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LUANA CARVALHO DA SILVA

MICROCÁPSULAS DE ÓLEO DE PEQUI OBTIDAS POR COACERVAÇÃO COMPLEXA: CARACTERIZAÇÃO, LIBERAÇÃO CONTROLADA E APLICAÇÃO EM IOGURTE

FORTALEZA - CEARÁ
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RESUMO

A adição de óleo vegetal em iogurte, como o óleo de pequi (*Caryocar coriaceum* Wittm.), tem se destacado para as indústrias alimentícias por estar associado ao aumento dos teores de ácidos graxos insaturados e adições de bioativos inexistentes, como os carotenoides. A microencapsulação pode ser usada para mascarar o forte sabor do óleo de pequi, além de proteger os compostos bioativos e promover sua liberação controlada. Por isso, o objetivo desse trabalho é caracterizar as microcápsulas de óleo de pequi, obtidas por coacervação complexa, utilizando como materiais de parede a goma de cajueiro/quitosana e goma de cajueiro/gelatina, e avaliar sua aplicação em iogurte. Ambos os materiais de parede obtiveram bons resultados em eficiência de encapsulamento (>80%), rendimento (> 50%) e capacidade de carga (<50%). O infravermelho por transformada de Fourier (FTIR) comprovou a interação eletrostática dos coacervados, confirmando a eficácia da encapsulação. As análises termogravimétricas (TGA) e calorimetria exploratória diferencial (DSC), além de Rancimat, mostraram a estabilidade da microcápsula. As microcápsulas apresentaram melhor resistência à desintegração na faixa de pH de 4 a 5 e em temperaturas abaixo de 80 °C, sendo assim foi escolhida a matriz alimentar de iogurte para aplicação das microcápsulas. A presença do óleo de pequi levou ao um aumento significativo de ácidos graxos insaturados no perfil de ácidos graxos do iogurte, e a etapa de resfriamento do iogurte a mais adequada para adição das microcápsulas. Posteriormente, foi avaliada a influência das microcápsulas de óleo de pequi nas análises de pH, sólidos totais, sinérese, cor, morfologia, textura e viscosidade, durante o tempo de armazenamento de 28 dias. Nos 28 dias de armazenamento, os valores de pH diminuíram de 4,5 para 4,32 para todas as formulações, mas os valores de sólidos totais aumentaram (>11%) e a sinérese diminuiu (<50%) devido à retenção de água causada pelos biopolímeros das microcápsulas. No perfil de liberação pela digestão *in vitro*, houve uma liberação gradativa do óleo, fato que aumentou a bioacessibilidade do beta-caroteno. Com isso, conclui-se que as microcápsulas de goma de cajueiro/quitosana proporcionaram uma matriz mais densa, protegendo melhor o óleo, e ao aplicar em iogurte, melhorou o perfil de ácidos graxos e promoveu a adição de um importante composto bioativo, o beta-caroteno, sendo que as formulações com as microcápsulas obtiveram melhor estabilidade e bioacessibilidade do beta-caroteno.

Palavras-chaves: Óleo de pequi; Goma de cajueiro; Quitosana; Gelatina; Microencapsulação; Iogurte; Beta-caroteno.

ABSTRACT

The addition of vegetable oil to yogurt, such as pequi oil (*Caryocar coriaceum* Wittm.), has stood out for the food industries because it is associated with an increase in unsaturated fatty acids and non-existent additions of bioactive, such as carotenoids. Microencapsulation can be used to mask the strong flavor of pequi oil, in addition to protecting bioactive compounds and promoting their controlled release. Therefore, the objective of this work is to characterize pequi oil microcapsules, obtained by complex coacervation, using cashew gum/chitosan and cashew gum/gelatin as wall materials, and to evaluate their application in yogurt. Both wall materials had good results in encapsulation efficiency (>80%), yield (>50%), and load capacity (<50%). Fourier transform infrared (FTIR) confirmed the electrostatic interaction of coacervates, confirming the efficacy of encapsulation. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC), in addition to Rancimat, showed the stability of the microcapsule. The microcapsules showed better resistance to disintegration in the pH range from 4 to 5 and at temperatures below 80 °C, so the yogurt food matrix was chosen for application of the microcapsules. The presence of pequi oil led to a significant increase in unsaturated fatty acids in the fatty acid profile of yogurt, and the yogurt cooling step was the most suitable for the addition of microcapsules. Subsequently, the influence of pequi oil microcapsules in the analysis of pH, total solids, syneresis, color, morphology, texture, and viscosity, during a storage time of 28 days, was evaluated. At 28 days of storage, pH values decreased from 4.5 to 4.32 for all formulations, but total solids values increased (>11%) and syneresis decreased (<50%) due to water retention caused by the biopolymers of the microcapsules. In the release profile by in vitro digestion, there was a gradual release of oil, a fact that increased the bioaccessibility of beta-carotene. Thus, it is concluded that the microcapsules of cashew gum/chitosan provided a denser matrix, better protecting the oil, and when applied to yogurt, it improved the fatty acid profile and promoted the addition of an important bioactive compound, beta-carotene, and the formulations with microcapsules had better stability and bioaccessibility than beta-carotene.

Keywords: Pequi oil; Cashew gum; Chitosan; Gelatin; Microencapsulation; Yogurt, Beta-carotene.

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FT-IR	Fourier Transform Infrared Spectroscopy
TGA	Thermogravimetric Analysis
DSC	Differential Scanning Calorimetry
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
CG	Cashew Gum (goma de cajueiro)
CT	Chitosan (quitosana)
GE	Gelatin (gelatina)
PO	Pequi Oil (óleo de pequi)
CCP	Microcápsulas de óleo de pequi, utilizando a goma de cajueiro/quitosana
CGP	Microcápsulas de óleo de pequi, utilizando a goma de cajueiro/gelatina
Y-C	Iogurte controle
Y-PO	Iogurte adicionado com óleo de pequi puro
Y-CCP	Iogurte adicionado com microcápsulas CCP
Y-CGP	Iogurte adicionado com microcápsulas CGP
EE	Encapsulation Efficiency
TO	Total Oil
SO	Surface Oil
FAME	Fatty Acid Methyl Esters
CG-FID	Gas Chromatography apparatus with Flame Ionization Detector
HPLC	High Performance Liquid Chromatography
LC	Loading Capacity
TPA	Texture Profile Analysis
BC	Beta-carotene
SGF	Simulated Gastric Fluid
SIF	Simulated Intestinal Fluid

SUMÁRIO

1 INTRODUÇÃO

Os iogurtes são produtos derivados do leite mais popular, sendo resultantes da acidificação química ou fermentação láctica lenta da lactose do leite por bactérias iniciadoras, *Streptococcus thermophilus* e *Lactobacillus delbrueckii* subsp. *bulgaricus* (GHARIBZAHEDI; CHRONAKIS, 2018; LADJEVARDI; GHARIBZAHEDI; MOUSAVI, 2015). Devido ao seu alto consumo, pode ser um bom veículo para garantir e melhorar a ingestão diária de nutrientes, o que pode prevenir doenças, e trazer impactos positivos na saúde dos consumidores (SILVA et al., 2019). Assim, a incorporação de ingredientes funcionais em matrizes alimentares, como iogurte, tem sido realizada com o objetivo de fortificar, e aumentar o valor agregado do produto.

Dentre os componentes bioativos que possuem baixa concentração no iogurte, pode-se destacar os ácidos graxos insaturados, porém existem poucos estudos que melhoram o perfil de ácidos graxos do iogurte, sendo os óleos vegetais e de peixes os mais utilizados (BABA et al., 2018; BELLO et al., 2015; NIEUWENHOVE et al., 2019; RIBEIRO, B.; BONIF, 2021).

O óleo de pequi tem se destacado como matéria-prima para a indústria alimentícia e farmacêutica pela excelente qualidade associada a altos teores de ácidos graxos insaturados, predominantemente oleico (60,6%) (PESSOA et al., 2015), e carotenoides, como o beta-caroteno, um precursor da vitamina A (PINTO et al., 2018). O beta-caroteno, encontrado no óleo de pequi (cerca de 270 μL g-1 de óleo), tem um papel crucial na saúde humana, como melhorar a imunidade, diminuir o risco de doenças cardíacas, e aumentar as funções gastrointestinais (GENG et al., 2022; XIE et al., 2021). No entanto, os carotenoides têm uma estrutura apolar e moléculas altamente insaturadas, tornando-os suscetíveis à oxidação e isomerização durante o processamento ou armazenamento (ŠEREGELJ et al., 2021). O óleo de pequi já é utilizado na indústria de alimentos na produção de margarinas (OLIVEIRA et al., 2011) e queijos (BENEVIDES et al 2009), conferindo ao produto uma especificidade característica desejável pelos fabricantes. Porém algumas vezes, dependendo da aplicação, mascarar características naturais de sabor e aroma são tão importantes quanto sua estabilidade química. A utilização dos métodos de microencapsulação pode mascarar o forte sabor do óleo de pequi, além de proteger e promover sua liberação controlada.

Dos métodos de microencapsulação usados, a coacervação complexa tem sido particularmente bem-sucedida na estabilização de lipídios insaturados, e no fornecimento de um ingrediente com um prazo de validade sensorial prolongado (BARROW; NOLAN; JIN, 2007). Por ser um método bastante recomendado para a microencapsulação de substâncias lipofílicas, vários trabalhos o utilizam para encapsular óleos vegetais. O método consiste em

um fenômeno de separação de fase líquido-líquido que ocorre entre biopolímeros de cargas opostas por meio de interação eletrostática (EGHBAL; CHOUDHARY, 2018). A coacervação complexa funcionará impedindo que os compostos interajam fisicamente e/ou quimicamente com o ambiente externo, criando uma barreira ao redor do material ativo (SOUZA et al., 2020).

No presente estudo foram utilizadas duas combinações de diferentes materiais de parede: goma de cajueiro/quitosana e goma de cajueiro/gelatina. Estudos anteriores mostraram o potencial da goma de caju como substituto da goma arábica na microencapsulação do óleo de pequi (SILVA et al., 2018; NASCIMENTO et al., 2020). Uma seleção cuidadosa do material da parede é vital, pois impõe a eficiência de encapsulação e a estabilidade da microcápsula. O material de parede mais adequado para aplicações em alimentos deve ter as seguintes propriedades: não reativo com o núcleo, capaz de selar e reter o núcleo intacto dentro da cápsula, capaz de proteger eficazmente o núcleo contra condições ambientais adversas, isento de qualquer sabor desagradável, ser comestível e não tóxico, e economicamente viável (DHAKAL; HE, 2020; MCCLEMENTS; DECKER; WEISS, 2007; NAZZARO et al., 2012).

Com isso foi realizada aplicação dessas microcápsulas de óleo de pequi no iogurte com o objetivo de enriquecê-lo com os componentes bioativos do óleo. A adição do óleo de pequi no iogurte tem como principais funções aumentar o teor de ácidos graxos insaturados, como também a melhora nutricional proporcionada pelo beta-caroteno presente no óleo, que é precursor da pró-vitamina A. Testes de caracterização e liberação controlada das microcápsulas foram realizados previamente para a escolha da melhor matriz alimentar.

2 OBJETIVOS

2.1 Geral

Caracterizar as microcápsulas de óleo de pequi, obtidas por coacervação complexa, utilizando a goma de cajueiro/quitosana e goma de cajueiro/gelatina como materiais de parede, e avaliar sua aplicação em iogurte.

2.2 Específicos

- Produzir microcápsulas de óleo de pequi por coacervação complexa seguida da secagem por liofilização, caracterizar as microcápsulas pela eficiência de encapsulação, rendimento, capacidade de carga, morfologia e FT-IR, e estudar a estabilidade térmica (TGA e DSC) e oxidativa acelerada (Rancimat);
- Verificar o perfil de liberação sobre as condições *in vitro* ao longo do tempo, e em diferentes faixas de pH e temperatura, e calcular o perfil cinético de liberação a partir dos resultados obtidos na liberação *in vitro*;
- Realizar as análises de liberação e perfil de ácidos graxos do óleo de pequi e das microcápsulas aplicadas em matriz alimentar de iogurte.
- Analisar as propriedades do iogurte (pH, cor, sólidos totais, sinérese, textura viscosidade e morfologia) e estudar a estabilidade do óleo por meio da quantificação do beta-caroteno durante o tempo de armazenamento de 28 dias.
- Quantificar o beta-caroteno presente no óleo de pequi (encapsulado e não-encapsulado) adicionado em iogurte durante a digestão *in vitro* e analisar sua bioacessibilidade após completa digestão.

3 REVISÃO DE LITERATURA

3.1 Pequi (*Caryocar coariceum* Wittm.)

O gênero Caryocar é amplamente difundido na América do Sul e compreende mais de vinte espécies. No Brasil, considerando uma área que inclui os Estados de Amazonas, Pará, Maranhão, Piauí, Goiás, Bahia, Ceará, São Paulo e Minas Gerais, as principais espécies são *Caryocar brasiliense* Cambess. e *Caryocar coriaceum* Wittm. (SENA et al., 2010).

O pequizeiro (*C. coriaceum*) (Figura 1) é uma espécie lenhosa perene nativa do nordeste do Brasil, ocorrendo em áreas de cerrado e cerradão (vegetação savana) na Chapada do Araripe no sul do Ceará. Segundo Sousa Júnior (2012), a Chapada do Araripe, em função de suas características edafoclimáticas, sendo mais úmida e chuvosa em relação a outras áreas do Nordeste, se apresenta como uma região favorável à produção do pequi. O pequizeiro pode ser classificado como frutífera ou oleaginosa, em razão das suas características e formas de utilização.

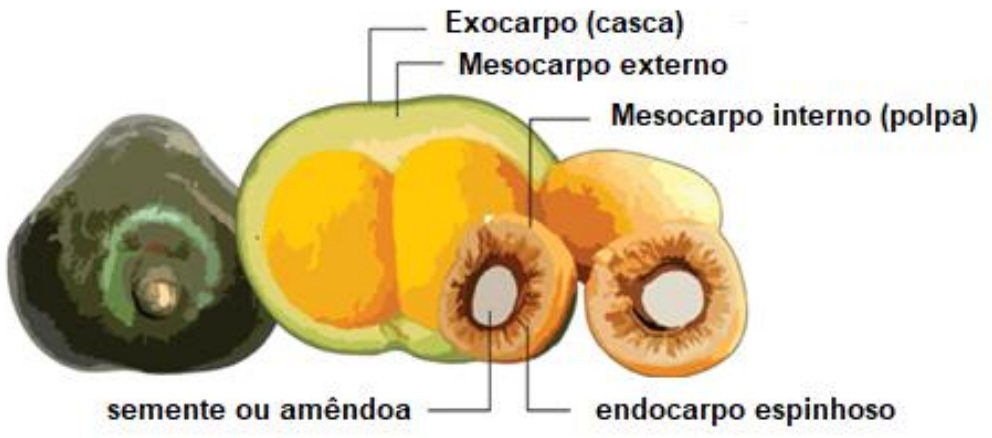
Figura 1: Pequizeiro (*Caryocar coriaceum* Wittm.).

Fonte: próprio autor.

O pequi, também conhecido como pequi, piquiá, piqui-do-cerrado, piquiá bravo, pequerim, amendoâ-de-espinho, grão-de-cavalo e suari, tem como palavra de origem o tupi, e

significa casca espinhosa (SANTOS et al., 2013). O fruto (Figura 2) é constituído pelo exocarpo ou pericarpo, de coloração esverdeada ou marrom-esverdeada, mesocarpo externo, polpa branca com coloração pardo-acinzentada e mesocarpo interno, que constitui a porção comestível do fruto, possuindo coloração amarelada, e separa-se facilmente do mesocarpo externo quando maduro. O endocarpo, que é espinhoso, protege a semente ou amêndoa, que é revestida por um tegumento fino e marrom, sendo também uma porção comestível (NASCIMENTO-SILVA; NAVES, 2019).

Figura 2: Constituição esquemática do pequi (fruto).



Fonte: adaptado de Nascimento-Silva & Naves, 2019.

As cascas (exocarpo e mesocarpo) representam aproximadamente 80% da massa do fruto inteiro, e apresentam alto teor de fibras alimentares (45%). Além disso, a avaliação do perfil de monossacarídeos revelou a presença de uma grande quantidade de polissacarídeos pécticos (56%) (LEÃO et al., 2018). As cascas do pequi também possuem bioativos como polifenóis e carotenoides que proporcionam altos valores de atividade antioxidante (CANGUSSU et al., 2021; CALDEIRA et al., 2021).

A polpa do fruto de pequi é rica em lipídios (33,4%), é fonte importante de fibra alimentar (10,02%) e possui 3% de proteínas. Os quatro componentes majoritários da amêndoa de pequi são lipídios (51,51%), proteínas (25,27%), carboidratos (8,33%) e fibra alimentar (2,2%), apresentando baixo teor de umidade. (LIMA et al., 2007; PESSOA et al., 2015; TRAESEL et al., 2016). Tanto na polpa como na amêndoa do pequi, predominam os ácidos graxos insaturados com 61,35% e 52,17%, respectivamente, e teor elevado de minerais (cálcio,

ferro, zinco, fósforo, magnésio e potássio) (JOHNER et al., 2018). O ácido oleico está presente em maior concentração na polpa (55,87%), seguido pelo ácido palmítico (35,17%) (MIRANDA-VILELA et al., 2009).

O valor energético da amêndoa do pequi é considerado alto (aproximadamente 600 kcal/100 g), bem como o teor de lipídios (cerca de 50%), e de proteínas (acima de 25%) (TORRES et al., 2016). Os ácidos palmítico e oleico são predominantes, com em quantidades semelhantes, 43,76% e 43,59%, respectivamente. Também estão presentes o ácido linoleico (5,51%), esteárico (2,04%), e palmitoleico (1,23%), além de outros ácidos graxos em menores quantidades. Assim, tanto a polpa como a amêndoa do pequi possuem ácidos graxos importantes para compor uma dieta saudável (LIMA et al., 2007).

3.1.1 Óleo da polpa de pequi

A polpa do pequi apresenta aproximadamente 60% de óleo comestível, sendo que o processo tradicional de extração consiste no cozimento intensivo em água, e posterior retirada do sobrenadante. As principais desvantagens desse método de extração são o baixo rendimento, a necessidade de alta temperatura, e o fato de o óleo não ser filtrado. Para superar essas limitações, a busca de métodos que utilizem temperaturas amenas, e solventes não tóxicos, vem sendo estudados.

A extração do óleo por meio da separação física de fases por centrifugação, possibilita a obtenção de um produto de alta qualidade, já que não utiliza solventes orgânicos e altas temperaturas. Resumidamente, o processo para obtenção do óleo, partindo da polpa do pequi, baseia-se em aquecer a polpa entre 45 °C e 55 °C para facilitar a extração do óleo dos tecidos, centrifugar, e obter o óleo que fica sobrenadante (LIMA et al., 2019). Outro método de extração vantajoso, é a extração com CO_2 supercrítico, que utiliza temperaturas mais amenas (40–60 °C), e apresenta alto desempenho de extração, separação fácil de soluto do solvente, solvente não tóxico, e alta pureza (JOHNER et al., 2018).

O perfil de ácidos graxos presentes no óleo da polpa de pequi apresenta diferenças nas proporções de ácidos graxos saturados e insaturados em função das características do solo onde a árvore foi plantada e, também do processo de extração usado (Tabela 1). De maneira geral, o óleo de pequi apresenta altos de teores de ácidos graxos palmítico (maior que 35%) e oleico (maior que 49%) e baixíssimos teores de ácido linolênico, perfil interessante para as indústrias de alimentos, devido a maior estabilidade oxidativa frente ao aquecimento. Segundo Sprecher (1981), o ácido oleico participa do metabolismo humano e desempenha papel

importante na síntese de hormônios, sendo essencial na nutrição humana, além de ajudar a diminuir os triglicerídeos, colesterol LDL, colesterol total e o índice glicêmico. A presença de ácido oleico também aumenta a estabilidade à oxidação do óleo vegetal (MEZZOMO et al., 2010).

Tabela 1: Perfil de ácidos graxos do óleo da polpa de pequi obtidos por diferentes métodos de extração.

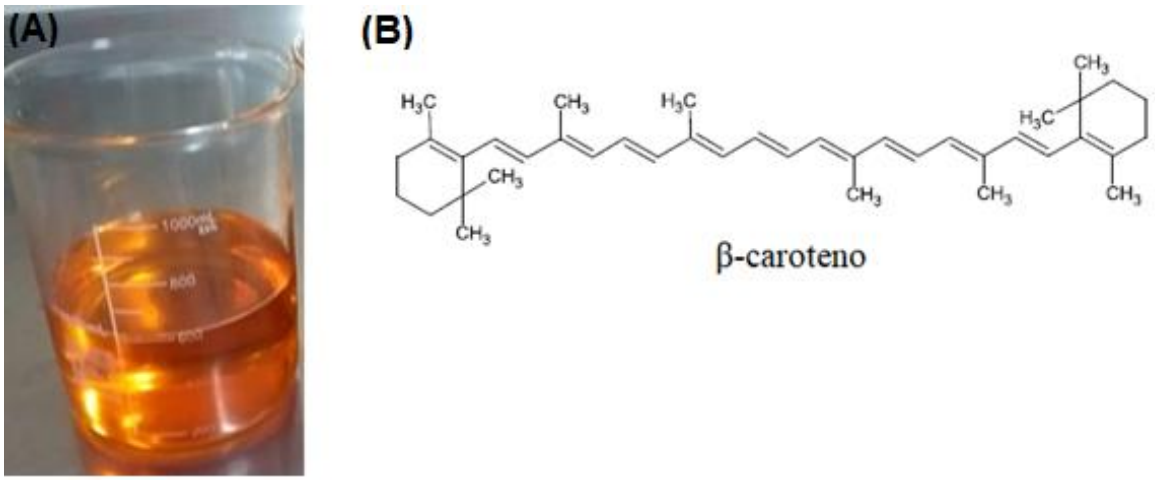
Ácidos graxos	Extração por cozimento em água (FIGUEIREDO et al., 2016)	Extração por solvente (SENA et al., 2010)	Extração fluido supercrítico (PESSOA et al., 2015)	Extração por separação física de fases (*)
Palmítico (C16:0)	34,1	34,18	35,34	32,81
Palmitoleico (C16:1)	0,3	0,27	0,55	0,41
Esteárico (C18:0)	1,7	1,73	1,83	1,58
Oleico (C18:1)	55,8	55,79	58,98	61,41
Linoleico (18:2)	1,8	1,80	2,38	2,32
Linolênico (18:3)	---	--	0,34	0,34

*Resultados obtidos pelo próprio autor.

