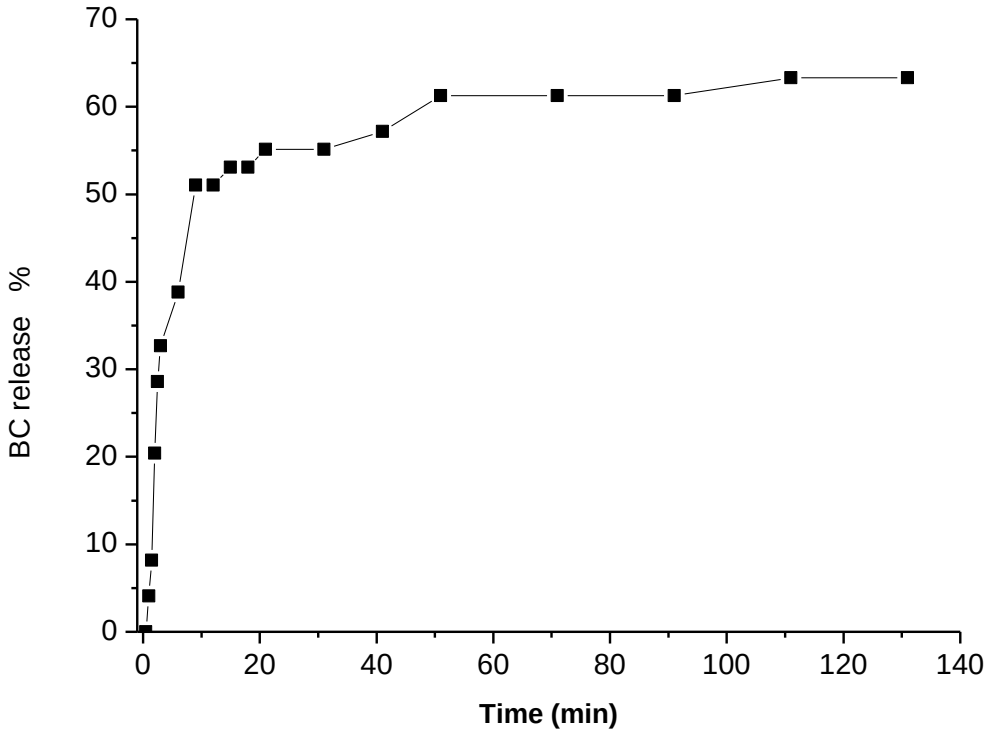

Fig.7

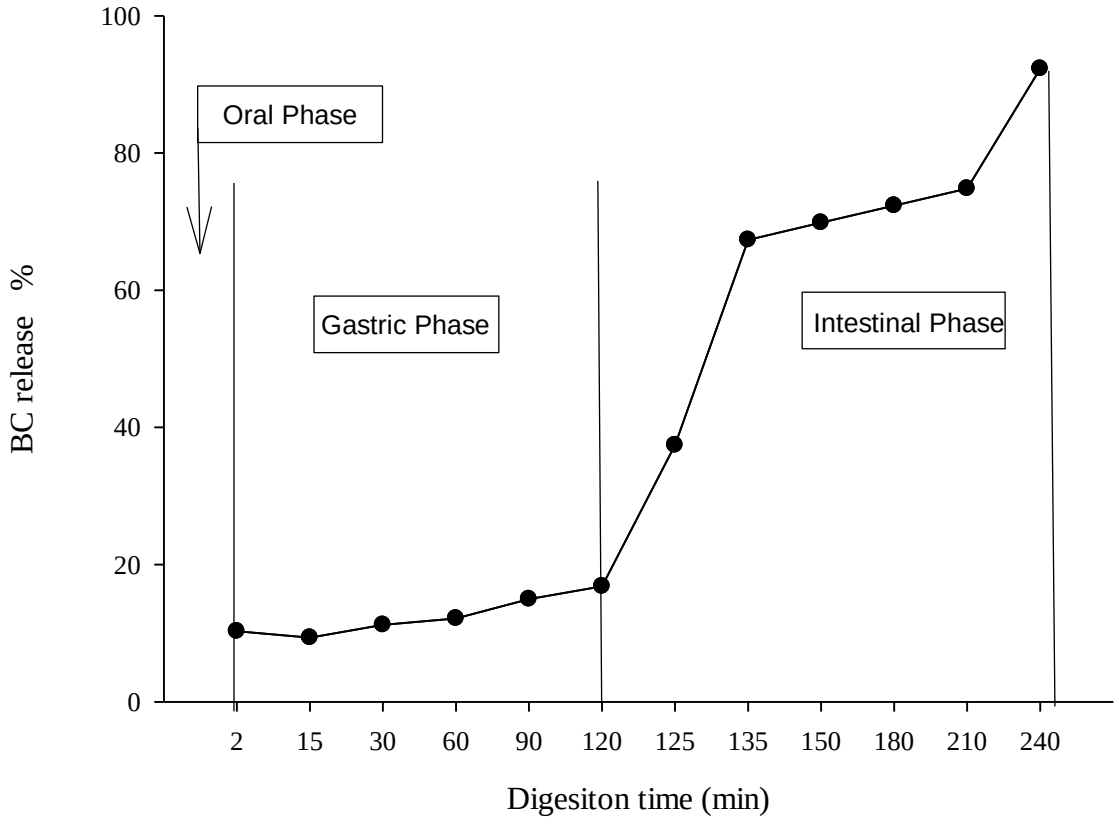
100
80
60
40
20
0
BC release %
Oral Phase
Gastric Phase
Intestinal Phase
2
15
30
60
90
120
125
135
150
180
210
240
Digesiton time (min)