Fonte: elaborada pela autora.

Precursor da pró-vitamina A, o beta-caroteno é o carotenoide responsável pela pigmentação característica do óleo de pequi (Figura 3) e age como um antioxidante natural presente no óleo, sendo o principal carotenoide encontrado (50% do conteúdo total). Ribeiro et al. (2012) explicaram que o maior teor de beta-caroteno é obtido com a exposição do óleo à temperaturas baixas durante o processo de extração, pois esta condição favorece a preservação dos carotenoides no óleo. As características benéficas dos carotenoides à saúde tornam essencial o consumo de alimentos considerados fontes desses compostos. Todavia, os carotenoides são compostos altamente insaturados e instáveis, o que os torna passíveis de degradação durante o processamento e armazenamento, diminuindo o valor nutricional dos alimentos (MATTA, 2013).

Figura 3: (A) Óleo extraído da polpa do pequi e (B) estrutura química do beta-caroteno.



Fonte: elaborada pela autora.

O óleo, tanto da polpa quanto da amêndoa, pelo seu sabor distinto, e por ser fonte de lipídios e vitaminas antioxidantes (A e E), é bastante utilizado na alimentação, e no preparo de molhos e temperos, acompanhando pratos regionais, e substituindo outras fontes de lipídios (FIGUEIREDO et al., 2016). Devido ao alto teor de ácidos graxos monoinsaturados (ácido oleico), a sua adição na dieta possui efeitos hipolipemiantes, reduzindo o risco de desenvolver doenças cardiovasculares.

O óleo de pequi vem sendo usado na medicina popular para o tratamento de resfriados e gripes, reumatismo, úlceras externas, dores musculares, e inflamação (SERRA et al., 2020). Suas propriedades terapêuticas se devem ao alto teor de fenóis totais, bem como aos seus ácidos graxos, importantes contribuintes para sua atividade antioxidante, e anti-inflamatória (FIGUEIREDO et al., 2016; SARAIVA et al., 2011; SENA et al., 2010). Acredita-se que os ácidos graxos essenciais tenham importante efeito anti-inflamatório no organismo, sendo usados como tratamentos nutricionais para doenças de pele, artrite e doenças respiratórias, como asma (HÄSSIG; LIANG; STAMPFLI, 2000).

3.2 Microencapsulação

A tecnologia de microencapsulação foi apresentada pela primeira vez por Green e Schleicher na década de 1950 com um registro de patente para a preparação de cápsulas contendo corantes, que foram desenvolvidas para serem incorporadas ao papel para fins de cópia (PAULO; SANTOS, 2017). É uma técnica na qual um ou mais compostos (núcleo, fase

interna ou material ativo) são circundados ou imobilizados por um ou mais materiais (casca, matriz encapsulante, material de suporte ou parede) para serem protegidos de fatores externos, como luz, alta concentração de oxigênio, calor, umidade, impedindo a evaporação de compostos voláteis, mascarando sabores e odores desagradáveis, e desenvolvendo valor agregado (COMUNIAN; FAVARO-TRINDADE, 2016). As microcápsulas são geralmente partículas de tamanho variando de 1 a 1000 μm (YE; GEORGES; SELOMULYA, 2018).

A técnica de microencapsulação pode facilitar o manuseio conveniente de materiais, permitindo a conversão de um material líquido/gasoso em sólido (HUANG; YU; RU, 2010). Um benefício adicional da microencapsulação é fornecer uma concentração adequada e dispersão consistente do material do núcleo. Essas funcionalidades têm sido amplamente exploradas na distribuição de medicamentos e vacinas nos setores farmacêuticos, e estão cada vez mais sendo utilizadas para agregar valor a novos produtos na indústria de alimentos (DHAKAL; HE, 2020).

A microencapsulação é um método multidisciplinar que envolve conhecimentos e de física, físico-química, química de polímeros, química de coloides, bioquímica, biotecnologia e ciência dos materiais (DHAKAL; HE, 2020). Existem diversos métodos de microencapsulação, e podem ser divididos em (OZKAN et al., 2019): métodos físicos (spray drying, spray chilling, liofilização e leito fluidizado) métodos físico-químicos (coacervação simples, coacervação complexa, gelificação iônica e deposição eletrostática camada-por-camada, evaporação de solvente, envolvimento lisossômico) e químicos (polimerização interfacial, polimerização *in situ*, e inclusão molecular).

Esta abordagem tecnológica tem sido explorada pelas indústrias farmacêutica (68%), alimentícia (13%), cosmética (8%), têxtil (5%), biomédica (3%), agrícola (2%) e eletrônica (PAULO; SANTOS, 2017). A microencapsulação visa aumentar a eficácia de substâncias selecionadas na indústria, e vários autores têm discutido as principais vantagens da sua aplicação em diferentes setores da indústria. No entanto, as indústrias farmacêutica e alimentícia são as principais forças motrizes nos avanços da microencapsulação.

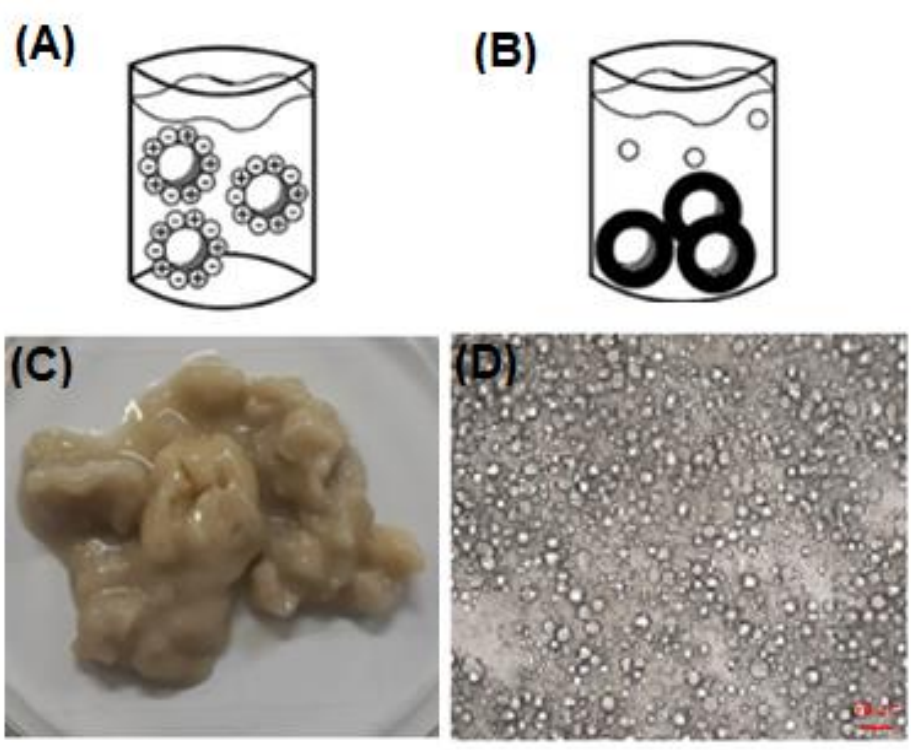
3.2.1 Coacervação complexa

Coacervação é um termo usado na química coloidal para denotar o processo de separação de fase associativo, induzido pela modificação do ambiente do meio (pH, força iônica, temperatura, solubilidade), sob condições controladas (TIMILSENA et al., 2017, 2019). A coacervação complexa é um fenômeno de separação de fase líquido-líquido que ocorre entre

biopolímeros de cargas opostas, por meio de interação eletrostática (EGHBAL; CHOUDHARY, 2018), impedindo que os compostos interajam fisicamente e/ou quimicamente com o ambiente externo, criando uma barreira ao redor do material ativo (SOUZA et al., 2020).

O processo de coacervação complexa (Figura 4) é um sistema trifásico que envolve o solvente, o material ativo, e o material de revestimento. Em geral, este processo envolve quatro etapas: (i) preparação de uma solução aquosa de dois ou mais polímeros e mistura da fase hidrofóbica com a solução aquosa de um polímero (geralmente solução proteica) e homogeneização da mistura resultante, de modo a produzir uma emulsão estável; (ii) mudança de pH, onde cada polímero irá assumir as respectivas cargas efetivas; (iii) mudança de temperatura para separação de fases; e (iv) endurecimento dos polímeros utilizando temperatura elevada, agente de dessolvatação ou reticulador (DESAI; PARK, 2005; TIMILSENA et al., 2019).

Figura 4: (A) Esquema representativo, onde (A) é interação eletrostática para formação das microcápsulas pelo método de coacervação complexa e (B) é a precipitação dos coacervados. (C) Imagem real dos cocervados formados e (D) micrografias das microcápsulas formadas.



Fonte: elaborada pela autora.

A coacervação complexa é conhecida por sua simplicidade, baixo custo, escalabilidade, e reprodutibilidade no encapsulamento de ingredientes alimentícios, o que resulta em alta eficiência de encapsulação, mesmo com capacidade de carga muito alta (até 99%). Timilsena et al. (2019) relataram que as características das matrizes encapsulantes usadas no processo de encapsulamento afetam significativamente a estabilidade do material ativo, a eficiência do processo, e o grau de proteção dos componentes ativos.

Vários parâmetros operacionais influenciam a complexação dos polímeros por meio de interações eletrostáticas (força iônica), pH, razão entre matrizes encapsulantes, concentração do material da parede, distribuição de carga, homogeneização, solubilidade das macromoléculas, peso molecular dos biopolímeros, e propriedades físicas e químicas das microcápsulas. Portanto, uma maior compreensão desses parâmetros é fundamental para uma melhor coacervação, e sua aplicação de forma mais eficiente (BEN AMARA et al., 2017; EGHBAL; CHOUDHARY, 2018; MA et al., 2019; RIOS-MERA et al., 2019; ZHANG, G. et al., 2009). Embora a força eletrostática entre macromoléculas com carga oposta seja a principal força motriz, outras forças como ligações intermoleculares de van der Walls, e interações hidrofóbicas nas proteínas, também afetam o complexo processo de coacervação (TIMILSENA et al., 2017; TURGEON; SCHMITT; SANCHEZ, 2007).

Essa técnica de microencapsulação tem sido particularmente bem-sucedida na estabilização de lipídios insaturados, e no fornecimento de um ingrediente com maior prazo de validade sensorial (BARROW; NOLAN; JIN, 2007). Por ser um método bastante recomendado para a microencapsulação de substâncias lipofílicas, vários trabalhos utilizam este método para encapsular óleos vegetais (tabela 2).

Tabela 2: Trabalhos recentes de microencapsulação de óleo vegetais por coacervação complexa.

Material encapsulante	Material ativo	Finalidade	Referência
Goma de cajueiro/gelatina	Óleo de pequi	Otimizar os parâmetros do processo de formação das microcápsulas	(NASCIMENTO et al., 2020)
Ovalbumina/alginato de sódio	Óleo de sacha inchi	Proteger os compostos bioativos e estudo térmico	(SOARES et al., 2019)
Goma arábica/gelatina	Óleo de pequi	Avaliar a influência da temperatura, velocidade de agitação com o objetivo de preservar os carotenoides presentes no óleo	(JUSTI; SANJINEZ-ARGANDOÑA; MACEDO, 2018)
Goma de cajueiro/quitosana	Óleo de pequi	Otimizar os parâmetros do processo de formação das microcápsulas	(SILVA et al., 2018)
Quitosana/xantana e quitosana/pectina	Óleo de palma	Aplicar as micropartículas ricas em carotenoides em alimento (pão e iogurte)	(RUTZ et al., 2017)
Gelatina/alginato	Óleo de buriti	Estudar influência da velocidade da agitação no tamanho das microcápsulas	(LEMOS; MARIANO MARFIL; NICOLETTI, 2017)
Isolado de proteína de linhaça/goma de linhaça	Óleo de linhaça	Comparar os métodos de secagem na estabilidade oxidativa	(KAUSHIK et al., 2016)
Proteína da semente de chia/Goma de semente de chia	Óleo de chia	Melhorar a estabilidade oxidativa do óleo	(TIMILSENA et al., 2016)

Fonte: elaborada pela autora.

3.2.2 Secagem por liofilização

A liofilização é uma das técnicas comumente utilizadas para secagem de microcápsulas termossensíveis produzidas por coacervação complexa, proporcionando para as micropartículas alta qualidade de estabilidade térmica e oxidativa, e estendendo seu tempo de estocagem (BALLESTEROS et al., 2017; GONZÁLEZ et al., 2016). Durante o processo de liofilização, a temperatura do material é reduzida abaixo do seu ponto de congelamento, e a água é removida por sublimação de gelo em vapor de água, a pressões inferiores às do ponto triplo da água (FENG et al., 2020).

O processo de liofilização pode ser dividido em três etapas: congelamento, secagem primária, e secagem secundária (ASSEGEHEGN et al., 2019). Durante a etapa de congelamento a maior parte da água é convertida em sólido, onde se formam redes de cristais de gelo. É nessa etapa que é determinada a morfologia do material, o tamanho, e a distribuição do tamanho dos cristais de gelo, que por sua vez influenciam vários parâmetros críticos, como resistência do produto seco, taxas de secagem primária e secundária, extensão da cristalinidade do produto, área de superfície, e reconstituibilidade do produto seco (ASSEGEHEGN et al., 2019, 2020; SEARLES; CARPENTER; RANDOLPH, 2001).

Durante a secagem primária, a água livre congelada é removida pela conversão do gelo em vapor (sublimação). O processo de secagem é em função da temperatura da prateleira e da pressão da câmara, e a escolha apropriada desses dois parâmetros pode encurtar essa fase (BJELOŠEVIĆ et al., 2020). A última etapa do processo de liofilização é a secagem secundária, onde ocorre a remoção da água absorvida do produto. Durante a secagem secundária, a temperatura da prateleira aumenta ainda mais, enquanto a pressão é mantida constante ou, em alguns casos, é menor, e então a água sorvida na matriz do soluto é reduzida por dessorção (ISHWARYA; ANANDHARAMAKRISHNAN; STAPLEY, 2015; LIAPIS; BRUTTINI, 2009).

A principal vantagem do processo de liofilização é a obtenção de um produto de alta qualidade, devido à baixa indução de degradação térmica, à retenção de materiais voláteis responsáveis pelo aroma e sabor, e à estrutura rígida do material seco (LIAPIS; LITCHFIELD, 1979). O produto liofilizado possui uma textura porosa, sendo prontamente reconstituído quando imerso em água, ao seu respectivo tamanho e forma originais e possui estabilidade durante o armazenamento (MARQUES; SILVEIRA; FREIRE, 2006).

Dentre as desvantagens verificadas estão os custos elevados, devido ao processo de congelamento, a utilização de pressão reduzida, e o tempo maior que demanda em relação a outras técnicas, tornando o uso da liofilização na indústria de alimentos limitada aos produtos de alto valor agregado (RATTI, 2001).

3.2.3 Liberação controlada

O mecanismo de liberação do núcleo no local, e no momento adequado, é tão importante quanto a capacidade de proteção (DHAKAL; HE, 2020). As propriedades particulares da rede polimérica, tais como comprimento da cadeia, flexibilidade e mobilidade, absorção de água e comportamento de dilatação, extensão da plastificação, ou potenciais interações entre o polímero e o agente ativo, afetarão a taxa de difusão através da matriz polimérica e, portanto, a liberação do núcleo (MARTINS et al., 2014).

Os mecanismos de liberação variam com o tipo de matriz encapsulante usado para inserir ou encapsular o agente ativo, o método de preparação e o ambiente onde ocorre a liberação. Nos mecanismos de liberação, processos de difusão, biodegradação, dilatação (com formação de gel e inchaço) e osmose são os mais comumente estudados (ESTEVINHO et al., 2013). A classificação dos mecanismos de liberação é baseada nos fenômenos físico-químicos que promovem a liberação do agente ativo. Eles podem atuar individualmente ou combinados e incluem sistemas controlados por difusão, por barreira, ativados por pressão, por solvente, controlados osmoticamente, por pH, sensíveis à temperatura, ativados por fusão e combinados (GONÇALVES; ESTEVINHO; ROCHA, 2016).

Na área alimentícia os mecanismos de liberação mais relevantes são por ação do solvente ou por difusão, sendo este último considerado um dos mais relevantes (AZEREDO, 2005). A difusão consiste no deslocamento do agente ativo através da matriz, passando de uma região mais concentrada para uma de menor concentração (RODRÍGUEZ et al., 2016) e ocorre especialmente quando a micropartícula está intacta (SILVA; VIEIRA; HUBINGER, 2014), sendo controlada pelas limitações da transferência de massa na camada entre a matriz polimérica e o meio.

A ordem e o mecanismo de liberação das micropartículas podem ser descritos através da cinética de liberação, e avaliados na forma de diversos modelos matemáticos, sendo que as microcápsulas que tem como mecanismo de liberação a difusão, têm como modelos mais comumente utilizados, os de ordem zero, primeira ordem, Higuchi e Korsmeyer-Peppas (ANSARIFAR et al., 2017; CAMPELO-FELIX et al., 2017; ZANDI et al., 2017). Esses

modelos matemáticos se baseiam na lei de difusão de Fick e, dependendo do valor de n (coeficiente de difusão), podemos classificar o mecanismo de liberação nos seguintes casos: caso I, onde $n < 0,43$ ocorre liberação de acordo com a lei de difusão de Fick, seguindo um gradiente de potencial químico; caso II, onde $0,43 < n < 0,85$ o sistema de liberação é anômalo (não seguindo a lei de Fick); caso III, onde $n > 0,85$ ocorre uma liberação acerelada pela erosão ou intumescimento (molhabilidade) dos polímeros (CAMPELO et al., 2018).

3.3 Matriz encapsulante

Uma seleção cuidadosa do material da parede é vital, pois impõe a eficiência de encapsulação e a estabilidade da microcápsula. O material encapsulado mais adequado para aplicações em alimentos deve ter as seguintes propriedades: não reativo com o núcleo, capaz de selar e reter o núcleo intacto dentro da cápsula, capaz de proteger eficazmente o núcleo contra condições ambientais adversas, sem qualquer sabor desagradável, comestível e não tóxico e economicamente viável (DHAKAL; HE, 2020; MCCLEMENTS; DECKER; WEISS, 2007; NAZZARO et al., 2012). Dentre os materiais mais utilizados como matriz encapsulante estão os polissacarídeos e as proteínas:

- Polissacarídeos: compostos de fibra vegetal (inulina, pectina, β-glucana, polissacarídeo solúvel de soja), fibra animal (quitosana), gomas (guar, locusta, xantana, carragena, ágar, furcelanos, alginatos, gelana, curdlana, arábica, acácia e cajueiro), amido e derivados.
- Proteínas: proteína de soro de leite, gelatina, proteínas de sementes oleaginosas (de soja, canola, gergelim, girassol), zeína, proteína de ervilha, proteínas de arroz e glúten de trigo.
- Outros: fermento, ceras, lipídios.

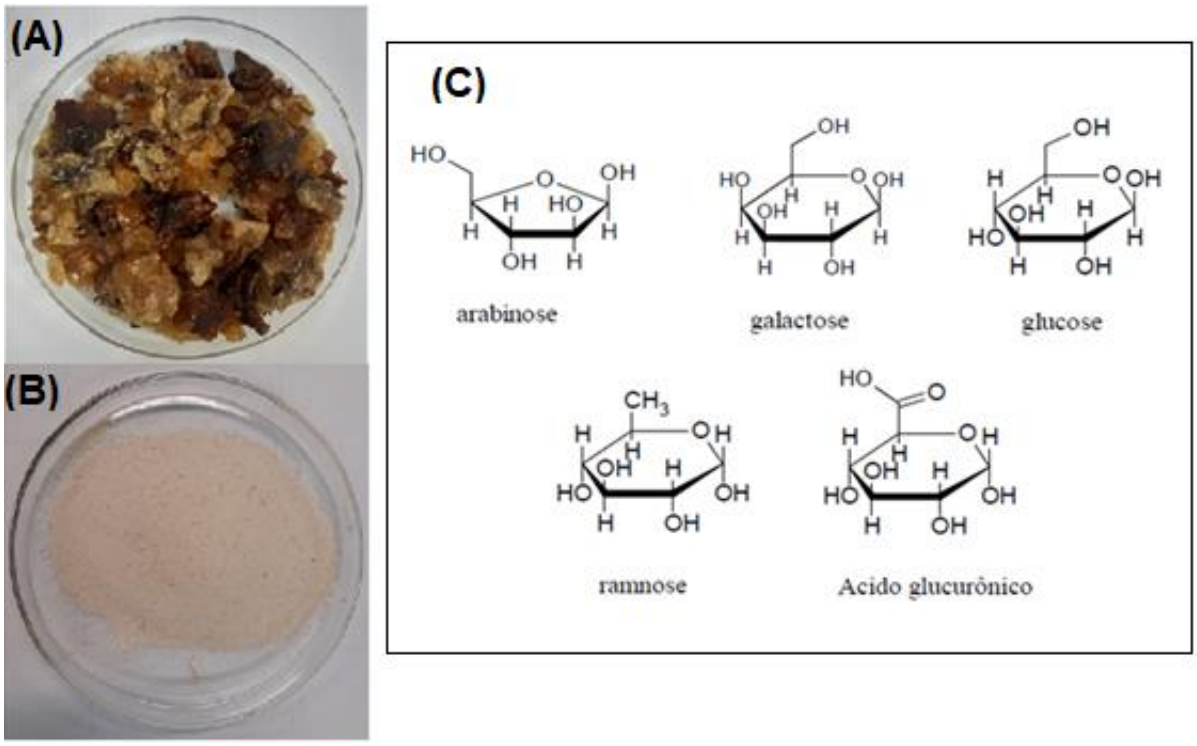
Nos últimos anos, o desejo dos pesquisadores de usar materiais alternativos em vez de materiais convencionais de parede, especialmente maltodextrina e goma arábica, levou ao uso de novos grupos de materiais de parede, que podem ser categorizados como materiais de parede “verdes”. Na verdade, o uso desses componentes oferece oportunidades para valorizar os subprodutos/resíduos alimentares de alto valor agregado. A utilização de subprodutos/resíduos para a produção desses compostos está de acordo com a tendência de produção sustentável. Além disso, alguns novos métodos de produção foram desenvolvidos para produzir componentes verdes a partir do subproduto/resíduo, levando em consideração as

questões ambientais e energéticas, que contribuem para a sustentabilidade de tais abordagens (SAMBORSKA et al., 2021).

3.3.1 Goma de cajueiro

A goma de cajueiro (figura 5) é produzida a partir do exsudato extraído da *Anarcadium occidentale* L., uma árvore popular conhecida como cajueiro. O cajueiro é uma árvore rústica que pode atingir 3,6 metros de altura e é facilmente encontrada em países tropicais como o Brasil, principalmente nas regiões Norte e Nordeste, em Estados como Piauí, Ceará e Rio Grande do Norte (RIBEIRO, A. J. et al., 2016).

Figura 5: (A) Exsudato (goma bruta), (B) polissacarídeo isolado (goma de cajueiro) e (C) principais estruturas químicas constituintes.



Fonte: elaborada pela autora.

O exsudato do cajueiro é obtido naturalmente pelas células epiteliais da casca após incisões ou em resposta a ataques de insetos e patógenos no tronco e nos ramos das árvores, produzindo assim a goma ou resina. Sua produção média é de 700 g por árvore/ano, com potencial de produção anual de até 38.000 t. A obtenção do exsudato pode ocorrer em todas as

partes da árvore e sua composição qualitativa e quantitativa depende da maturidade da árvore e das condições ambientais (KUMAR et al., 2012). A extração é realizada fisicamente por meio de incisões na casca ou quimicamente pela introdução na casca de substâncias como óxido de etileno, derivados do ácido benzóico e ácido 2-cloroetilfosfônico.

O exsudato, uma massa resinosa marrom, pode ser refinada por meio de solubilização, centrifugação, filtração e precipitação em etanol (TORQUATO et al., 2004). Em seguida, ele passa por uma etapa de secagem resultando em um pó amarelo rico em polissacarídeos. É um heteropolissacarídeo complexo constituído de uma cadeia principal de D-galactose (72%) com ramificação de D-glucose (14%), arabinose (4,6%), ramnose (3,2%) e ácido glucurônico (4,7%) (PAULA; RODRIGUES, 1995; PAULA; HEATLEY; BUDD, 1998).

As vantagens das gomas naturais incluem ser barato, biocompatível, processamento ecologicamente correto e alta biodisponibilidade (KUMAR et al., 2012). A goma de cajueiro tem sido estudada por suas propriedades funcionais, incluindo ser não tóxica, biodegradável e biocompatível (DIAS et al., 2016). Essa goma também exibe comportamento terapêutico, incluindo atividade anti-inflamatória (CARVALHO et al., 2015), efeitos antimicrobianos (CAMPOS et al., 2012; TORQUATO et al., 2004), cura natural (ARAGÃO-NETO et al., 2017; MONTEIRO et al., 2007), um aglutinante para comprimidos farmacêuticos (GOWTHAMARAJAN et al., 2011) e um imobilizador de enzima (SILVA et al., 2010). Além disso, a goma de cajueiro tem sido usada na indústria de alimentos e bebidas como um agente espessante e estabilizador coloidal (GOMEZ-ESTACA et al., 2016; KUMAR et al., 2012; PORTO; CRISTIANINI, 2014).

Nos últimos anos, a goma de cajueiro mostrou um potencial promissor nas indústrias farmacêutica e de biotecnologia (FORATO et al., 2015; PRAJAPATI et al., 2013). No campo biomédico e nanotecnologia, a goma de cajueiro tem sido usada para montar filmes (DIAS et al., 2016), sensores ultrafinos (BITTENCOURT et al., 2016; TEIXEIRA et al., 2015) e entrega de drogas sistemas (CORDEIRO et al., 2017; MONTEIRO et al., 2007). Em relação a este último, a goma de cajueiro tem sido utilizada como aglutinante para a produção de formulações farmacêuticas adesivas bucais, evitando o metabolismo hepático, melhorando a biodisponibilidade dos princípios ativos (GOWTHAMARAJAN et al., 2011).

A goma de cajueiro também já vem sendo estudada nas tecnologias de microencapsulação, principalmente como potencial substituto da goma arábica. A tabela 3, apresenta os principais trabalhos encontrados na literatura que utilizam a goma de cajueiro em diferentes técnicas de microencapsulação.

Tabela 3: Artigos científicos que utilizam a goma de cajueiro na tecnologia de microencapsulação.

Material encapsulante	Material ativo	Método de encapsulação	Objetivo	Referência
Goma de cajueiro	Beta-caroteno dissolvido no óleo de rícino	Eletropulveriza-ção	Produzir e caracterizar com futuro potencial em aplicar nas áreas agrícolas, alimentícias e farmacêuticas	(VÁZQUEZ-GONZÁLEZ et al., 2021)
Goma de cajueiro/gelatina	Óleo de café verde	Coacervação complexa	Produzir, caracterizar e aplicar em sucos	(DE OLIVEIRA, W. Q. et al., 2020)
Gelatina/ (goma arábica, pectina, goma de cajueiro, carboximetilcelulose e κ-carragenina)	Extrato de canela	Coacervação complexa	Caracterizar (estabilidade dos compostos fenólicos) e aplicar em sorvetes (mascarar sabor e adstringência)	(SOUZA et al., 2020)
Goma de cajueiro/gelatina	Óleo de pequi	Coacervação complexa	Otimizar os parâmetros do processo de formação das microcápsulas	(NASCIMENTO et al., 2020)
Goma de cajueiro e maltodextrina	Extrato de chá verde	Spray drying	Proteger os compostos bioativos e potencial como ingrediente alimentar	(SILVA, F. et al., 2018)

Goma de cajueiro	d- limoneno	Emulsão (produzidas por DHP)	Estudar o efeito do DHP nas propriedades da emulsão e de encapsulação	(PORTO; CRISTIANINI, 2018)
Goma de cajueiro/quitosana	Óleo de pequi	Coacervação complexa	Otimizar os parâmetros do processo de formação das microcápsulas	(CARVALHO DA SILVA et al., 2018)
Goma de cajueiro, amido modificado e goma arábica	Óleo de peixe	Spray drying	Avaliar a goma de caju em comparação com os materiais convencionais em relação às propriedades e estabilidade oxidativa do óleo	(BOTREL et al., 2017)
Goma de cajueiro/gelatina	Extrato lipídico contendo astaxantina de resíduos de camarão	Coacervação complexa	Caracterizar e estudar a capacidade como corante em aplicações em Iogurtes	(GOMEZ-ESTACA et al., 2016)
Goma de cajueiro e inulina	Óleo essencial de gengibre	Spray drying	Avaliar o efeito da substituição parcial da goma de caju pela inulina sobre as características do óleo essencial	(FERNANDES et al., 2016)

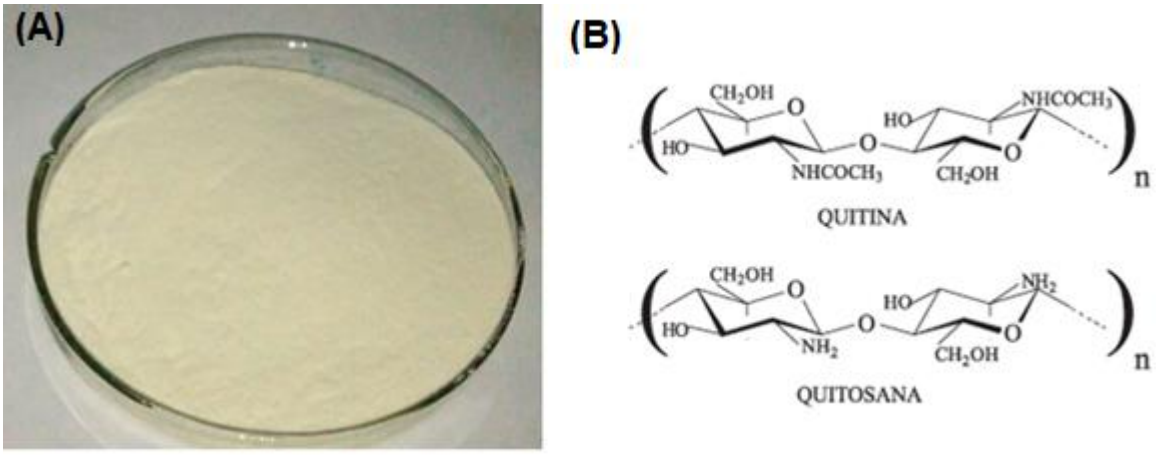
Alginato/goma de cajueiro	Óleo essencial de Lippia sidoides	Spray drying	Produzir, caracterizar e estudar a cinética de liberação	(OLIVEIRA, E. F.; PAULA; PAULA, 2014)
Alginato/goma de cajueiro	Óleo essencial de Lippia sidoides	Gelificação ionotrópica	Produzir, caracterizar e estudar a cinética de liberação in vitro e in vivo	(PAULA, H. C. B. et al., 2012)
Goma de cajueiro	Extrato de café	Spray drying	Proteger os compostos voláteis	(RODRIGUES; GROSSO, 2008)

Fonte: elaborada pela autora.

3.3.2 Quitosana

Quitosana é segundo polímero natural mais obtido na natureza, depois da celulose, e é um polissacárido linear de amino de composto β-1,4 ligado a 2-(acetilamino)-2-desoxi-D-glucose (WANG et al., 2021). Obtido pela desacetilição da quitina extraída do exoesqueleto de crustáceos, insetos e parede celular de fungos, a quitosana (Figura 6) é um amino polissacarídeo natural de carga positiva (WANG et al., 2021). Esse polímero tem sido amplamente estudado como material de parede para microencapsulação de compostos que deveriam ter liberação controlada, como fármacos, óleos e compostos bioativos, com destaque para aqueles de caráter lipofílico. É usado sozinho ou em combinação com outros polímeros, como alginato, goma xantana, ciclodextrinas, oligossacarídeos e proteínas de soro de leite (CHANG et al., 2020; JANTARATHIN; BOROMPICHAICHARTKUL; SANGUANDEEKUL, 2017; RIBEIRO, J. S.; VELOSO, 2021; RUDKE et al., 2019).

Figura 6: (A) Quitosana obtida pela desasetilação da quitina e (B) estrutura química da quitina e da quitosana.



Fonte: elaborada pela autora.

A quitosana consiste em resíduos de glucosamina e N-acetil glucosamina. Tem uma excelente capacidade de formação de complexos, que pode ser atribuída principalmente à disponibilidade de grupos -NH_2 livres distribuídos ao longo da cadeia principal da quitosana (YU et al., 2021). A abundância do grupo amina faz com que o polímero forme uma rede semicristalina emaranhada de ligações hidrofóbicas e de hidrogênio no estado sólido e contribui para uma boa solubilidade em condições ácidas (LIM; HWANG; LEE, 2021).

A molécula de quitosana não é anfifílica típica, mas os componentes da molécula são desacetilados (hidrofílicos) e resíduos de D-glucosamina acetilados (hidrofóbicos) que definem as propriedades ativas de superfície da quitosana. Dependendo do grau de desacetilização, a molécula fica mais ou menos hidrofóbica. A presença de porções hidrofóbicas e hidrofílicas na estrutura molecular favorece interações entre polímeros (MILKOVA, 2021).

A quitosana tem muitas aplicações potenciais na indústria por causa de propriedades físico-químicas e biológicas únicas. Dentre elas as mais estudadas são sua biodegradabilidade, biocompatibilidade, capacidade de quelação de íons de metais pesados, propriedades de formação de gel, baixa toxicidade e baixo custo (BUDINČIĆ et al., 2021), e bioatividades, como antioxidante, antimicrobiana, antifúngica, antitumoral, antialérgica, ativadora do sistema imunológico, anti-hipertensiva e redutora do colesterol. Essas propriedades funcionais podem depender de características estruturais, como peso molecular médio, grau de desacetilação, padrão de acetilação, polidispersidade e pureza. Além disso, a quitosana pode ser facilmente derivatizada para potencializar suas propriedades, como

aumentar a estabilidade em solução, atividades antimicrobianas e antioxidantes (ROCHA; COIMBRA; NUNES, 2017).

Em comparação com os polímeros sintéticos orgânicos na área têxtil e médica, a quitosana tem propriedades diferenciadas, como excelente aceitabilidade ambiental e não corrosividade. Além disso, a quitosana tem excelente desempenho de formação de filme e é atualmente aplicada na distribuição de drogas e nos campos alimentícios (HUO et al., 2018). A quitosana também tem sido amplamente utilizada na indústria farmacêutica e alimentícia como um transportador para a entrega de medicamentos. O uso de microesferas de quitosana como sistemas de liberação controlada de fármacos convencionais, fármacos protéicos e compostos bioativos, vem atraindo cada vez mais atenção desde o início dos anos 90 (ESTEVINHO et al., 2013).

A quitosana é considerada um polímero promissor como material de revestimento para encapsulamento com potencial aplicação (ROCHA; COIMBRA; NUNES, 2017). Tem sido frequentemente usada como um material de parede, bem como um emulsificante para microencapsulação de óleos insaturados (SHEN et al., 2010). Foi observado que, sendo um polissacarídeo policatiônico, a quitosana envolve as gotículas da emulsão óleo-em-água (o/w) e repele metais pró-oxidantes no ambiente circundante (KLINKESORN et al., 2005). Curiosamente, a quitosana também é um polissacarídeo natural biofuncional, muitos pesquisadores relataram que a suplementação dietética de quitosana pode resultar em efeitos de promoção da saúde, como atividades hipolipidêmicas e cardioprotetoras (ANANDAN et al., 2015; VISHNU et al., 2017; ZHANG, W. et al., 2013).

3.3.3 Gelatina

A gelatina é uma mistura de vários polipeptídeos de alto peso molecular obtidos por meio da hidrólise controlada do colágeno fibroso insolúvel em ossos, couros, peles, tendões de animais (ARSYANTI et al., 2018; JANNAT et al., 2020). Devido às suas características tecnológicas e funcionais únicas, a gelatina é amplamente utilizada nas indústrias alimentícia, farmacêutica, médica, cosmética e fotográfica, como espessante, agente clarificante, emulsificante e formador de gel. A produção mundial anual de gelatina foi de aproximadamente 326.000 toneladas em 2008, e a demanda global por gelatina ainda está aumentando (JANNAT et al., 2020). Os recursos mais importantes da produção de gelatina são pele de porco (46%), pele de gado (29%), e ossos de porco e gado (23%) (AYKIN-DINÇER; KOÇ; ERBAS, 2017).

Durante a extração da gelatina, os tecidos animais crus são submetidos a sucessivos tratamentos com ácido ou base diluída, resultando na clivagem parcial das ligações cruzadas das proteínas. A conversão do colágeno em gelatina gera moléculas de diferentes massas, devido à clivagem das ligações cruzadas covalentes inter-cadeias, e à quebra desfavorável de algumas ligações peptídicas intracadeia (ZHOU; MULVANEY; REGENSTEIN, 2006). O grau de conversão é dependente da intensidade do pré-tratamento ou da extração com água quente (SAMBORSKA et al., 2021).

O ponto isoelétrico da gelatina pode variar dependendo da fonte obtida, devido a pequenas variações dos grupos amida. A gelatina tipo A, que é fabricada a partir da hidrólise ácida, apresenta um valor do ponto isoelétrico próximo da condição alcalina (pH 7 a 9). Pelo contrário, a gelatina tipo B, fabricada a partir da hidrólise alcalina, mostra o valor do ponto isoelétrico próximo da condição ácida (pH 4 a 5) (ALI; KISHIMURA; BENJAKUL, 2018; COMUNIAN; FAVARO-TRINDADE, 2016).

As propriedades reológicas e funcionais da gelatina, como força do gel, viscosidade, gelificação e ponto de fusão, estão relacionadas às suas características químicas (ZHANG, G. et al., 2009). As propriedades físico-químicas mais importantes da gelatina são a força do gel e a viscosidade. A qualidade da gelatina é medida como a força do gel ou valor de Bloom, que pode ser classificado como baixo (<150), médio (150–220) e alto Bloom (220–300) (KAEWRUANG et al., 2014).

A gelatina tem sido amplamente utilizada como ingrediente em cápsulas moles e duras e como suplementos alimentares (YAP; GAM, 2019). Ainda é a principal escolha comercial como material de parede devido à sua excelente solubilidade em água, capacidade de emulsificação e espessamento, e alta atividade de reticulação devido à presença de grupos amino primários (PAULA, D. de A. et al., 2019).

3.4 Aplicação da microencapsulação na área de alimentos

Uma variedade de alimentos funcionais e suplementos dietéticos foram introduzidos no mercado nos últimos anos, oferecendo excelentes perspectivas para a melhoria da saúde pública. No entanto, a maior preocupação é que os compostos bioativos podem não ser totalmente absorvidos pelo corpo porque foram degradados antes de chegarem ao órgão-alvo. Por exemplo, a eficácia dos compostos bioativos pode ser reduzida devido à sua instabilidade causada por condições ácidas severas, reações enzimáticas, tempo de residência insuficiente e baixa permeabilidade no trato gastrointestinal (LEE et al., 2012). Por isso, para melhoria da

estabilidade do composto bioativo e controle da sua liberação, vem sendo empregada a tecnologia de encapsulação.

Para aplicações em alimentos, há muitos desafios no desenvolvimento de sistemas de microencapsulação eficientes e adequados. Por isso alguns critérios são utilizados para seleção da matriz encapsulante, assim como da técnica de microencapsulação, como por exemplo (DHAKAL; HE, 2020):

- Capacidade de proteger os núcleos de elementos ambientais no processamento de alimentos, incluindo alta temperatura, pressão, tensões mecânicas, oxigênio, acidez e alcalinidade, umidade e outros compostos reativos;
- Não utilização de calor e estresse mecânico;
- Digestível no sistema digestivo humano;
- Seguro para consumo humano, idealmente derivado de ingredientes comestíveis naturais;
- Facilidade na produção em escala industrial;
- Idealmente inodoro e incolor;
- Boa solubilidade em água;
- Viabilidade econômica.

Já foram relatados diversos usos da microencapsulação na aplicação em alimentos. Nesse sentido, pode-se citar uso de microcápsulas em bala de geleia (MOURA et al., 2019), sucos (OLIVEIRA, et al., 2020), biscoitos (GÓMEZ-MASCARAQUE et al., 2017), licores (JIANG; ZHU, 2019), chá (HERNÁNDEZ-BARRUETA et al., 2020) e iogurtes (COMUNIAN et al., 2016; RUTZ et al., 2017).

Os produtos lácteos são uma excelente fonte de proteínas de alta qualidade, cálcio, potássio, fósforo, magnésio, zinco e vitaminas B, como riboflavina, niacina, vitamina B6 e vitamina. As proteínas do iogurte são de excelente qualidade biológica, assim como as do leite, porque o valor nutritivo de suas proteínas é bem preservado durante o processo de fermentação (SENGUPTA et al., 2016). Os iogurtes são os produtos derivados do leite mais populares, produzidos por acidificação do leite ou por fermentação da lactose por bactérias iniciadoras, nomeadamente *Streptococcus thermophilus* e *Lactobacillus delbrueckii* subsp. *bulgaricus* (GHARIBZAHEDI; CHRONAKIS, 2018; LADJEVARDI; GHARIBZAHEDI; MOUSAVI, 2015).

O mercado global de iogurtes foi avaliado em 2019 em US $ 99.553,38 milhões, e está estimado em US $ 141.829,95 milhões até 2025, com taxa de crescimento anual composta

(CAGR) de 6,25%, de 2020 a 2025. O mercado de iogurtes tem visto um crescimento significativo devido ao aumento de consumidores preocupados com a saúde. Consequentemente, o mercado de iogurtes tem apresentado várias opções saudáveis, e sabores para satisfazer as preferências do consumidor (RIBEIRO, B.; BONIF, 2021). Um grande número de estudos mostrou que há muitos benefícios para a saúde com o consumo de iogurtes, incluindo melhora na tolerância à lactose e função imunológica, redução dos níveis de colesterol sérico e pressão arterial, controle de infecções gastrointestinais e sintomas do intestino irritável, aumento de nutrientes 'biodisponibilidade e indução de atividades anticarcinogênicas, antimicrobianas, antioxidantes e hipoalergênicas (YU et al., 2021).