Fig.8

Table 1: Composition of the formulations and encapsulation efficiency (EE) of the capsules produced by complex coacervation

Samples	Core (g)	Wall (g)				Concentration of Biopolymers (%)	Core:wall ratio	EE%
		WPIn (g)	CMC (g)	Transglutaminase(g)	Total (g)			
S1	0.35	0.15	0.0025	0.125	0.175	0.5	2:1	82.46 ± 3.73^{cd}
S2	0.175	0.15	0.0025	0.125	0.175	0.5	1:1	93.54 ± 2.13^{ab}
S3	0.0875	0.15	0.0025	0.125	0.175	0.5	1:2	91.69 ± 1.07^{abc}
S4	0.7	0.3	0.05	0.125	0.35	1.0	2:1	68.92 ± 4.16^{e}
S5	0.35	0.3	0.05	0.125	0.35	1.0	1:1	77.95 ± 3.55^{d}
S6	0.175	0.3	0.05	0.125	0.35	1.0	1:2	76.92 ± 5.33^{d}
S7	1.4	0.6	0.1	0.125	0.7	2.0	2:1	75.38 ± 5.33^{d}
S8	0.7	0.6	0.1	0.125	0.7	2.0	1:1	96.21 ± 3.39^{a}
S9	0.35	0.6	0.1	0.125	0.7	2.0	1:2	83.69 ± 1.07^{bcd}

The analyses were performed in three replicates. The same letters in the same column do not differ significantly by Tukey test with a probability of 5%’

Table 2: Kinetics constant of the β-C in β-SIO microcapsules release profile in food model.

	Higuchi		**First order**		**Rigger-Peppas**		
Food Model	K	R^2	K	R^2	K	n	R^2
Soybean	20.49	0.79	20.13	0.77	34.77	0.13	0.99

CONCLUSÃO GERAL

Diante do trabalho realizado podemos concluir que se formaram complexos coacervados entre o isolado proteico do soro aquecido e a Carboximetilcelulose. As características químicas e morfológicas dos complexos coacervados demonstraram as alterações produzidas pela interação eletrostática. As nanopartículas fabricadas através do tratamento térmico do isolado proteico do soro produziram cápsulas na escala menor de 100nm.

Os biopolímeros e agente reticulante utilizado foram eficazes na proteção do β-caroterno presente no óleo de sacha inchi apresentando cápsulas com alta eficiência de encapsulação (>90%). As cápsulas produzidas por IPS_n/CMC apresentaram resistência durante simulação oral e gástrica e considerável estabilidade após simulação gastrointestinal e baixa liberação do β-caroterno em alimentos com base lipídica. Além disso, a liberação do β-caroterno ocorreu por difusão Fickian de acordo com modelo de Rigger-Peppas.

Por fim, as cápsulas produzidas poderiam ser utilizadas para o transporte de ingredientes bioativos, futuros estudos sobre a aplicação do óleo essencial de pimenta preta encapsulado em produtos alimentícios podem ser avaliados, assim como sua atividade antimicrobiana e antioxidante após encapsulação.