Na última década, houve um interesse notável em fortificar leite e produtos lácteos com uma ampla variedade de ingredientes bioativos. O iogurte é um produto alimentar muito popular, destacando-se como um dos produtos lácteos fermentados mais consumidos no mundo. Devido ao seu alto consumo, pode ser um bom veículo para garantir e melhorar a ingestão diária de nutrientes, o que pode prevenir doenças e trazer impactos positivos na saúde dos consumidores (SILVA, et al., 2019). Assim, a incorporação de ingredientes funcionais em matrizes alimentares, como iogurte, tem sido realizada para fins selecionados, como a incorporação de curcumina (E100) (ALMEIDA et al., 2018), óleo de echium encapsulado, fitoesteróis e ácido sinápico (COMUNIAN et al., 2017), óleo de peixe encapsulado (GHORBANZADE et al., 2017), e aditivos naturais de camomila e erva-doce (CALEJA et al., 2016).

Dentre os componentes alimentares que menos estão presentes no iogurte, destacam-se a fibra alimentar, os compostos fenólicos, e os ácidos graxos insaturados. Dietas com alto teor desses componentes desempenham um papel significativo na prevenção de várias doenças (ROMÁN et al., 2019). Como resultado, fontes naturais como frutas e cereais têm sido usadas para fortificar iogurtes com fenólicos (HELAL; TAGLIAZUCCHI, 2018) e fibra alimentar (HASHEMI GAHRUIE et al., 2015). No entanto, foram relatadas pesquisas limitadas sobre a fortificação de iogurte com ácidos graxos insaturados (monoinsaturados e/ou poli-insaturados) para melhorar seu perfil lipídico (BABA et al., 2018; BELLO et al., 2015). Entre os poucos estudos relatados para melhorar o perfil de ácidos graxos de iogurtes foram geralmente usados óleos vegetais e óleos de peixe (BABA et al., 2018; BELLO et al., 2015; NIEUWENHOVE et al., 2019; RIBEIRO, B.; BONIF, 2021).

4 ARTIGOS

4.1 CAPÍTULO I - Microencapsulation methods for vegetable oils: principles, stability, and applications - a review

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Microencapsulation methods for vegetable oils: principles, stability, and applications - a review

Running head: Microencapsulation methods for vegetable oils

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SUMMARY

In addition to being used in food, fuel, and lubricants, vegetable oils are promising in many additional applications such as food additives, nutritional supplements, cosmetics, and biomedicine; however, their low oxidative stability can limit their use. Microencapsulation is a well-established methodology for the preservation of oils against degradation, controlled release of active ingredients, protection against external factors during storage, and enhanced durability. In this article, microencapsulation methods for vegetable oils are reviewed, including physical methods (spray drying and freeze-drying), physical-chemical methods (complex coacervation, ionic gelation and electrostatic layer-by-layer deposition), and chemical methods (interfacial/*in situ* polymerization). This article also provides information on the principles, parameters, advantages, disadvantages, and applications of these methods.

Key words: Microparticles; vegetable oils; oxidative stability; fatty acids; controlled release.

INTRODUCTION

Vegetable oils are gaining a lot of attention in commercial development these days because of their availability, low price, biodegradability, and mild environmental impact. Vegetable oils contain, as their main components, triglycerides-esters of glycerol with three long-chain fatty acids *(1, 2)*. The fatty acids can be the same or different in terms of hydrocarbon chain formed from 10 to 22 carbon

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atoms. In addition, the location and number of double bonds in the fatty acid chains cause these compounds to exhibit different physical and chemical properties *(2)*. One of the most important parameters that influence lipid oxidation is the degree of unsaturation of the fatty acids *(3)*. Oxidation caused by several external factors such as temperature, light, presence of oxygen and humidity can lead to the formation of unpleasant flavors and odors, reduction in the product shelf life, and generation of free radicals, which can have negative physiological effects on the body *(4)*. The reason to use microencapsulation methods is to protect the oil against these external factors, along with the possibilities of masking its odors and flavors and providing release control.

Microencapsulation is a technique in which one or more substances (e.g., a core substance, an active material, a separate phase in a mixture) are surrounded or immobilized by one or more materials (e.g., a shell, a polymer matrix, a support, or wall material) and protected from biotic and abiotic factors *(5)*. It is an effective technology to protect fatty acids and associated vitamins from the oxidative degradation process *(6)*. The shell and core characteristics are important factors that play a critical role in determining the encapsulation efficiency, core stability, and other microencapsulation physicochemical characteristics *(7)*.

The several methods of microencapsulation *(8)* can be divided into: physical methods (spray drying and freeze-drying) physical-chemical methods (complex coacervation, ionic gelation and electrostatic layer-by-layer deposition), and chemical methods (interfacial polymerization and *in situ* polymerization). The choice of a microencapsulation method will depend on the active material and encapsulating matrix, as well as the possible application area (Table 1).

Table 1: Recent representative articles on the methods, core substances, encapsulating matrices, and (suggested) applications for microencapsulated vegetable oils.

Methods	Vegetable Oil	Encapsulating Matrix	(Suggested) Applications	References
Spray-drying	Linseed oil	Different combinations of maltodextrin, gum arabic, whey protein and methyl cellulose	Food application: bread	Gallardo et al. *(9)*
	Linseed oil	Modified starch	Suggested application in food	Barroso et al. *(10)*
	Green coffee oil	Different combinations of modified starch, gum arabic and maltodextrin	Suggested application in food	Silva, Vieira and Hubinger *(11)*

	Green coffee oil	Gum arabic	Suggested application in cosmetics	Nosari et al. *(12)*
	Cress seed oil	Whey protein	Food application: biscuit	Umesha et al. *(13)*
Freeze-drying	Olive oil	Different combinations of maltodextrin, carboxymethylcellulose and lecithin	Suggested application in food	Calvo et al. *(14)*
	Walnut oil	Different combinations of sodium caseinate, maltodextrin, lecithin and carboxymethylcellulose	Suggested application in food	Calvo et al. *(15)*
Complex coacervation	Corn oil	Xylitol and gelatin	Suggested application in food	Santos et al. *(16)*
	Palm oil	Chitosan/xanthan and chitosan/pectin	Food application: yogurt and bread	Rutz et al. *(17)*
	Pequi oil	Cashew gum/chitosan	Suggested application in cosmetics	Silva et al. *(18)*
	Pomegranate seed oil	Whey protein/gum arabic	Suggested application in food	Costa et al. *(19)*
	Green coffee oil	Cashew gum/gelatin	Food application: juice	Oliveira et al. *(20)*
Ionic gelation	Chia oil	Sodium alginate and calcium chloride	Food application: hamburger	Heck et al. *(20, 21)*
Electrostatic layer-by-layer deposition	Linseed oil	Bovine serum albumin (emulsifier), poly-L-arginine and dextran sulfate	Suggested application in food	Lomova, Sukhorukov and Antipina *(23)*
	Sunflower oil	Bovine serum albumin, poly (sodium 4-styrenesulfonate) and poly (allylamine hydrochloride)	Suggested application in food	Sadovoy et al. *(24)*
	Green coffee oil	Lecithin and chitosan	Suggested application in cosmetics	Carvalho, Silva and Hubinger *(25)*
	Chia oil	Modified sunflower lecithin, chitosan, and maltodextrin	Suggested application in food	Julio et al. *(26)*
Polymerization *in situ*	Neem oil	Phenol formaldehyde	Insecticide	Bagle et al. *(27)*

This review aims to present the methods commonly used in the microencapsulation of vegetable oils. The principles of each method, operating parameters, advantages, and disadvantages will first be

presented. Then, the representative studies will be discussed, highlighting their main objectives and possible applications.

MICROENCAPSULATION METHODS FOR VEGETABLE OIL

Physical methods

Spray drying

Spray drying is one of the most common methods used in microencapsulation of vegetable oil. The method consists of a process capable of transforming solutions, suspensions, or emulsions into a solid product. The spray drying process (Fig. 1) can be defined as an operation in which a liquid stream pumped into an atomizer is constantly divided into very fine droplets inside the drying chamber. A polymer, which serves as the encapsulant, is usually dissolved in the solution or in the continuous phase of a suspension or emulsion. In the drying chamber, the fine droplets come into contact with hot air, which by convection provides energy to heat and vaporize most of the solvent present in the droplets, forming dust particles. These are separated from the drying gas using a cyclone or a filter bag *(28, 29)*.

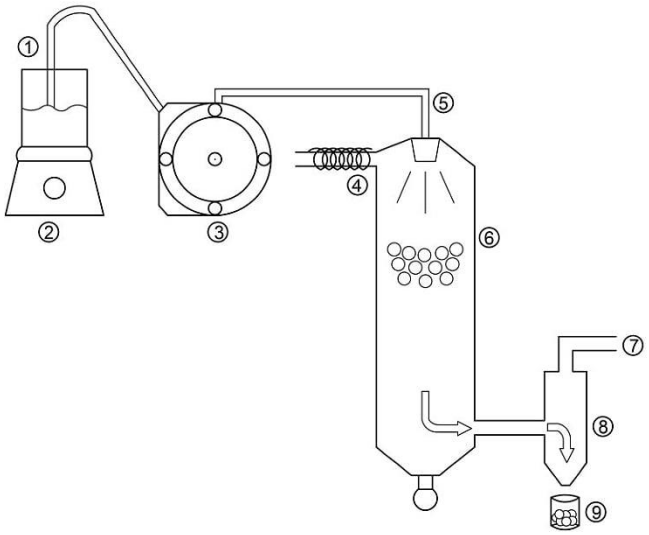

Fig. 1 Representative scheme for the spray drying microencapsulation process, where: 1) feeder; 2) magnetic stirrer; 3) peristaltic pump; 4) hot air/gas inlet; 5) atomizer nozzle; 6) drying chamber; 7) gas extractor; 8) cyclone; and 9) particle collector

Physical properties of emulsions and atomization parameters are important factors used to define the droplet formation and other parameters such as retention/encapsulation efficiency, physical-chemical properties, yield and storage stability *(4, 11, 30-32)*.

Atomization parameters are related to the spray drying equipment: inlet/outlet temperature, feeding flow, atomizer gas flow, atomizer gas type, and nozzle size. *(30, 31, 33, 34)*. Laboratory-scale spray-dryer usually has settings that allow the operator to vary the particles properties. The process can be modified in terms of its cycle mode, atomizer types, and airflow rate. However, this modularity becomes limited on an industrial scale due to financial and technological difficulties. For example, while

a variation in atomizer or airflow is viable on an industrial scale, the change of the cyclone or the drying chamber geometry can be very expensive *(35).*

This process has some advantages over other methods, such as the availability of large equipment, the possibility of using a wide variety of encapsulating materials, potentially large-scale production, simple equipment, high efficiency, and low process cost *(36).*

Many vegetable oils have been microencapsulated using the spray drying method. Bae and Lee *(37)* microencapsulated avocado oil in whey protein and maltodextrin matrix and obtained good results in improving the oil oxidative stability in different matrix concentrations. Other similar studies were reported emphasizing improvements in the oxidative stability of microencapsulated oil by spray drying *(38-40).*

Nosari et al. *(12)* observed the antioxidant activity of microencapsulated green coffee oil in gum arabic matrix under the effect of light, heat and oxygen and verified better stability results in comparison with non-encapsulated oil, even better than with the addition of alpha-tocopherol, an antioxidant widely used in the cosmetic and food industry. Gomes and Kurozawa *(41)* evaluated the potential antioxidant use of hydrolyzed rice protein in the microencapsulation of linseed oil and reported a reduction in the lipid oxidation of linseed oil during storage due to the greater antioxidant capacity of the protein hydrolysate. Oliveira et al. *(42)* evaluated the effect of a partial replacement of the whey protein isolate by maltodextrin and inulin on the pequi oil microcapsules and reported on the degradation of the bioactive compounds.

Freeze-drying

Freeze-drying or lyophilization is one of the microencapsulation methods most used in thermosensitive molecules, being a good alternative to the spray drying method *(43).* Microparticles with high resistance to thermal and oxidative degradation and good encapsulation efficiency are produced *(38, 44, 45).* During the freeze-drying process, the material temperature is reduced below its freezing point and the water is removed by sublimation at pressures below that of the triple point of water *(46).*

Freeze-drying process can be divided into three stages: freezing (solidification), primary drying (ice sublimation) and secondary drying (thawed water desorption) *(47–50).* During the freezing stage most of the water is converted into a solid, where ice crystal networks are formed. It is at this stage that the morphology of materials, the size and the size distribution of the ice crystals are determined, which in turn influence several critical parameters, such as dry product resistance, primary and secondary drying rates, extent of product crystallinity, surface area, and dry product reconstitutability *(47, 50–52).*

Primary drying is the second stage of the freeze-drying process, and this is closely related to the previous freezing stage *(53).* The sublimation of the ice starts from the top surface of the sample and continues to the bottom. For samples that were quickly frozen and formed small ice crystals that prevent

the mass transfer of sublimated water vapor through the dry layer, the primary drying can take a long time. On the other hand, slow freezing forms large ice crystals that facilitate the movement of water vapor (the mass transfer rate is high) and, as a result, the primary drying time is reduced *(54)*. During primary drying, the free frozen water is removed by converting the ice to vapor (sublimation). The drying process depends on the shelf temperature and the chamber pressure, and the appropriate choice of these two parameters can shorten this phase *(53)*.

The last stage of freeze-drying is secondary drying, where the water absorbed from the product is removed. This is the water that has not formed ice during freezing and not sublimated *(47)*. During secondary drying, the shelf temperature increases even more, while the pressure is kept constant or, in some cases, is lower than that of primary drying *(53)*. The sorbed water that remains in the solute matrix (the freeze-frozen phase) is then further reduced by desorption *(55, 56)*.

Some recent studies have used freeze-drying as a microencapsulation method of vegetable oil. Özbek and Ergönül *(57)* evaluated the interaction effect of whey protein, maltodextrin, and gum arabic in microencapsulation of pumpkin seed oil in relation to emulsion stability, encapsulation efficiency, solubility, wetting time, total polyunsaturated fatty acid content, and total saturated fatty acid content. Calvo et al. *(15)* evaluated the influence of microencapsulation on the chemical composition of extra virgin olive oil and its oxidative stability, which prolonged the microencapsulated oils shelf life.

Comunian et al. *(58)* studied the co-encapsulation of pequi and buriti oils using whey protein to obtain better carotenoid retention and oxidative stability and compared the emulsion with and without the freeze-drying process. The freeze-dried samples showed the best carotenoid retention and oxidative stability, indicating that emulsification of the oils followed by freeze-drying can serve as effective carriers of bioactive compounds. Souza et al. *(59)* microencapsulated the chia oil, rich in polyunsaturated fatty acid (omega-3 and omega-6), to increase its stability during processing and storage.

Physical-chemical methods

Complex coacervation

Coacervation is a term used in colloidal chemistry to denote the process of formation of an associative phase induced by the environment modification (pH, ionic strength, temperature, solubility) under controlled conditions *(6, 60)*. Complex coacervation is a phenomenon of liquid-liquid phase separation that occurs between oppositely charged polymers through electrostatic interactions *(61)*. Complex coacervation functions in microencapsulation by creating a barrier around the active material and preventing active compounds from interacting physically and/or chemically with the external environment *(62)*. It is a classic method of microencapsulation with great advantages, including more moderate reaction conditions during processing, lower equipment cost and greater loading capacity *(5)*. This technique of microencapsulation has been particularly successful in stabilizing unsaturated lipids and providing a product with a consistent sensory shelf life *(63)*.

Complex coacervation (Fig. 2) comprises a three-phase system that involves the solvent, the active material and the coating material. In general, this process for emulsions involves four steps: (i) preparing an aqueous solution of two or more polymers and mixing the hydrophobic phase with the aqueous solution of a polymer, often a protein solution, and homogenizing the resulting mixture, in order to produce a stable emulsion; (ii) pH change, where each polymer assumes its respective effective charges; (iii) change in temperature to a certain level necessary to induce coacervation and phase separation; and (iv) polymer hardening using high temperature, desolvation agent or crosslinker *(60, 64)*.

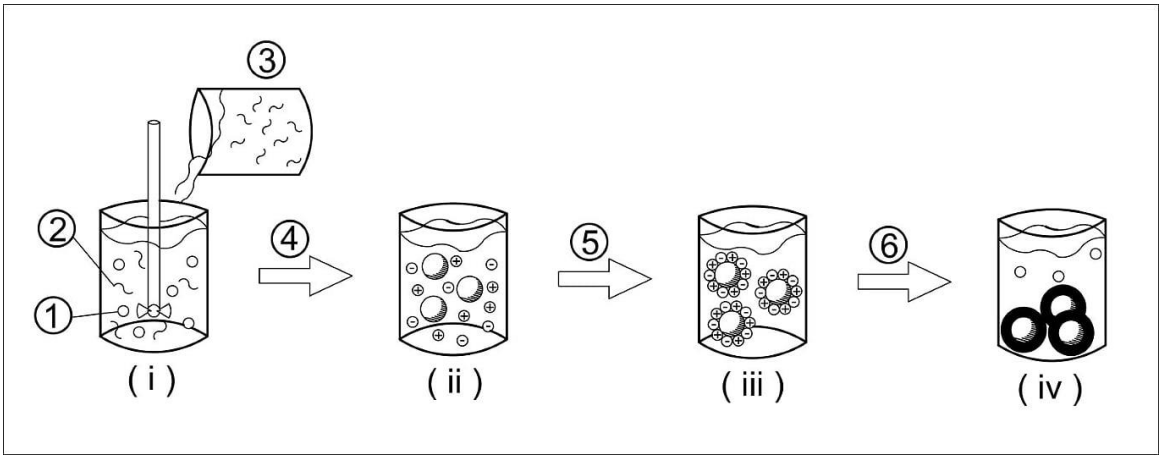

Fig. 2 Representative scheme for the complex coacervation process, where: 1) oil dispersed in the emulsion; 2) first solubilized polymer; 3) second solubilized polymer; 4) pH adjustment; 5) refrigeration; and 6) precipitation of coacervates.

Several operational parameters influence the complexation of the encapsulation procedure through electrostatic interactions (ionic strength), pH, composition of the encapsulating matrix, matrix concentration, charge distribution, homogenization, macromolecules solubility, and other molecular properties related to physical and chemical conditions of the solution. Therefore, a greater understanding of these parameters is fundamental for a better coacervation and its more efficient application *(61, 65–68)*. Although the electrostatic force between oppositely charged macromolecules is the main driving force, van der Walls intermolecular forces and hydrophobic interactions in proteins also affect the complex coacervation process *(6, 69)*.

After the coacervate formation process, the microcapsules can be dried, to improve storage and increase their shelf life. Among the drying methods, the most used are spray drying *(70–72)* and freeze-drying *(62, 73–76)*. The choice of the drying method will depend on the nature of the encapsulating matrix and the active material.

Complex coacervation is a highly recommended method for the microencapsulation of lipophilic substances. Several studies use this method to encapsulate vegetable oils. Soares *(76)*

microencapsulated the sacha inchi oil (*Plukenetia volubilis* L.), rich in omega-3, in ovalbumin matrix and sodium alginate, obtaining microcapsules with good thermal behavior and protection for the bioactive compounds in the oil. Rutz et al. *(17)* studied the palm oil microencapsulation, rich in carotenoids, in chitosan/xanthan and chitosan/pectin matrices followed by spray drying and freeze-drying. Linseed oil, a rich source of omega-3 fatty acids was microencapsulated by Kaushik et al. *(77)* in a matrix formed by complex coacervation of linseed protein isolate and inseed gum. This matrix was cross-linked with glutaraldehyde and spray dried or freeze-dried; better results on oxidative stability was obtained than the non-encapsulated oil. Lemos, Marfil and Nicoletti *(73)* investigated the influence of hydrodynamic conditions in the buriti oil coacervation, rich in carotenoids, using gelatin/alginate as matrix and identified the agitation speed as having a strong influence on the microcapsules size. Justi, Sanjinez-Argandoña and Macedo *(78)* studied the pequi oil microencapsulation using gelatin/gum arabic as encapsulating matrices to improve the oil stability. In that study, the influence of temperature, agitation speed and core material on oil coacervation was evaluated in the preservation of carotenoids present in the oil. Timilsena et al. *(7)* obtained chia seed oil microcapsules in chia seed protein/chia seed gum matrix to improve the oil oxidative stability.

A few studies carried out the optimization of microcapsule formation parameters by complex coacervation. Devi et al. *(79)* microencapsulated the olive oil in gelatin/sodium alginate matrix and optimized the proportions of the biopolymers and pH. Silva et al. *(18)* studied cashew gum/chitosan for pequi oil microencapsulation and optimized the formation parameters for coacervates (viz., biopolymer charge, pH, and matrices ratios). A similar study was conducted by Nascimento et al. *(80)* with the cashew gum/gelatin matrix.

Ionic gelation

Ionic or ionotropic gelation is a microencapsulation process that starts from an aqueous polymeric solution, where ions with a low molecular weight interact with oppositely charged polyelectrolytes forming an insoluble gel as shown in Fig. 3 *(81)*. It can be used to encapsulate hydrophilic or hydrophobic compounds *(82–84)*. The active material to be encapsulated is dissolved/dispersed or emulsified in the polymeric solution. The drops of polymer solution that reach the ionic solution stimulate the formation of spherical gel structures, which contain the active material dispersed throughout the polymer matrix *(85–88)*.

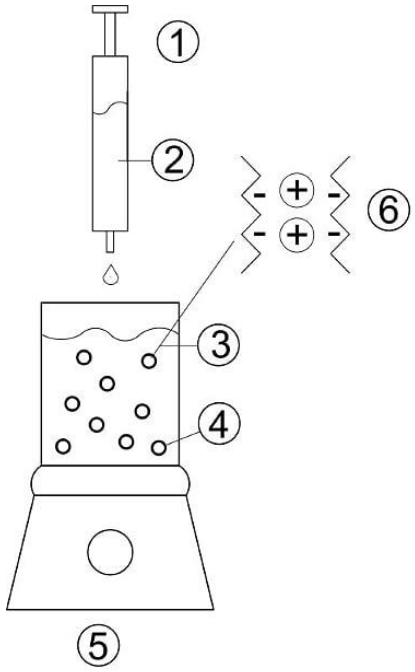

Fig. 3 Representative scheme for the ionic gelation process, where: 1) syringe; 2) emulsion (polymer + active material); 3) cross-linking solution; 4) microparticles; 5) magnetic stirrer; and 6) microparticle wall formation

This technique was adopted for natural polysaccharides to produce biocompatible and biodegradable products *(89, 90).* Sodium alginate is the most used biopolymer for ionic gelation due to its gelling property and chemical structure. Having carboxylic groups, alginates readily form gels in the presence of calcium ions or other divalent or trivalent cations, and a high guluronate content in alginate can produce stronger gels *(91).*

This technique is simple, easy to encapsulate substances, relatively low cost, and does not require specialized equipment, high temperature or an organic solvent *(5, 84, 85).* In addition, the ionic gelation technique has several more advantages, such as the use of aqueous solutions, small particle size, better control of particle size through variations in the precursor concentration, and the possibility of encapsulating a wide variety of substances *(92–94).*

Ionic gelation can be used in association with the nozzle vibration technique (NVT) that is featured in some equipment. NVT causes the formation of droplets of the same size by superimposing vibrations on a fluid jet through a precisely drilled nozzle. The vibration frequency selected determines the amount of droplets produced *(95).* This technology has gained significant interest for its ability to produce reproducible microspheres with defined sizes, thereby generating uniform and monodisperse particles *(96).* For the optimization of dispersed droplets, some parameters must be well defined, such as feed flow rate, vibration frequency, vibration amplitude and electrode voltage *(97–99).*

This technique is also applicable for microencapsulation of vegetable oil. Because it does not entail thermal stress, some recent studies used this technique to improve the oxidative stability of the encapsulated active ingredients. Menin et al. *(90)* employed ionic gelation associated with NVT and obtained particles with oxidative stability up to 13 times greater than the non-encapsulated oil. Sathasivam et al. *(100)* used ionic gelation/NVT in the microencapsulation of red palm oil with carboxymethylcellulose matrix and obtained capsules with good thermal stability.

Heck et al. *(21)* used ionic gelation to microencapsulate the chia oil enriched with rosemary that is targeted to replace 50% fat in hamburgers. The hamburgers produced with microparticles of chia oil enriched with rosemary showed greater oxidative stability, especially after cooking. In addition, the incorporation of rosemary antioxidants into chia oil reduced the sensory defects caused by lipid oxidation.

Electrostatic layer-by-layer deposition

In electrostatic layer-by-layer deposition (Fig. 4), an ionic emulsifier that quickly adsorbs on the surface of lipidic droplets during homogenization is used to produce a primary emulsion containing small droplets; then, an oppositely charged polyelectrolyte is added to the system which adsorbs on the droplet surfaces and produces a secondary emulsion containing droplets coated with a two-layer interface. This procedure can be repeated to form oil droplets coated by interfaces containing three or more layers *(101–105).* The deposited polymeric coatings allow substances to withstand various environmental stresses, such as pH, ionic strength, freezing and heating and show improved performance relative to uncoated ones *(106, 107).*

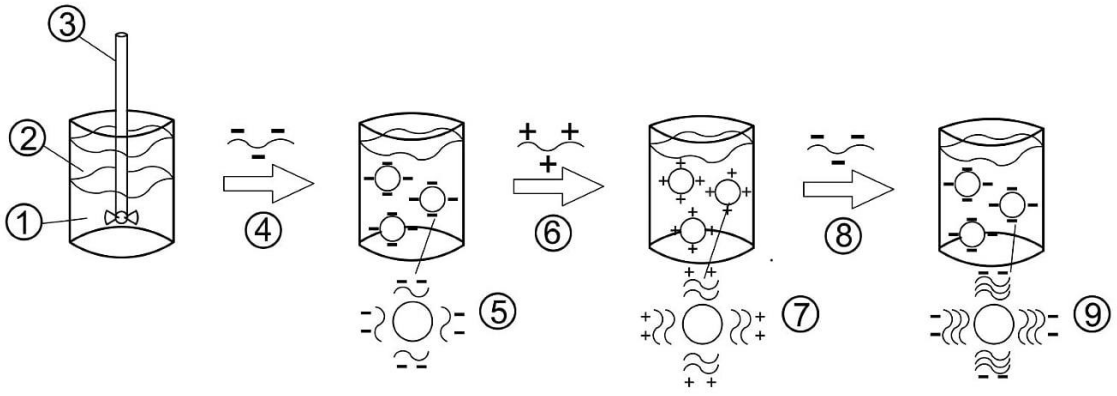

Fig. 4 Representative scheme for the electrostatic layer-by-layer process, where: 1) water; 2) oil; 3) agitation; 4) emulsifier; 5) primary emulsion; 6) polymer I; 7) secondary emulsion; 8) polymer II; and 9) tertiary emulsion

Layer-by-layer technology is simple, versatile and may be used to develop multilayer coatings when needed *(108).* The ionic characteristic (electric charge) must be evaluated as a function of the pH

between emulsifier and each biopolymer used in the system to determine the conditions for best electrostatic attraction between them *(103, 109).* The effectiveness can often be optimized by controlling the number, type and sequence of biopolymers layers used to coat the lipid droplets, as this allows the control of the thickness, composition, charge, permeability, and integrity of the interfaces *(110).*

Multilayer emulsions may have potential applications in the food or cosmetic industry, as this process offers advantages such as protecting emulsion droplets from oxidation or lipid aggregation, controlling or releasing active materials, and improving stability against environmental agents due to the thicker interfacial layers *(25, 111).* The electrostatic deposition method is, therefore, a versatile tool for modulating the functional performance of emulsion-based delivery systems by altering their interfacial properties *(112).*

Drying methods are recommended after the electrostatic layer-by-layer deposition process to transform the material into powder, with the objective of improving handling and storage. Freeze-drying *(26, 113)* and spray drying *(25, 114–116)* are the most used for drying of the particles. Carvalho, Silva and Hubinger *(25)* microencapsulated green coffee oil using emulsions stabilized by lecithin and chitosan through electrostatic layer-by-layer deposition followed by spray drying. Microparticles thus obtained exhibited better results for sun protection and oxidative stability than the free oil, with potential applications in cosmetics and personal care. Noello et al. *(115)* produced chia oil microparticles using both emulsions stabilized by whey protein concentrate and pectin through the electrostatic layer-by-layer deposition and emulsions prepared only with whey protein concentrate. The microparticles were dried by spray drying and showed higher oxidative stability than the pure oil. The emulsions obtained by electrostatic deposition had smaller droplet diameters and greater stability than emulsions produced only with whey. Julio et al. *(26)* did the physical-chemical characterization of chia oil microparticles produced from mono- and bi-layer emulsions using modified sunflower lecithin, chitosan and maltodextrin as matrix. The two-layer microparticles with modified sunflower lecithin were effective in protecting the chia oil against oxidative deterioration, being an adequate delivery system for the ω-3 fatty acids in this oil for the functional food application.

Fioramonti et al. *(111)* investigated the influence of acid pH and sodium alginate concentration on the flaxseed oil properties in water emulsions stabilized by whey protein isolate. Fioramonti et al. *(113)* also conducted another study about dehydration of microparticles. Freeze-drying was chosen due to the bioactive compounds present in linseed oil. However, the techniques applied to obtain microparticles by ultrasonic emulsification and freeze-drying processes contributed significantly to the oil oxidation, as high input energies during ultrasonic emulsification promoted an increase in lipid oxidation rates, with the consequent formation of highly reactive peroxides, whose concentration was beyond the limit allowed for food matrices. Fioramonti et al. *(116)* also evaluated the influence of homogenization pressure and spray drying. The experiments showed that it was possible to transform a

highly oxidizable ingredient of liquid phase into a solid, easy-to-handle powder with peroxide values within the permitted range for food.

Chemical methods

Interfacial/*in situ* polymerization

Microencapsulation of an active material by interfacial polymerization involves the oil-soluble phase dispersion in a continuous aqueous phase or an aqueous phase dispersion in an organic phase, depending on the solubility medium of the encapsulated core substance and the conditions for the precipitation of polymeric materials at the drop interface. Each phase contains a specific dissolved monomer suitable to react with the other monomer present in the other phase. The dispersed phase acts as a good solvent for the monomers, but it also serves as a non-solvent for the polymer produced in the reaction. Therefore, during polymerization, the system is composed of three mutually immiscible phases. Once the various monomers are added in drops to the system, reactions take place at the interface, resulting in the formation of insoluble oligomers in each drop with the tendency to precipitate at the interface to form a primary shell around the drop. The polymer formed is not soluble in the dispersed phase or in the continuous phase and precipitates *(117, 118).*

The interfacial polymerization technique has potential advantages, including possible control of microcapsule average size and shell thickness, high load of the active compound, versatile and stable mechanical and chemical properties of the shell, low cost, easy scale-up, simplicity and reliability of the process *(119, 120).* On the other hand, there are also some factors that limit the application of this technique, such as the production of a large oil-water interface, where proteins or enzymes are prone to inactivation, altering the biological activities of proteins during the polymerization reaction *(8).* Another negative point is that the resulting microcapsules usually have certain unreacted shell monomers, which can react with the core material and potentially cause its deactivation or other unwanted consequences *(121).*

In situ polymerization is similar to interfacial polymerization; however, the reagents involved in the synthesis of the active matrix are obtained from both dispersed and continuous phases. During in situ polymerization (Fig. 5), oil-in-water emulsions or water-in-oil emulsions are first produced under vigorous stirring or sonication of a biphasic liquid. The monomers and initiators used to build the capsule matrix are dissolved in the dispersed or continuous phase. As the polymer synthesized from the monomers is insoluble in the emulsion, polymerization usually occurs on the core material droplets surface, and the resulting polymer accumulates on the droplets surface, generating microcapsules with the desired core material *(121–123).* The controlled deposition and polymer precipitation occurs at the interface using precipitants, or a change in pH, temperature, or solvent quality *(122).*

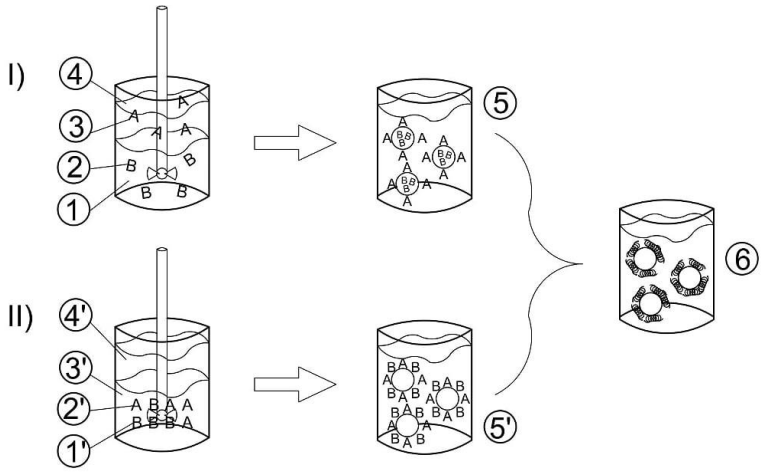

Fig. 5 Representative scheme for microencapsulation by polymerization: I) interfacial, where: 1) aqueous solution (hydrophilic phase); 2) monomer B; 3) monomer A; 4) oil (lipophilic phase); 5) diffusion of monomers to the interface; and 6) polymerization reaction and matrix formation. II) *in situ*, where 1') monomer B; 2') monomer A; 3') aqueous solution (hydrophilic phase); 4') oil (lipophilic phase); 5') dissolving the monomers in the continuous phase; and 6) polymerization reaction and matrix formation

In situ polymerization requires longer reaction times compared to other encapsulation techniques. However, this technique offers some advantages, such as low cost, ease of industrial manufacturing, and simplicity of the procedure *(124)*. It should be noted that the microcapsules preparation by in situ polymerization depends not only on the core materials and the encapsulating matrix, but also on the reaction conditions, i.e., the type of emulsifier, agitation speed, core/matrix mass ratio, pH value, reaction temperature, and hydrophobic surface of the core *(125, 126)*.

Moser et al. *(127)* studied the formation of microcapsules produced by the interfacial polymerization of chickpea protein and pectin in buriti oil emulsions and dried via spray drying. The microencapsulation and drying process did not influence the lipid oxidation, showing that the emulsions produced by interfacial polymerization protected the oil from the high temperatures of spray drying. Suryanarayana, Rao and Kumar *(128)* prepared linseed oil microcapsules coated by in situ polymerization of urea-formaldehyde resin. These microcapsules were incorporated into a paint formulation. The mechanical stability was studied and the microcapsules showed sufficient strength to withstand the shear generated during mixing and application of the paint.

CONCLUSIONS

In this review, several techniques commonly used in the microencapsulation of vegetable oil were presented. The advantages and disadvantages of each technique were discussed to assist in the choice of an appropriate approach for the encapsulation of the vegetable oil. Because the needs are

different for the food, cosmetic, personal care, and pharmaceutical industry, it is useful to have all these techniques available for use. From the publications in the literature, it is clear that this field is still growing and evolving, and we expect further developments and improvements in the future.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

All authors contributed to the study conception and design. The idea for the article were performed by [Luana Carvalho da Silva] and [Roselayne Ferro Furtado]. The literature search and data analysis were realized by [Luana Carvalho da Silva] and [Rachel Menezes Castelo]. The critically revised were performed by [Huai N. Cheng], [Atanu Biswas], [Roselayne Ferro Furtado] and [Carlucio Roberto Alves]. The first draft of the manuscript was written by [Luana Carvalho da Silva] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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REFERENCES

1. Miao S, Wang P, Su Z, Zhang S. Vegetable-oil-based polymers as future polymeric biomaterials. Acta Biomater. 2014;10(4):1692–704.

https://doi.org/10.1016/j.actbio.2013.08.040

2. Ataei S, Khorasani SN, Neisiany RE. Biofriendly vegetable oil healing agents used for developing self-healing coatings: A review. Prog Org Coatings. 2019;129:77–95.
https://doi.org/10.1016/j.porgcoat.2019.01.012

3. Frega N, Mozzon M, Lercker G. Effects of free fatty acids on oxidative stability of vegetable oil. JAOCS. 1999;76(3):325–9.
https://doi.org/10.1007/s11746-999-0239-4

4. Alcântara MA, Lima AEA, Braga ALM, Tonon RV, Galdeano MC, Mattos MC, et al. Influence of the emulsion homogenization method on the stability of chia oil microencapsulated by spray drying. Powder Technol. 2019;354:877–85.
https://doi.org/10.1016/j.powtec.2019.06.026

5. Comunian TA, Favaro-Trindade CS. Microencapsulation using biopolymers as an alternative to produce food enhanced with phytosterols and omega-3 fatty acids: A review. Food Hydrocoll. 2016;61:442–57.
https://doi.org/10.1016/j.foodhyd.2016.06.003

6. Timilsena YP, Wang B, Adhikari R, Adhikari B. Advances in microencapsulation of polyunsaturated fatty acids (PUFAs)-rich plant oils using complex coacervation: A review. Food Hydrocoll. 2017;69:369–81.
https://doi.org/10.1016/j.foodhyd.2017.03.007

7. Timilsena YP, Adhikari R, Barrow CJ, Adhikari B. Microencapsulation of chia seed oil using chia seed protein isolate-chia seed gum complex coacervates. Int J Biol Macromol. 2016;91:347–57.
https://doi.org/10.1016/j.ijbiomac.2016.05.058

8. Ozkan G, Franco P, Marco I, Xiao J, Capanoglu E. A review of microencapsulation methods for food antioxidants: Principles, advantages, drawbacks and applications. Food Chem. 2019;272(July 2018):494–506.
https://doi.org/10.1016/j.foodchem.2018.07.205

9. Gallardo G, Guida L, Martinez V, López MC, Bernhardt D, Blasco R, Pedroza-Islas R, Hermida LG. Microencapsulation of linseed oil by spray drying for functional food application. Food Res Int. 2013;52(2):473–82.
https://doi.org/10.1016/j.foodres.2013.01.020

10. Barroso AKM, Pierucci APTR, Freitas SP, Torres AG, Rocha-Leão MHM. Oxidative stability and sensory evaluation of microencapsulated flaxseed oil. J Microencapsul. 2014;31(2):193–201.
https://doi.org/10.3109/02652048.2013.824514

11. Silva VM, Vieira GS, Hubinger MD. Influence of different combinations of wall materials and homogenisation pressure on the microencapsulation of green coffee oil by spray drying. Food Res Int. 2014;61:132–43.

https://doi.org/10.1016/j.foodres.2014.01.052

12. Nosari ABFL, Lima JF, Serra OA, Freitas LAP. Improved green coffee oil antioxidant activity for cosmetical purpose by spray drying microencapsulation. Brazilian J Pharmacogn. 2015;25(3):307–11.
https://doi.org/10.1016/j.foodres.2014.01.052

13. Umesha SS, Manohar RS, Indiramma AR, Akshitha S, Naidu KA. Enrichment of biscuits with microencapsulated omega-3 fatty acid (Alpha-linolenic acid) rich Garden cress (Lepidium sativum) seed oil: Physical, sensory and storage quality characteristics of biscuits. LWT - Food Sci Technol. 2015;62(1):654–61.
https://doi.org/10.1016/j.foodres.2014.01.052

14. Calvo P, Hernández T, Lozano M, González-Gómez D. Microencapsulation of extra-virgin olive oil by spray-drying: Influence of wall material and olive quality. Eur J Lipid Sci Technol. 2010;112(8):852–8.
https://doi.org/10.1002/ejlt.201000059

15. Calvo P, Castaño ÁL, Lozano M, González-Gómez D. Influence of the microencapsulation on the quality parameters and shelf-life of extra-virgin olive oil encapsulated in the presence of BHT and different capsule wall components. Food Res Int. 2012;45(1):256–61.
https://doi.org/10.1016/j.foodres.2011.10.036

16. Santos MG, Bozza FT, Thomazini M, Favaro-Trindade CS. Microencapsulation of xylitol by double emulsion followed by complex coacervation. Food Chem. 2015;171:32–9.
https://doi.org/10.1016/j.foodchem.2014.08.093

17. Rutz JK, Borges CD, Zambiazi RC, Crizel-Cardozo MM, Kuck LS, Noreña CPZ. Microencapsulation of palm oil by complex coacervation for application in food systems. Food Chem. 2017;220:59–66.
https://doi.org/10.1016/j.foodchem.2016.09.194

18. Silva LC, Nascimento MA, Mendes LG, Furtado RF, Costa JMC, Cardoso ALH. Optimization of cashew gum and chitosan for microencapsulation of pequi oil by complex coacervation. J Food Process Preserv. 2018;42(3):1–8.
https://doi.org/10.1016/j.foodchem.2016.09.194

19. Costa AMM, Moretti LK, Simões G, Silva KA, Tonon RV, Torres AG. Microencapsulation of pomegranate (Punica granatum L.) seed oil by complex coacervation: Development of a potential functional ingredient for food application. LWT - Food Sci Technol. 2020;109519.
https://doi.org/10.1016/j.foodchem.2016.09.194

20. Oliveira WQ, Wurlitzer NJ, Araújo AWO, Comunian TA, Bastos MSR, Oliveira AL, et al. Complex coacervates of cashew gum and gelatin as carriers of green coffee oil: The effect of microcapsule application on the rheological and sensorial quality of a fruit juice. Food Res Int. 2020;131:109047.

https://doi.org/10.1016/j.foodchem.2016.09.194

21. Heck RT, Lucas BN, Santos DJP, Pinton MB, Fagundes MB, Araújo Etchepare M, et al. Oxidative stability of burgers containing chia oil microparticles enriched with rosemary by green-extraction techniques. Meat Sci. 2018;146:147–53.
https://doi.org/10.1016/j.meatsci.2018.08.009

22. Heck RT, Fagundes MB, Cichoski AJ, Menezes CR, Barin JS, Lorenzo JM, et al. Volatile compounds and sensory profile of burgers with 50% fat replacement by microparticles of chia oil enriched with rosemary. Meat Sci. 2019;148:164–70.
https://doi.org/10.1016/j.meatsci.2018.08.009

23. Lomova MV, Sukhorukov GB, Antipina MN. Antioxidant coating of micronsize droplets for prevention of lipid peroxidation in oil-in-water emulsion. ACS Appl Mater Interfaces. 2010;2(12):3669–76.
https://doi.org/10.1021/am100818j

24. Sadovoy AV, Kiryukhin MV, Sukhorukov GB, Antipina MN. Kinetic stability of water-dispersed oil droplets encapsulated in a polyelectrolyte multilayer shell. Phys Chem Chem Phys. 2011;13(9):4005–12.
https://doi.org/10.1039/c0cp01762k

25. Carvalho AGS, Silva VM, Hubinger MD. Microencapsulation by spray drying of emulsified green coffee oil with two-layered membranes. Food Res Int. 2014;61:236–45.
https://doi.org/10.1016/j.foodres.2013.08.012

26. Julio LM, Copado CN, Crespo R, Diehl BWK, Ixtaina VY, Tomás MC. Design of microparticles of chia seed oil by using the electrostatic layer-by-layer deposition technique. Powder Technol. 2019;345:750–7.
https://doi.org/10.1016/j.powtec.2019.01.047

27. Bagle AV, Jadhav RS, Gite VV, Hundiwale DG, Mahulikar PP. Controlled release study of phenol formaldehyde microcapsules containing neem oil as an insecticide. Int J Polym Mater Polym Biomater. 2013;62(8):421–5.
https://doi.org/10.1080/00914037.2012.719142

28. Lisboa HM, Duarte ME, Cavalcanti-Mata ME. Modeling of food drying processes in industrial spray dryers. Food Bioprod Process. 2018;107:49–60.
https://doi.org/10.1016/j.fbp.2017.09.006

29. Gil-Chávez J, Padhi SSP, Hartge U, Heinrich S, Smirnova I. Optimization of the spray-drying process for developing aquasolv lignin particles using response surface methodology. Adv Powder Technol. 2020;31(6):2348-56.
https://doi.org/10.1016/j.apt.2020.03.027

30. Moser P, Ferreira S, Nicoletti VR. Buriti oil microencapsulation in chickpea protein-pectin matrix as affected by spray drying parameters. Food Bioprod Process. 2019;117:183–93. https://doi.org/10.1016/j.fbp.2019.07.009

31. Koç M, Güngör Ö, Zungur A, Yalçın B, Selek İ, Ertekin FK, et al. Microencapsulation of Extra Virgin Olive Oil by Spray Drying: Effect of Wall Materials Composition, Process Conditions, and Emulsification Method. Food Bioprocess Technol. 2015;8(2):301–18. https://doi.org/10.1007/s11947-014-1404-9

32. Chang C, Varankovich N, Nickerson MT. Microencapsulation of canola oil by lentil protein isolate-based wall materials. Food Chem. 2016;212:264–73. https://doi.org/10.1016/j.foodchem.2016.05.136

33. Gharsallaoui A, Roudaut G, Chambin O, Voilley A, Saurel R. Applications of spray-drying in microencapsulation of food ingredients: An overview. Food Res Int. 2007;40(9):1107–21. https://doi.org/10.1016/j.foodres.2007.07.004

34. Ixtaina VY, Julio LM, Wagner JR, Nolasco SM, Tomás MC. Physicochemical characterization and stability of chia oil microencapsulated with sodium caseinate and lactose by spray-drying. Powder Technol. 2015;271:26–34. https://doi.org/10.1016/j.foodres.2007.07.004

35. Ziaee A, Albadarin AB, Padrela L, Femmer T, O'Reilly E, Walker G. Spray drying of pharmaceuticals and biopharmaceuticals: Critical parameters and experimental process optimization approaches. Eur J Pharm Sci. 2019;127:300–18. https://doi.org/10.1016/j.ejps.2018.10.026

36. Casanova F, Santos L. Encapsulation of cosmetic active ingredients for topical application-a review. J Microencapsul. 2016;33(1):1–17. https://doi.org/10.3109/02652048.2015.1115900

37. Bae EK, Lee SJ. Microencapsulation of avocado oil by spray drying using whey protein and maltodextrin. J Microencapsul. 2008;25(8):549–60. https://doi.org/10.1080/02652040802075682

38. González A, Martínez ML, Paredes AJ, León AE, Ribotta PD. Study of the preparation process and variation of wall components in chia (Salvia hispanica L.) oil microencapsulation. Powder Technol. 2016;301:868–75. https://doi.org/10.1016/j.powtec.2016.07.026

39. Le Priol L, Dagmey A, Morandat S, Saleh K, El Kirat K, Nesterenko A. (2019). Comparative study of plant protein extracts as wall materials for the improvement of the oxidative stability of sunflower oil by microencapsulation. Food Hydrocoll. 2019;95:105–115. https://doi.org/10.1016/j.foodhyd.2019.04.026

40. Carneiro HCF, Tonon RV, Grosso CRF, Hubinger MD. (2013). Encapsulation efficiency and oxidative stability of flaxseed oil microencapsulated by spray drying using different combinations of wall materials. J Food Eng. 2013;115(4):443–451.
https://doi.org/10.1016/j.jfoodeng.2012.03.033
41. Gomes MHG, Kurozawa LE. Improvement of the functional and antioxidant properties of rice protein by enzymatic hydrolysis for the microencapsulation of linseed oil. J Food Eng. 2020;267:109761.
https://doi.org/10.1016/j.jfoodeng.2012.03.033
42. Oliveira ÉR, Fernandes RVB, Botrel DA, Carmo EL, Borges S V., Queiroz F. Study of Different Wall Matrix Biopolymers on the Properties of Spray-Dried Pequi Oil and on the Stability of Bioactive Compounds. Food Bioprocess Technol. 2018;11(3):660–79.
https://doi.org/10.1007/s11947-017-2027-8
43. González-Ortega R, Faieta M, Di Mattia CD, Valbonetti L, Pittia P. Microencapsulation of olive leaf extract by freeze-drying: Effect of carrier composition on process efficiency and technological properties of the powders. J Food Eng. 2020;285:110089.
https://doi.org/10.1016/j.jfoodeng.2020.110089
44. Pasrija D, Ezhilarasi PN, Indrani D, Anandharamakrishnan C. Microencapsulation of green tea polyphenols and its effect on incorporated bread quality. LWT - Food Sci Technol. 2015;64(1):289–96.
https://doi.org/10.1016/j.lwt.2015.05.054
45. Ballesteros LF, Ramirez MJ, Orrego CE, Teixeira JA, Mussatto SI. Encapsulation of antioxidant phenolic compounds extracted from spent coffee grounds by freeze-drying and spray-drying using different coating materials. Food Chem. 2017;237:623–31.
https://doi.org/10.1016/j.foodchem.2017.05.142
46. Feng Y, Ping Tan C, Zhou C, Yagoub AEGA, Xu B, Sun Y, et al. Effect of freeze-thaw cycles pretreatment on the vacuum freeze-drying process and physicochemical properties of the dried garlic slices. Food Chem. 2020;324:126883.
https://doi.org/10.1016/j.foodchem.2020.126883
47. Abdelwahed W, Degobert G, Stainmesse S, Fessi H. Freeze-drying of nanoparticles: Formulation, process and storage considerations. Adv Drug Deliv Rev. 2006;58(15):1688–713.
https://doi.org/10.1016/j.addr.2006.09.017
48. Patel SM, Doen T, Pikal MJ. Determination of end point of primary drying in freeze-drying process control. AAPS PharmSciTech. 2010;11(1):73–84.
https://doi.org/10.1016/j.addr.2006.09.017
49. Sundaramurthi P, Suryanarayanan R. Calorimetry and complementary techniques to characterize frozen and freeze-dried systems. Adv Drug Deliv Rev. 2012;64(5):384–95.

https://doi.org/10.1016/j.addr.2011.12.004

50. Assegehegn G, Brito-de la Fuente E, Franco JM, Gallegos C. The Importance of Understanding the Freezing Step and Its Impact on Freeze-Drying Process Performance. J Pharm Sci. 2019;108(4):1378–95.
https://doi.org/10.1016/j.xphs.2018.11.039

51. Searles JA, Carpenter JF, Randolph TW. The ice nucleation temperature determines the primary drying rate of lyophilization for samples frozen on a temperature-controlled shelf. J Pharm Sci. 2001;90(7):860–71.
https://doi.org/10.1016/j.xphs.2018.11.039

52. Assegehegn G, Brito-de la Fuente E, Franco JM, Gallegos C. Use of a temperature ramp approach (TRA) to design an optimum and robust freeze-drying process for pharmaceutical formulations. Int J Pharm. 2020;578:119116.
https://doi.org/10.1016/j.ijpharm.2020.119116

53. Bjelošević M, Zvonar Pobirk A, Planinšek O, Ahlin Grabnar P. Excipients in freeze-dried biopharmaceuticals: Contributions toward formulation stability and lyophilisation cycle optimisation. Int J Pharm. 2020;576:119029.
https://doi.org/10.1016/j.ijpharm.2020.119029

54. Morais ARDV, Alencar ÉDN, Xavier Júnior FH, Oliveira CM De, Marcelino HR, Barratt G, et al. Freeze-drying of emulsified systems: A review. Int J Pharm. 2016;503(1–2):102–14.
https://doi.org/10.1016/j.ijpharm.2016.02.047

55. Liapis AI, Bruttini R. A mathematical model for the spray freeze drying process: The drying of frozen particles in trays and in vials on trays. Int J Heat Mass Transf. 2009;52(1–2):100–11.
https://doi.org/10.1016/j.ijheatmasstransfer.2008.06.026

56. Ishwarya SP, Anandharamakrishnan C, Stapley AGF. Spray-freeze-drying: A novel process for the drying of foods and bioproducts. Trends Food Sci Technol. 2015;41(2):161–81.
https://doi.org/10.1016/j.ijheatmasstransfer.2008.06.026

57. Aksoylu Özbek Z, Günç Ergönül P. Optimisation of wall material composition of freeze–dried pumpkin seed oil microcapsules: Interaction effects of whey protein, maltodextrin, and gum Arabic by D–optimal mixture design approach. Food Hydrocoll. 2020;107:105909.
https://doi.org/10.1016/j.ijheatmasstransfer.2008.06.026

58. Comunian TA, Silva MP, Moraes ICF, Favaro-Trindade CS. Reducing carotenoid loss during storage by co-encapsulation of pequi and buriti oils in oil-in-water emulsions followed by freeze-drying: Use of heated and unheated whey protein isolates as emulsifiers. Food Res Int. 2020;130:108901.
https://doi.org/10.1016/j.foodres.2019.108901

59. Souza MF, Francisco CRL, Sanchez JL, Guimarães-Inácio A, Valderrama P, Bona E, et al. Fatty acids profile of chia oil-loaded lipid microparticles. Brazilian J Chem Eng. 2017;34(3):659–69. https://doi.org/10.1590/0104-6632.20170343s20150669

60. Timilsena YP, Akanbi TO, Khalid N, Adhikari B, Barrow CJ. Complex coacervation: Principles, mechanisms and applications in microencapsulation. Int J Biol Macromol. 2019;121:1276–86. https://doi.org/10.1016/j.ijbiomac.2018.10.144

61. Eghbal N, Choudhary R. Complex coacervation: Encapsulation and controlled release of active agents in food systems. LWT - Food Sci Technol. 2018;90:254–64. https://doi.org/10.1016/j.lwt.2017.12.036

62. Brito de Souza V, Thomazini M, Chaves IE, Ferro-Furtado R, Favaro-Trindade CS. Microencapsulation by complex coacervation as a tool to protect bioactive compounds and to reduce astringency and strong flavor of vegetable extracts. Food Hydrocoll. 2020;98:105244. https://doi.org/10.1016/j.foodhyd.2019.105244

63. Barrow CJ, Nolan C, Jin Y. Stabilization of highly unsaturated fatty acids and delivery into foods. Lipid Technol. 2007;19(5):108–11. https://doi.org/10.1002/lite.200600037

64. Desai KGH, Park HJ. Recent developments in microencapsulation of food ingredients. Drying Technol. 2005;23:1361–1394. https://doi.org/10.1081/DRT-200063478

65. Liu S, Low NH, Nickerson MT. Effect of pH, salt, and biopolymer ratio on the formation of pea protein isolate gum arabic complexes. J Agric Food Chem. 2009;57(4):1521–6. https://doi.org/10.1021/jf802643n

66. Ben Amara C, Kim L, Oulahal N, Degraeve P, Gharsallaoui A. Using complexation for the microencapsulation of nisin in biopolymer matrices by spray-drying. Food Chem. 2017;236:32–40. https://doi.org/10.1016/j.foodchem.2017.04.168

67. Ma T, Zhao H, Wang J, Sun B. Effect of processing conditions on the morphology and oxidative stability of lipid microcapsules during complex coacervation. Food Hydrocoll. 2019;87:637–43. https://doi.org/10.1016/j.foodhyd.2018.08.053

68. Rios-Mera JD, Saldaña E, Ramírez Y, Auquiñivín EA, Alvim ID, Contreras-Castillo CJ. Encapsulation optimization and pH- and temperature-stability of the complex coacervation between soy protein isolate and inulin entrapping fish oil. LWT - J Food Sci Technol. 2019;116:108555. https://doi.org/10.1016/j.foodhyd.2018.08.053

69. Turgeon SL, Schmitt C, Sanchez C. Protein-polysaccharide complexes and coacervates. Curr Opin Colloid Interface Sci. 2007;12(4–5):166–78. https://doi.org/10.1016/j.cocis.2007.07.007

70. Amara C Ben, Eghbal N, Degraeve P, Gharsallaoui A. Using complex coacervation for lysozyme encapsulation by spray-drying. J Food Eng. 2016;183:50–7.
https://doi.org/10.1016/j.jfoodeng.2016.03.016
71. Timilsena YP, Vongsvivut J, Tobin MJ, Adhikari R, Barrow C, Adhikari B. Investigation of oil distribution in spray-dried chia seed oil microcapsules using synchrotron-FTIR microspectroscopy. Food Chem. 2019;275:457–66.
https://doi.org/10.1016/j.foodchem.2018.09.043
72. Tavares L, Zapata Noreña CP. Encapsulation of garlic extract using complex coacervation with whey protein isolate and chitosan as wall materials followed by spray drying. Food Hydrocoll. 2019;89:360–9.
https://doi.org/10.1016/j.foodhyd.2018.10.052
73. Lemos YP, Mariano Marfil PH, Nicoletti VR. Particle size characteristics of buriti oil microcapsules produced by gelatin-sodium alginate complex coacervation: Effect of stirring speed. Int J Food Prop. 2017;20:1438–47.
https://doi.org/10.1080/10942912.2017.1349139
74. Cruz MCR, Andreotti Dagostin JL, Perussello CA, Masson ML. Assessment of physicochemical characteristics, thermal stability and release profile of ascorbic acid microcapsules obtained by complex coacervation. Food Hydrocoll. 2019;87:71–82.
https://doi.org/10.1016/j.foodhyd.2018.07.043
75. Comunian TA, Nogueira M, Scolaro B, Thomazini M, Ferro-Furtado R, Castro IA, et al. Enhancing stability of echium seed oil and beta-sitosterol by their coencapsulation by complex coacervation using different combinations of wall materials and crosslinkers. Food Chem. 2018;252:277–84.
https://doi.org/10.1016/j.foodchem.2018.01.121
76. Soares BS, Siqueira RP, Carvalho MG, Vicente J, Garcia-Rojas EE. Microencapsulation of sacha inchi oil (Plukenetia volubilis L.) using complex coacervation: Formation and structural characterization. Food Chem. 2019;298:125045.
https://doi.org/10.1016/j.foodchem.2019.125045
77. Kaushik P, Dowling K, McKnight S, Barrow CJ, Adhikari B. Microencapsulation of flaxseed oil in flaxseed protein and flaxseed gum complex coacervates. Food Res Int. 2016;86:1–8.
https://doi.org/10.1016/j.foodres.2016.05.015
78. Justi PN, Sanjinez-Argandoña EJ, Macedo MLR. Microencapsulation of pequi pulp oil by complex coacervation. Rev Bras Frutic. 2018;40(2):e-874.
https://doi.org/10.1590/0100-29452018874

79. Devi N, Hazarika D, Deka C, Kakati DK. Study of complex coacervation of gelatin a and sodium alginate for microencapsulation of olive oil. J Macromol Sci Part A Pure Appl Chem. 2012;49(11):936–45.
https://doi.org/10.1080/10601325.2012.722854
80. Nascimento MA, Silva LC, Mendes LG, Furtado RF, Costa JMC, Biswas A, et al. Pequi oil microencapsulation by complex coacervation using gelatin-cashew gum. Int J Food Stud. 2020;9(2020):SI97–109.
https://doi.org/10.7455/ijfs/9.SI.2020.a8
81. Kurozawa LE, Hubinger MD. Hydrophilic food compounds encapsulation by ionic gelation. Curr Opin Food Sci. 2017;15:50–5.
https://doi.org/10.1016/j.cofs.2017.06.004
82. Castro-Rosas J, Ferreira-Grosso CR, Gómez-Aldapa CA, Rangel-Vargas E, Rodríguez-Marín ML, Guzmán-Ortiz FA, et al. Recent advances in microencapsulation of natural sources of antimicrobial compounds used in food - A review. Food Res Int. 2017;102:575- 87.
https://doi.org/10.1016/j.foodres.2017.09.054
83. Pavelková M, Kubová K, Vysloužil J, Kejdušová M, Vetchý D, Celer V,et al. Biological Effects of Drug-Free Alginate Beads Cross-Linked by Copper Ions Prepared Using External Ionotropic Gelation. AAPS PharmSciTech. 2017;18(4):1343–54.
https://doi.org/10.1208/s12249-016-0601-4
84. Karimirad R, Behnamian M, Dezhsetan S. Bitter orange oil incorporated into chitosan nanoparticles: Preparation, characterization and their potential application on antioxidant and antimicrobial characteristics of white button mushroom. Food Hydrocoll. 2020;100(September 2019):105387.
https://doi.org/10.1016/j.foodhyd.2019.105387
85. Souza FN, Gebara C, Ribeiro MCE, Chaves KS, Gigante ML, Grosso CRF. Production and characterization of microparticles containing pectin and whey proteins. Food Res Int. 2012;49(1):560–6.
https://doi.org/10.1016/j.foodres.2012.07.041
86. Leong JY, Lam WH, Ho KW, Voo WP, Lee MFX, Lim HP, et al. Advances in fabricating spherical alginate hydrogels with controlled particle designs by ionotropic gelation as encapsulation systems. Particuology. 2016;24:44–60.
https://doi.org/10.1016/j.partic.2015.09.004
87. Rozman NAS, Tong WY, Leong CR, Anuar MR, Karim S, Ong SK, et al. Homalomena pineodora essential oil nanoparticle inhibits diabetic wound pathogens. Sci Rep. 2020;10(1):1–11.
https://doi.org/10.1038/s41598-020-60364-0

88. Attallah OA, Shetta A, Elshishiny F, Mamdouh W. Essential oil loaded pectin/chitosan nanoparticles preparation and optimization: via Box-Behnken design against MCF-7 breast cancer cell lines. RSC Adv. 2020;10(15):8703–8.
https://doi.org/10.1039/c9ra10204c

89. Patel MA, AbouGhaly MHH, Schryer-Praga J V., Chadwick K. The effect of ionotropic gelation residence time on alginate cross-linking and properties. Carbohydr Polym. 2017;155:362–71.
https://doi.org/10.1016/j.carbpol.2016.08.095

90. Menin A, Zanoni F, Vakarelova M, Chignola R, Donà G, Rizzi C, et al. Effects of microencapsulation by ionic gelation on the oxidative stability of flaxseed oil. Food Chem. 2018;269:293–9.
https://doi.org/10.1016/j.foodchem.2018.06.144

91. Fernando IPS, Lee WW, Han EJ, Ahn G. Alginate-based nanomaterials: Fabrication techniques, properties, and applications. Chem Eng J. 2019;123823.
https://doi.org/10.1016/j.cej.2019.123823

92. Fàbregas A, Miñarro M, García-Montoya E, Pérez-Lozano P, Carrillo C, Sarrate R, et al. Impact of physical parameters on particle size and reaction yield when using the ionic gelation method to obtain cationic polymeric chitosan-tripolyphosphate nanoparticles. Int J Pharm. 2013;446(1–2):199–204.
https://doi.org/10.1016/j.ijpharm.2013.02.015

93. Wang M, Doi T, McClements DJ. Encapsulation and controlled release of hydrophobic flavors using biopolymer-based microgel delivery systems: Sustained release of garlic flavor during simulated cooking. Food Res Int. 2019;119:6–14.
https://doi.org/10.1016/j.foodres.2019.01.042

94. Wonnie Ma IA, Ammar S, Bashir S, Selvaraj M, Assiri MA, Ramesh K, et al. Preparation of Hybrid Chitosan/Silica Composites Via Ionotropic Gelation and Its Electrochemical Impedance Studies. Prog Org Coatings. 2020;145:105679.
https://doi.org/10.1016/j.porgcoat.2020.105679

95. Dorati R, Genta I, Modena T, Conti B. Microencapsulation of a hydrophilic model molecule through vibration nozzle and emulsion phase inversion technologies. J Microencapsul. 2013;30(6):559–70.
https://doi.org/10.3109/02652048.2013.764938

96. Gu M, Zhang Z, Pan C, Goulette TR, Zhang R, Hendricks G, et al. Encapsulation of Bifidobacterium pseudocatenulatum G7 in gastroprotective microgels: Improvement of the bacterial viability under simulated gastrointestinal conditions. Food Hydrocoll. 2019;91:283–9
https://doi.org/10.1016/j.foodhyd.2019.01.040

97. Lascol M, Bourgeois S, Barratier C, Marote P, Lantéri P, Bordes C. Development of pectin microparticles by using ionotropic gelation with chlorhexidine as cross-linking agent. Int J Pharm. 2018;542(1–2):205–12.
https://doi.org/10.1016/j.ijpharm.2018.03.011

98. Moura SCSR, Berling CL, Garcia AO, Queiroz MB, Alvim ID, Hubinger MD. Release of anthocyanins from the hibiscus extract encapsulated by ionic gelation and application of microparticles in jelly candy. Food Res Int. 2019;121:542–52.
https://doi.org/10.1016/j.foodres.2018.12.010

99. Moura SCSR, Schettini GN, Garcia AO, Gallina DA, Alvim ID, Hubinger MD. Stability of Hibiscus Extract Encapsulated by Ionic Gelation Incorporated in Yogurt. Food Bioprocess Technol. 2019;12(9):1500–15.
https://doi.org/10.1007/s11947-019-02308-9

100. Sathasivam T, Muniyandy S, Chuah LH, Janarthanan P. Encapsulation of red palm oil in carboxymethyl sago cellulose beads by emulsification and vibration technology: Physicochemical characterization and in vitro digestion. J Food Eng. 2018;231:10–21.
https://doi.org/10.1016/j.jfoodeng.2018.03.008

101. Aoki T, Decker EA, McClements DJ. Influence of environmental stresses on stability of O/W emulsions containing droplets stabilized by multilayered membranes produced by a layer-by-layer electrostatic deposition technique. Food Hydrocoll. 2005;19(2):209–20.
https://doi.org/10.1016/j.foodhyd.2004.05.006

102. Guzey D, McClements DJ. Formation, stability and properties of multilayer emulsions for application in the food industry. Adv Colloid Interface Sci. 2006;128–130(2006):227–48.
https://doi.org/10.1016/j.cis.2006.11.021

103. Burgos-Díaz C, Wandersleben T, Marqués AM, Rubilar M. Multilayer emulsions stabilized by vegetable proteins and polysaccharides. Curr Opin Colloid Interface Sci. 2016;25:51–7.
https://doi.org/10.1016/j.cocis.2016.06.014

104. Burgos-Díaz C, Hernández X, Wandersleben T, Barahona T, Medina C, Quiroz A, et al. Influence of multilayer O/W emulsions stabilized by proteins from a novel lupin variety AluProt-CGNA and ionic polysaccharides on D-limonene retention during spray-drying. Colloids Surfaces A Physicochem Eng Asp. 2018;536:234–41.
https://doi.org/10.1016/j.colsurfa.2017.04.032

105. Fang S, Zhao X, Liu Y, Liang X, Yang Y. Fabricating multilayer emulsions by using OSA starch and chitosan suitable for spray drying: Application in the encapsulation of β-carotene. Food Hydrocoll. 2019;93(February):102–10.
https://doi.org/10.1016/j.foodhyd.2019.02.024

106. Chun JY, Choi MJ, Min SG, Weiss J. Formation and stability of multiple-layered liposomes by layer-by-layer electrostatic deposition of biopolymers. Food Hydrocoll. 2013;30(1):249–57. https://doi.org/10.1016/j.foodhyd.2012.05.024

107. Griffin K, Khouryieh H. Influence of electrostatic interactions on the formation and stability of multilayer fish oil-in-water emulsions stabilized by whey protein-xanthan-locust bean complexes. J Food Eng. 2020;277:109893. https://doi.org/10.1016/j.jfoodeng.2019.109893

108. Jeon S, Yoo CY, Park SN. Improved stability and skin permeability of sodium hyaluronate-chitosan multilayered liposomes by Layer-by-Layer electrostatic deposition for quercetin delivery. Colloids Surfaces B Biointerfaces. 2015;129:7–14. https://doi.org/10.1016/j.colsurfb.2015.03.018

109. Burgos-Díaz C, Gallardo M, Morales E, Piornos JA, Marqués AM, Rubilar M. Utilization of proteins from AluProt-CGNA (a novel protein-rich lupin variety) in the development of oil-in-water multilayer emulsion systems. Eur J Lipid Sci Technol. 2016;118(7):1104–12. https://doi.org/10.1002/ejlt.201500260

110. Liu C, Tan Y, Xu Y, McCleiments DJ, Wang D. Formation, characterization, and application of chitosan/pectin-stabilized multilayer emulsions as astaxanthin delivery systems. Int J Biol Macromol. 2019;140:985–97. https://doi.org/10.1016/j.ijbiomac.2019.08.071

111. Fioramonti SA, Martinez MJ, Pilosof AMR, Rubiolo AC, Santiago LG. Multilayer emulsions as a strategy for linseed oil microencapsulation: Effect of pH and alginate concentration. Food Hydrocoll. 2015;43:8–17. https://doi.org/10.1016/j.foodhyd.2014.04.026

112. Mundo JLM, Zhou H, Tan Y, Liu J, Julian D. Stabilization of soybean oil-in-water emulsions using polypeptide multilayers : Cationic polylysine and anionic polyglutamic acid. Food Res Int. 2020;109304. https://doi.org/10.1016/j.foodhyd.2014.04.026

113. Fioramonti SA, Rubiolo AC, Santiago LG. Characterisation of freeze-dried flaxseed oil microcapsules obtained by multilayer emulsions. Powder Technol. 2017;319:238–44. https://doi.org/10.1016/j.powtec.2017.06.052

114. Klinkesorn U, Sophanodora P, Chinachoti P, Decker EA, McClements DJ. Characterization of spray-dried tuna oil emulsified in two-layered interfacial membranes prepared using electrostatic layer-by-layer deposition. Food Res Int. 2006;39(4):449–57. https://doi.org/10.1016/j.foodres.2005.09.008

115. Noello C, Carvalho AGS, Silva VM, Hubinger MD. Spray dried microparticles of chia oil using emulsion stabilized by whey protein concentrate and pectin by electrostatic deposition. Food Res Int. 2016;89:549–57.
https://doi.org/10.1016/j.foodres.2016.09.003
116. Fioramonti SA, Stepanic EM, Tibaldo AM, Pavón YL, Santiago LG. Spray dried flaxseed oil powdered microcapsules obtained using milk whey proteins-alginate double layer emulsions. Food Res Int. 2019;119:931–40.
https://doi.org/10.1016/j.foodres.2018.10.079
117. Salaün F, Bedek G, Devaux E, Dupont D, Gengembre L. Microencapsulation of a cooling agent by interfacial polymerization: Influence of the parameters of encapsulation on poly(urethane-urea) microparticles characteristics. J Memb Sci. 2011;370(1–2):23–33.
https://doi.org/10.1016/j.memsci.2010.11.033
118. Kamble V, Sawant M, Mahanwar P. Microencapsulation of Cypermethrin Via Interfacial Polymerization for Controlled Release Application. Mater Today Proc. 2018;5(10):22621–9.
https://doi.org/10.1016/j.matpr.2018.06.636
119. Perignon C, Ongmayeb G, Neufeld R, Frere Y, Poncelet D. Microencapsulation by interfacial polymerisation: Membrane formation and structure. J Microencapsul. 2015;32(1):1–15.
https://doi.org/10.3109/02652048.2014.950711
120. Marcela F, Lucía C, Eva B, David G, Ángeles BM, Luis B. Microencapsulation of Essential Oils by Interfacial Polimerization Using Polyurea as a Wall Material. J Encapsulation Adsorpt Sci. 2015;5(4):165–77.
165–177.
https://doi.org/10.4236/jeas.2015.54014
121. Zhu DY, Rong MZ, Zhang MQ. Self-healing polymeric materials based on microencapsulated healing agents: From design to preparation. Prog Polym Sci. 2015;49–50:175–220.
https://doi.org/10.1016/j.progpolymsci.2015.07.002
122. Nguon O, Lagugné-Labarthet F, Brandys FA, Li J, Gillies ER. Microencapsulation by in situ Polymerization of Amino Resins. Polym Rev. 2018;58(2):326–75.
123. Zhang Y, Mustapha AN, Zhang X, Baiocco D, Wellio G, Davies T, Zhang Z, Li Y. Improved volatile cargo retention and mechanical properties of capsules via sediment-free in situ polymerization with cross-linked poly(vinyl alcohol) as an emulsifier. J Colloid Interface Sci. 2020;568:155–64.
https://doi.org/10.1016/j.jcis.2020.01.115
124. Khorasani SN, Ataei S, Neisiany RE. Microencapsulation of a coconut oil-based alkyd resin into poly(melamine–urea–formaldehyde) as shell for self-healing purposes. Prog Org Coatings. 2017;111(February):99–106.
https://doi.org/10.1016/j.porgcoat.2017.05.014

125. Kage H, Kawahara H, Hamada N, Kotake T, Oe N, Ogura H. Effects of core material, operating temperature and time on microencapsulation by in situ polymerization method. Adv Powder Technol. 2002;13(4):377–94.
https://doi.org/10.1163/156855202320536025
126. Han S, Chen Y, Lyu S, Chen Z, Wang S, Fu F. Effects of processing conditions on the properties of paraffin/melamine-urea-formaldehyde microcapsules prepared by in situ polymerization. Colloids Surfaces A Physicochem Eng Asp. 2020;585:124046.
https://doi.org/10.1016/j.colsurfa.2019.124046
127. Moser P, Nicoletti VR, Drusch S, Brückner-Gühmann M. Functional properties of chickpea protein-pectin interfacial complex in buriti oil emulsions and spray dried microcapsules. Food Hydrocoll. 2020;107:105929.
https://doi.org/10.1016/j.foodhyd.2020.105929
128. Suryanarayana C, Rao KC, Kumar D. Preparation and characterization of microcapsules containing linseed oil and its use in self-healing coatings. Prog Org Coatings. 2008;63(1):72–8.
https://doi.org/10.1016/j.porgcoat.2008.04.008

4.2 CAPÍTULO II - Characterization and controlled release of pequi oil microcapsules for yogurt application

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Characterization and controlled release of pequi oil microcapsules for yogurt application

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Abstract: Pequi oil is a valuable product for food industries due to its high levels of unsaturated fatty acids and carotenoids. This work used the complex coacervation technique with cashew gum/chitosan and cashew gum/gelatin matrices to encapsulate pequi oil in the production of enriched yogurt. Both wall materials achieved good results in encapsulation efficiency (>80%), yield (>50%), and loading capacity (<50%). The FTIR data demonstrated the presence of electrostatic interactions, thereby confirming encapsulation, and TGA, DSC and Rancimat data verified the microcapsule's stability. The acid release and temperature resistance results showed greater stability at pH's close to the optimum condition of the coacervation process and at temperatures below 80 °C. Finally, the potential for its application in yogurts was realized, showing that the preparation procedure of yogurt influenced the oil release rate and fatty acid profile, and that the addition of microcapsules should be optimally done during the cooling step.

Keywords: Polysaccharide; Protein; Complex coacervation; Pequi oil; Yogurt

1. INTRODUCTION

Pequi tree is native to Brazil and belongs to the *Caryocaraceae* family. Pequi oil has stood out as a useful raw material for the food and pharmaceutical industries due to its high levels of unsaturated fatty acids, predominantly oleic (60.6%), and carotenoids, such as beta-carotene, a precursor to vitamin A (Pinto et al., 2018). Oleic acid participates in human metabolism and plays an important role in hormone synthesis. In addition, it may improve heart conditions and reduce triglycerides, LDL cholesterol, total cholesterol, and the glycemic index (Mezzomo et al., 2010). Pequi oil is currently used in the food industry, despite its strong taste and smell, which give the product a distinctive characteristic noted by the manufacturers. In food formulations, sometimes masking the natural characteristics of taste and smell is as important as its chemical stability. The use of microencapsulation methods may mask the strong flavor and taste of the pequi oil, besides protecting against oxidation and facilitating its controlled release.

Oxidation can lead to unpleasant flavor and odor formation, reducing the product shelf life and promoting free radical generation, which can have negative physiological effects on the body (Alcântara et al., 2019). Complex coacervation is one of the most often used methods for lipid microencapsulation (Alexandre et al., 2019; Soares, Siqueira, Carvalho, Vicente, & Garcia-Rojas, 2019; Oliveira et al., 2020; Nascimento et al., 2020). This process entails moderate process conditions, low cost, and high encapsulation efficiency. It results from liquid-liquid phase separation that occurs between polymers of opposite charges through electrostatic interactions (Eghbal & Choudhary, 2018). This technique demands no sophisticated equipment, is effective, and can be easily adapted to protect essential fatty acids from the oxidative degradation process (Timilsena, Wang, Adhikari, & Adhikari, 2017).

Among the polysaccharides used as wall materials, gum arabic is one of the most often found in the food industry despite its high cost and problematic availability (Ghafarloo, Jouki, & Tabari, 2020). Recent studies have attempted to replace gum arabic by other types of plant-based gums (Jouki, Khazaei, Rezaei, & Taghavian-Saeid, 2021; Jouki & Khazaei, 2021; Khodashenas & Jouki, 2020; Shariati, Jouki, & Rafiei, 2020). A good substitute is cashew gum, due to its physical-chemical characteristics and also because it is a low-value coproduct of cashew production (Oliveira et al., 2018). Previous studies have already shown the potential of cashew gum as a substitute for gum arabic in the microencapsulation of pequi oil (Silva et al., 2018; Nascimento et al., 2020).

Nowadays, there has been much interest in fortifying milk and dairy products with a wide variety of bioactive ingredients. Because of its popularity, yogurt can be a good vehicle to ensure and improve the daily intake of nutrients, which can prevent diseases and maintain the health of consumers (Soares et al., 2019). Among the fatty acids that are present in yogurt can be mentioned the low quantity of unsaturated fatty acids. In this connection, enrichment with oils of vegetable or animal origin is desirable to improve the nutritional characteristic of yogurts. However, only a few studies have attempted to improve the unsaturated fatty acid profile of yogurts. Among the few reported studies, vegetables and fish oils were generally used (Bello, Torri, Piochi, & Zeppa, 2015; Nieuwenhove et al., 2019; Ribeiro & Bonif, 2021). The microencapsulation technology in the application of triglyceride oils in yogurt has been little explored in the literature (Baba et al., 2018; Hinnenkamp, Reineccius, & Ismail, 2021). Because of its economic potential, encapsulation of probiotics has been previously studied (Nyanzi, Jooste, & Buys, 2021).

In this work, two combinations of wall materials, cashew gum/chitosan (CCP) and cashew gum/gelatin (CGP) were compared for pequi oil microencapsulation to produce oil-enriched yogurts. The addition of microcapsules to yogurt was evaluated in two steps during the manufacturing process of the dairy products. The microencapsulation process was characterized, and aspects of the oil-controlled release were also evaluated in this study.

2. MATERIALS AND METHODS

2.1. Materials

Cashew gum (CG) polysaccharides were obtained from the *Anacardium occidentale* L. exudate collected from cashew trees at the Experimental Pacajus Field of Embrapa Agroindustry Tropical. Low molecular weight and 75-85% deacetylated chitosan (CT) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Bovine gelatin (GE) 225H type B was provided by Rousselot® (The Netherlands). Methyl tricosanoate was purchased from Sigma-Aldrich. The microorganism culture was commercially acquired from Bio Rich® (*Lactobacillus acidophilus* LA-5 $1x10^6$ UFC/g, *Bifidobacterium* BB-12 $1x10^6$ UFC/g, and *Streptococcus thermophilus*).

2.2. Polysaccharides isolation from cashew gum

The isolation of polysaccharides was performed using the methodology previously described by Silva et al. (2018). Initially, the exudate collected was crushed in a knife mill.

Then, the sample was solubilized in water (300 g of the sample in one liter of water), filtered and centrifuged (15,303*xg* for 10 min at 25 °C). After removing the residues, the supernatant was precipitated in ethanol at a sample : ethanol ratio of 1:3 (v/v) for 24 h at 8 °C. The excess ethanol was removed, and the precipitate dried in an air circulation oven at 60 °C. Finally, the sample was ground, and the purified CG obtained.

2.3. Pequi pulp oil extraction

The pequi fruits (*Caryocar coriaceum* Wittm.) were washed, sanitized (chlorine at 200 g/L in water for 30 min), peeled and submitted to a hammer pulper (300 kg/h capacity). The pulp was stored in plastic containers and kept in a freezer. For pequi oil (PO) extraction, the pulp was heated to 45 °C and centrifuged at 4,500 rpm for 15 min to separate the oil (Lima et al., 2019).

2.4. Formation of microcapsules

The cashew gum/chitosan pequi oil-microcapsules (CCP) were prepared according to the methodology described by Silva et al. (2018). For the coacervation process, each emulsion was prepared from 100 mL CT (0.05 g/L) in deionized water (20 mL/L acetic acid) with 2 g PO; 100 mL of CG solution (0.11 g/L) in deionized water was added and homogenized using an ultra-turrax homogenizer for 3 min at 10,000 rpm. Then, 400 mL of water was added and homogenized in the ultra-turrax.

The cashew gum/gelatin pequi oil-microcapsules (CGP) were obtained using the methodology described by Nascimento et al. (2020). An emulsion was initially prepared to produce a solution containing 100 mL of GE (0.2 g/L) in deionized water and 2 g of PO; a solution of 100 mL of CG (0.4 g/L) in deionized water was then added and stirred in an ultra-turrax for 3 min at 10,000 rpm. Thereafter, 400 mL of water was added and homogenized again in the ultra-turrax.

Finally, after the emulsions of CCP and CGP were prepared, the pH was adjusted to 4.5, and cooled (5 °C) overnight. The solutions containing the microcapsules were centrifuged at 15,303*xg* for 10 min at 25 °C, the excess water was removed, and the rest was frozen in an ultra-freezer (-80 °C) for 24 hours. The frozen materials were taken to a freeze-dryer (Liotop LP 820) until completely dried and dispersed in an impact mill (IKA A11 basic).

2.5. Microcapsules characterization

2.5.1. Yield

The yield was calculated based on the weight ratio of freeze-dried microcapsules before the drying process, according to eq.1 (Jouki, Khazaei, Rashidi-alavijeh, & Ahmadi, 2021), where Y is the percentage yield, M_f is the value in grams of the final dry mass obtained after freeze-drying, and M_i is the initial mass used in preparing the solution for the coacervation process (biopolymers mass + oil mass) (Jouki, Khazaei, Rashidi-alavijeh, & Ahmadi, 2021; Huang, Sun, Xiao, & Yang, 2012).

$$Y(\%) = \frac{Mf}{Mi}\ x\ 100 \qquad \text{(eq.1)}$$

2.5.2. Encapsulation efficiency

The amounts of total and superficial oil present were quantified by the oleic acid peak areas obtained by gas chromatography and standardized using an internal standard C23:0 (methyl tricosanoate). The encapsulation efficiency (EE) was determined by eq. 2, where A_{so} is the oleic acid area standardized from the superficial oil, and A_{to} is the oleic acid area standardized from the total oil.

$$EE(\%) = \frac{Ato - Aso}{Ato}\ x\ 100 \qquad \text{(eq.2)}$$

2.5.2.1. Determination of total and superficial oil

For the determination of the total oil (TO), 25 mL of 4 mol/L HCl solution was added to 0.5 g and 0.25 g of CCP and CGP, respectively, and shaken at 100 rpm for 24 h. 50 mL of ethanol (for better separation of the oil from the polymers), and 5 mL of n-heptane were added and centrifuged (15,303xg for 10 min at 25 °C). The fraction containing heptane with the released oil was collected, transesterified with methanol, and taken to a gas chromatograph to obtain the oleic acid peak area.

For the determination of the superficial oil (SO), 0.5 g and 0.25 g of CCP and CGP, respectively, were weighed and 50 mL of ethanol and 5 mL of n-heptane were added and stirred for 1 min. The fraction containing heptane with oil-released microcapsules were collected, transesterified, and taken to a gas chromatograph to obtain the oleic acid peak area.

2.5.2.2. Gas chromatography analysis

The fatty acids were converted into fatty acid methyl esters (FAMEs). In a 5 mL fraction of n-heptane containing the released oil, 0.25 mL of the 2 mol/L NaOH solution in methanol was added and vortexed for 20 sec. After transesterification to obtain FAMEs, 1 mL of the supernatant was collected and evaporated for further analysis. The analysis of the FAMEs was carried out in a gas chromatography apparatus with flame ionization detector (CG-FID) (Shimadzu, model GC2010 Plus), equipped with an Automator, model AOC 5000. A capillary column (SP2560) with biscyanopropyl polydimethylsiloxane stationary phase (100 m × 0.25 mm, df 0.20 μm; Supelco Bellefonte, PA) was used to separate the compounds. The injection mode used was flow division (split 1:30), and the carrier gas was hydrogen with a constant flow of 1.5 mL/min. The injector and detector temperature were 220 °C. The programming of the chromatographic oven included an initial temperature of 80 °C, ascending with a heating ramp from 11 °C/min to 180 °C, increasing in a ramp from 5 °C/min to 220 °C, and maintaining for 23 minutes. The identification of the peaks in the chromatogram was performed by comparing their retention indexes with those of a standard solution of fatty acids (CRM47885, Supelco) previously injected, following the same methodology. An internal standard C23:0 (methyl tricosanoate), diluted in hexane, at the concentration of 2 μL/mL, was used, where 10 μL of the standard solution and 700 μL of n-hexane (HPLC grade) were added together with the sample for the calculation of the peak area for standardized oleic acid. For the milk and yogurt samples, the fat extraction was performed by the method of Bligh & Dyer (1959).

2.5.3. Loading capacity

The loading capacity (LC) of the microcapsules was calculated according to Eq. 3, where Mpo is the encapsulated PO mass calculated by the mass used initially concerning EE, and Mf is the value in grams of the final dry mass obtained after freeze-drying. According to the equation, the values were adjusted by the encapsulation efficiency.

$$LC(\%) = \frac{Mpo}{Mf}\ x\ 100 \qquad \text{(eq.3)}$$

2.5.4. Microcapsules morphology

The micrographs of the coacervates before freeze-drying were obtained via transmission electron microscopy (TEM) (Model Vega 3, Tescan). An aliquot was collected from the precipitated microcapsules, water was added and dispersed by vortexing, and a small drop was deposited in a carbon-coated copper grid and left to stand for 3 min. The excess sample was

removed with a filter paper. Then, the sample was stained with phosphotungstic acid (0.04 g/L) for 3 min and the excess was again removed with filter paper. The TEM analysis was carried out at an acceleration voltage of 30 kV.

The micrographs of freeze-dried microcapsules were obtained using a scanning electron microscope (SEM; Quanta 450 FEG System: FEI Company, USA) with a scanning voltage of 5 kV. The samples were metalized with a thin layer of silver produced by an evaporator (Emitech, k550).

2.5.5. Fourier transform infrared spectroscopy (FT-IR)

The polymers and freeze-dried microcapsules were mixed with potassium bromide (KBr) (1% w/w) and analyzed by Fourier transform infrared spectroscopy on a Spectrum Two spectrometer (Perkin-Elmer, USA). The scan was performed in the frequency range 400-4000 cm^{-1} with a resolution of 4 cm^{-1}. The oil samples were analyzed by transmission on KBr disks with 0.025 mm Teflon spacers (Castelo, Silva, Sousa, Magalhães, & Furtado, 2020).

2.6. Thermal analysis

The microcapsules were analyzed by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). PerkinElmer STA 6000 equipment was used for TGA analysis. The samples were weighed and scanned in the temperature range from 25 to 750 °C at a heating rate of 10 °C/min and a nitrogen flow rate of 20 mL/min. For DSC analysis (Shimadzu), previously weighed samples were heated to a temperature range of 25 to 300 °C with a heating rate of 10 °C/min and a flow of nitrogen gas at 50 mL/min flow rate.

2.7. Accelerated oxidative stability (Rancimat)

The PO oxidative stability was analyzed by the oxidative stability index performed by accelerated oxidation using the Rancimat equipment (Metrohm Rancimat 743 model 873). 3 g of PO and 1.5 g of microcapsules (with and without oil) were used in a constant airflow of 10 L/h and a fixed temperature of 110 °C. The sample induction time, measured in hours, was used as an oxidative stability index.

2.8. Oil release

2.8.1. Calculation of release kinetics

For the calculation of release kinetics, microcapsules were released in vitro under accelerated conditions as described by Jain, Thakur, Ghoshal, Katare, & Shivhare (2016) with adaptations. 25 mL of 0.1 mol/L HCl solution was added to 0.5 g and 0.25 g of CCP and CGP, respectively, with constant shaking in a shaker (100 rpm). The released oil was evaluated at 0, 15, 30, 60, 120, 240, 360 min. After the specified analysis time, 50 mL of ethanol and 5 mL of n-heptane were added and slightly agitated. The fraction containing heptane with released oil was collected, transesterified, and taken to the gas chromatograph to obtain the oleic acid peak area according to section 2.5.2.2.

For the assessment of the release mechanism, the data were fitted to mathematical models that describe zero order, first order, Higuchi, and Korsmeyer-Peppas kinetics (Eqs. 4, 5, 6, and 7) as described by Budinčić et al. (2021) with adaptations:

Zero order: $Q = Q_0 - K_0\, t$ (eq.4)

First order $lnQ = lnQ_0 - K_1\, t$ (eq.5)

Higuchi: $Q = K_H\, t^{1/2}$ (eq.6)

Korsmeyer-Peppas: $Q = K_{KP}\, t^n$ (eq.7)

where Q is the amount of oil released at time t, Q0 is the initial amount of oil in the solution, n is the diffusion exponent indicative of the active ingredient's transport mechanism, K0, K1, KH, and KKP are release rate constants for zero order, first order, and kinetic models of Higuchi and Korsmeyer-Peppas, respectively.

2.8.2. Oil release as functions of temperature and pH

For evaluation of the effect of temperature on oil release, 0.5 g and 0.25 g of CCP and CGP, respectively, were added to 25 mL preheated water at temperatures of 2, 20, 40, 60, and 80 °C and shaken at constant temperature in a shaker (100 rpm) for 5 minutes. The release was measured at different pH in the range of 2 - 7.

After the analysis time, 50 mL of ethanol and 5 mL of n-heptane were added to the sample and slightly agitated. The fraction containing heptane with the released oil was collected, transesterified and taken to the gas chromatograph to obtain the oleic acid peak area according to section 2.5.2.2.

2.9. Application of microcapsules in yogurt

2.9.1. Yogurt production

Yogurt was prepared according to Yu et al. (2021) with modifications. Initially, the milk was pasteurized at 70 °C for 30 min, followed by cooling to 43 °C, where the microorganism culture was added in the ratio of 400 mg for 1 L of milk. The milk was kept at 43 °C in an oven for 3-4 hours until clots were formed at pH 4.6. Finally, the yogurt was kept cooled overnight for further analysis of oil release and fatty acid profile. The PO microcapsules were added (1% w/v) under three different conditions: Y1: the PO microcapsules were added along with the microorganism culture, Y2: the PO microcapsules were added after kept cooled overnight, and Y3: the PO microcapsules were added after 2 hours of cooling and kept cooled overnight (Figure 1).

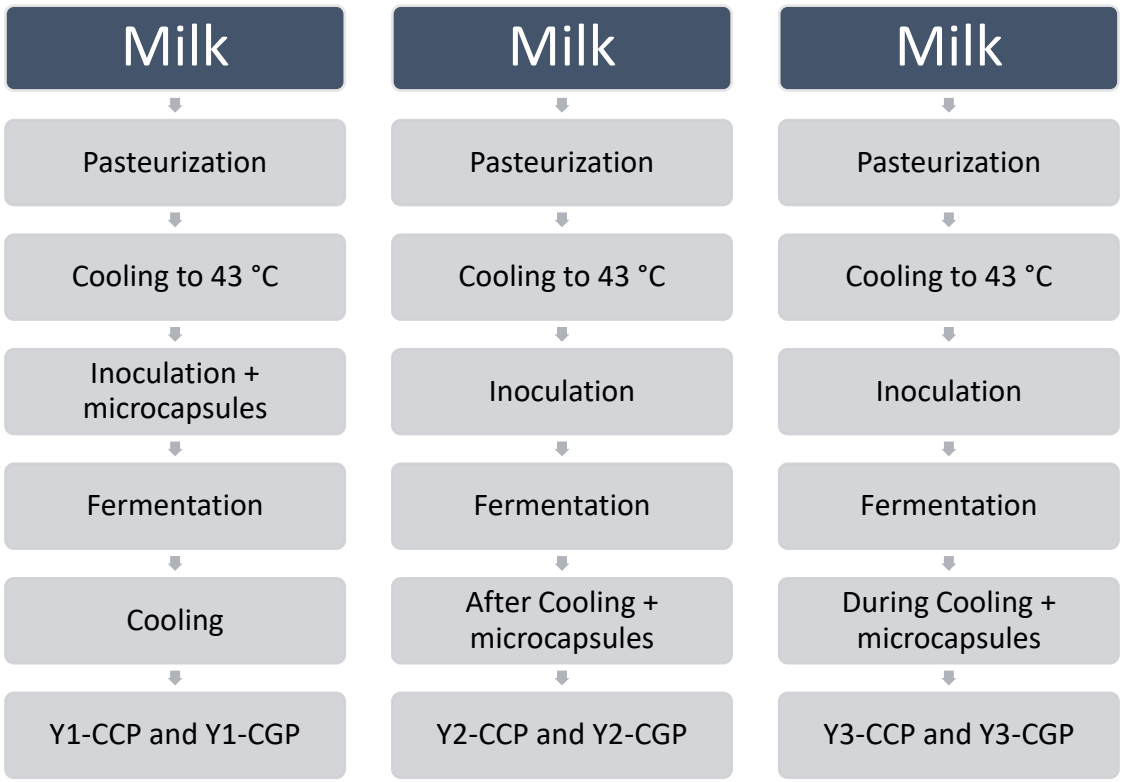

Figure 1: Flowchart representative of the preparation of yogurts enriched with microencapsulated pequi oil added in different stages.

2.10. Statistical analysis

The results for yogurt preparation were performed in triplicate and analyzed statistically using the Statistica 13.0 software. The values of efficiency, yield, and loading capacity were compared by Student's T-test.

3. RESULTS AND DISCUSSIONS

3.1. Microcapsules characterization

3.1.1. Encapsulation efficiency (EE), yield and loading capacity (LC)

Careful selection of wall material is vital, as it affects encapsulation efficiency as well as other characteristics of the microcapsules. Among the wall materials, the most used in complex coacervation are polysaccharides and proteins. The present work evaluates the combination of CCP and CGP, and the influence of these matrices on the EE, yield, and LC of PO microencapsulation.

The EE results differed significantly ($p>0.05$), with the CCP microcapsules (93.65 ± 0.37) exhibiting higher values than the CGP microcapsules (83.75 ± 2.88). A higher EE result indicates better trapping of the active material and a lower amount of surface oil that is more exposed to the external environment. Oliveira et al. (2020) encapsulated the green coffee oil by complex coacervation in a matrix of cashew gum and gelatin and obtained EE results above 85%. Other studies that used complex coacervation for vegetable oil microencapsulation also produced capsules with EE above 80% (Comunian et al., 2018; Lemos, Marfil, & Nicoletti, 2017; Timilsena, Adhikari, Barrow, & Adhikari, 2016).

The microcapsules indicated no significant difference ($p<0.05$) with respect to yield, being 53.29 ± 1.32 and 53.98 ± 1.15 for CCP and CGP microcapsules, respectively, showing that the change of wall material in this case had no detectable influence. Rutz et al. (2017) analyzed the yield results for different wall materials (chitosan/pectin and chitosan/xanthan), and also found no significant difference between the averages calculated. Alexandre et al. (2019) evaluated the yield of cashew gum and gelatin microcapsules crosslinked with tannic acid and obtained yield results ranging from 7.77% to 39.77% among the formulations attempted.

Regarding the LC, there was a significant difference between the results for CCP (25.71 ± 0.51) and CGP (39.30 ± 1.45) ($p>0.05$), with the better result for the CGP microcapsules. Other workers that used the complex coacervation as an encapsulation method also found LC about 30% (Oliveira et al., 2020; Timilsena et al., 2019). A higher LC decreases the thickness of the encapsulating matrix, which may cause a higher release rate (Devi, Hazarika, Deka, & Kakati, 2012) and influence the value of EE. This behavior was observed for CCP microcapsules that exhibited a higher EE and a lower LC, and for CGP that showed a lower EE and a higher LC.

3.1.2. Microcapsules morphology

Figures 2 A and B show the micrographs obtained by TEM of the CCP and CGP microcapsules, respectively. Both images revealed spherical micrometric shapes, with no signs of breaks and oil leaks. The particle size was heterogeneous as seen in Figures 2 C and D. In previous studies, the mean particle size of the CCP microcapsules was 4.8 µm (Silva et al., 2018) and 8.2 µm for the CGP microcapsules (Nascimento et al., 2020). The microcapsule density proved to be quite different, with the CCP having a darker tone, showing a denser matrix, which may rationalize the better result of EE. The CGP microcapsules, despite giving a better LC result, show a matrix with a lower quantity of biopolymers resulting in a less dense morphology, which makes the capsules more susceptible to ruptures caused by external factors.

Jeon, Yoo, & Park (2015) used TEM for the morphological characterization of particles obtained by multilayer electrostatic deposition with denser regions of 5 layers, with micrographs like those obtained with the CCP microcapsules. The studies carried out by Yu, Xue, & Zhang (2021), on the other hand, obtained micrographs in which the internal core and the external matrix can be viewed separately, typical of microcapsules obtained by complex coacervation, as can be observed in micrographs obtained from CGP microcapsules.

Micrographs of freeze-dried microcapsules were obtained by SEM. The agglomeration of microparticles (Figure 2 E and F) was also visualized by Cruz, Dagostin, Perussello, & Masson (2019) as a common feature of freeze-drying. The micrographs (Figure 2 G and H) revealed a homogeneous matrix without the presence of cracks and wrinkles, which indicates that the interactions between biopolymers occurred adequately without the need to add crosslinking materials as described by Soares et al. (2019).

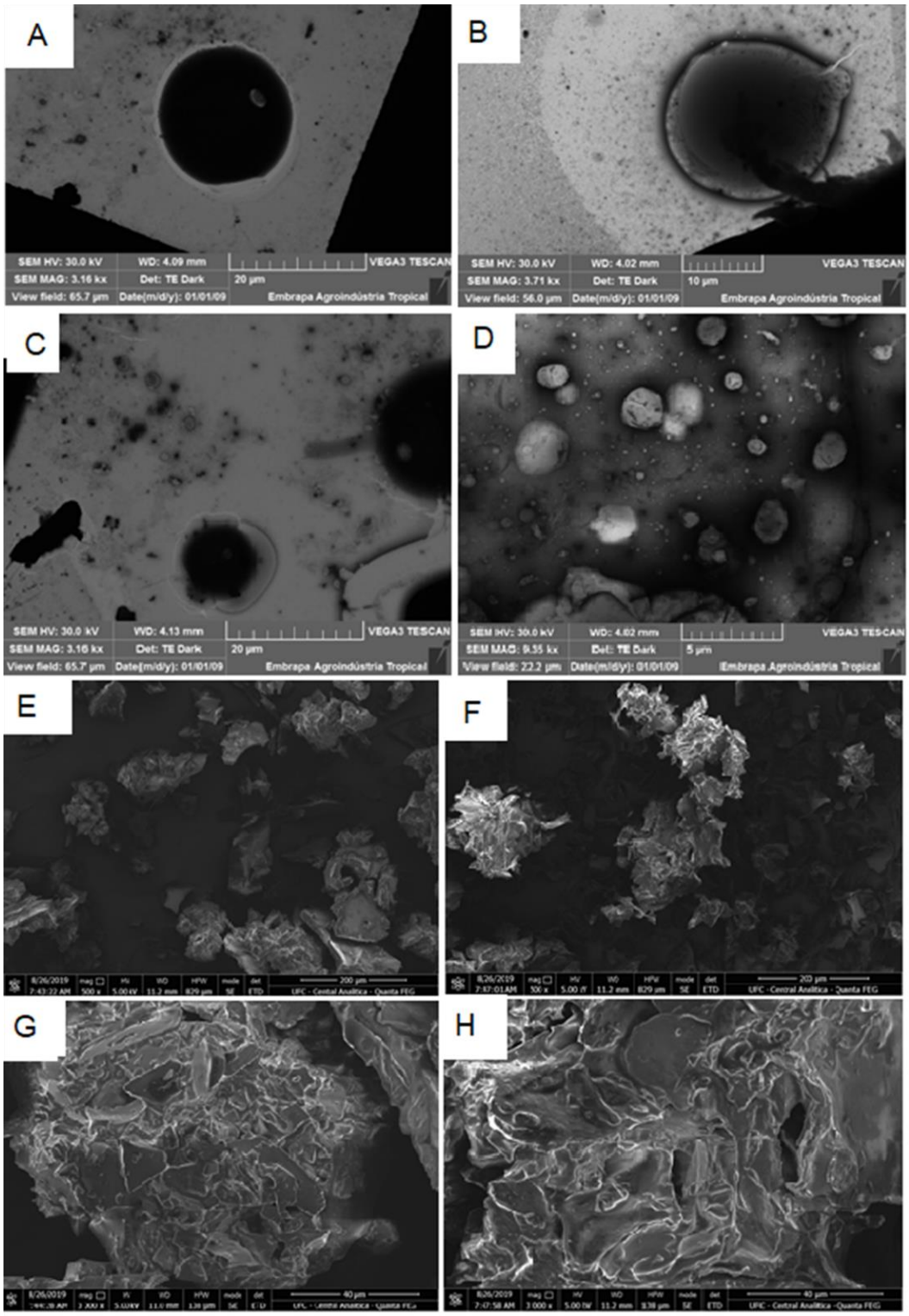

Figure 2: Transmission electron microscopy (TEM) micrographs of the microcapsules, where A and C correspond to CCP (cashew gum/chitosan-pequi oil) microcapsules, and B and D correspond to CGP (cashew gum/gelatin-pequi oil) microcapsules. Scanning electron microscopy (SEM) micrographs of the microcapsules are given in E and G corresponding to CCP microcapsules, and F and H corresponding to CGP microcapsules.

3.1.3. FT-IR

The FT-IR spectra for PO, CG, CT, and GE, as well as for CCP and CGP microcapsules, with and without the oil, are given in Figures 3 A and B. The PO showed strong characteristics of lipid bands in the 3050-2800 cm^{-1} region, which are associated with the C-H stretch of the methyl ($-CH_3$) and methylene ($-CH_2$) structures in the oils. This region is also associated with cis-alkene (-HC=CH-) present in unsaturated fatty acid molecules. The peak position can be used to determine the degree of unsaturation in oils because more double bonds lead to greater peak intensity (Ombredane et al., 2020; Timilsena et al., 2019). The large peak in the 1753 cm^{-1} region is attributed to the C=O bonds of in fatty acid esters. The peaks at 1467-1167 cm^{-1} are mainly from combinations of deformations of the groups $-CH_3$ and $-CH_2$. The bands in the region of 1237 and 1167 cm^{-1} are attributed to the symmetrical stretching (C-O-C) of triglycerides and phytosterol ester (Castelo et al., 2020) .

The CG peaks that appear with low intensities at about 2925 cm^{-1} are characteristic of O-H stretch in carboxyl groups. The CT spectrum (Figure 3 A) also showed the peaks of NH groups around 3300 cm^{-1}, and the amide bonds I, II and III are defined in angular deformation around 1656 and 1580 cm^{-1}, with greater intensity than GE. A low-intensity band around 1380 cm^{-1} was found for acetyl CH_3 groups remaining after deacetylation (Castelo et al., 2020). GE is a biodegradable amphoteric polymer composed of polypeptides with different molecular weights (Devi et al., 2012). In the GE spectrum (Figure 3 B), the strong absorption band around 3300 cm^{-1} was attributed to the amino groups, which have a positive charge in the acid medium (Cruz et al., 2019).

When comparing the spectra of the CCP (Figure 3 A) and CGP (Figure 3 B) microcapsules, each spectrum reveals distinct characteristics present in the biopolymers spectrum, as well as characteristic bands of PO. For both microcapsules, the cis-alkene band (-HC=CH-) at ca. 3000 cm^{-1} and C=O peak at 1753 cm^{-1} appear clearly and differently from the CCP and CGP microcapsules. The decreases in band intensity in the 1634 cm^{-1} range for CCP microcapsules and the 1649 cm^{-1} range for CGP microcapsules indicate that electrostatic interactions have occurred between the CT and GE amine groups ($-NH_3^+$) and CG carboxyl groups ($-COO^-$).

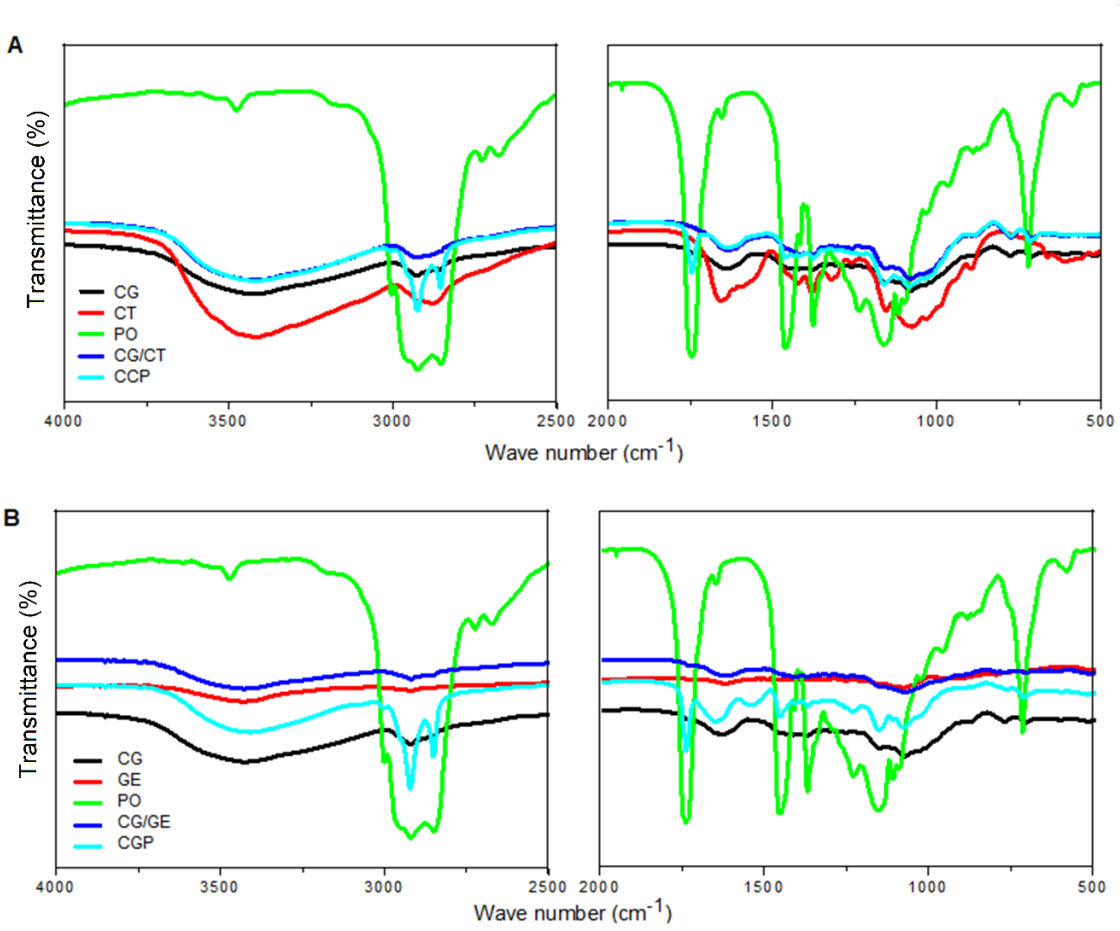

Figure 3: FT-IR spectra for A) cashew gum (CG), chitosan (CT), pequi oil (PO), cashew gum/chitosan (CG/CT) and cashew gum/chitosan-pequi oil (CCP); B) CG, gelatin (GE), PO, cashew gum/gelatin (CG/GE) and cashew gum/gelatin-pequi oil (CGP).

3.2. Thermal analysis and oxidative stability

TGA data (giving the percentage of weight loss) of the microcapsules during heating are illustrated in Figure 4 A and B. There was a slight weight loss from the CCP and CGP microcapsules up to about 150 °C, due to the loss of water. For the CCP microcapsules, three degradation peaks were presented: the first at 260 °C attributed to the degradation of chitosan (Chang, Varankovich, & Nickerson, 2016), the second at 319 °C referring to cashew gum, where polysaccharide degradation occurs (Silva, Feitosa, Paula, & Paula, 2009) and the third at 450 °C for PO degradation. For the CGP microcapsules, only the peaks for the degradation of the biopolymers were observed, indicating better stability, probably due to interpolymer affinity as a result of electrostatic interactions between them (Oliveira, Paula, & Paula, 2014).

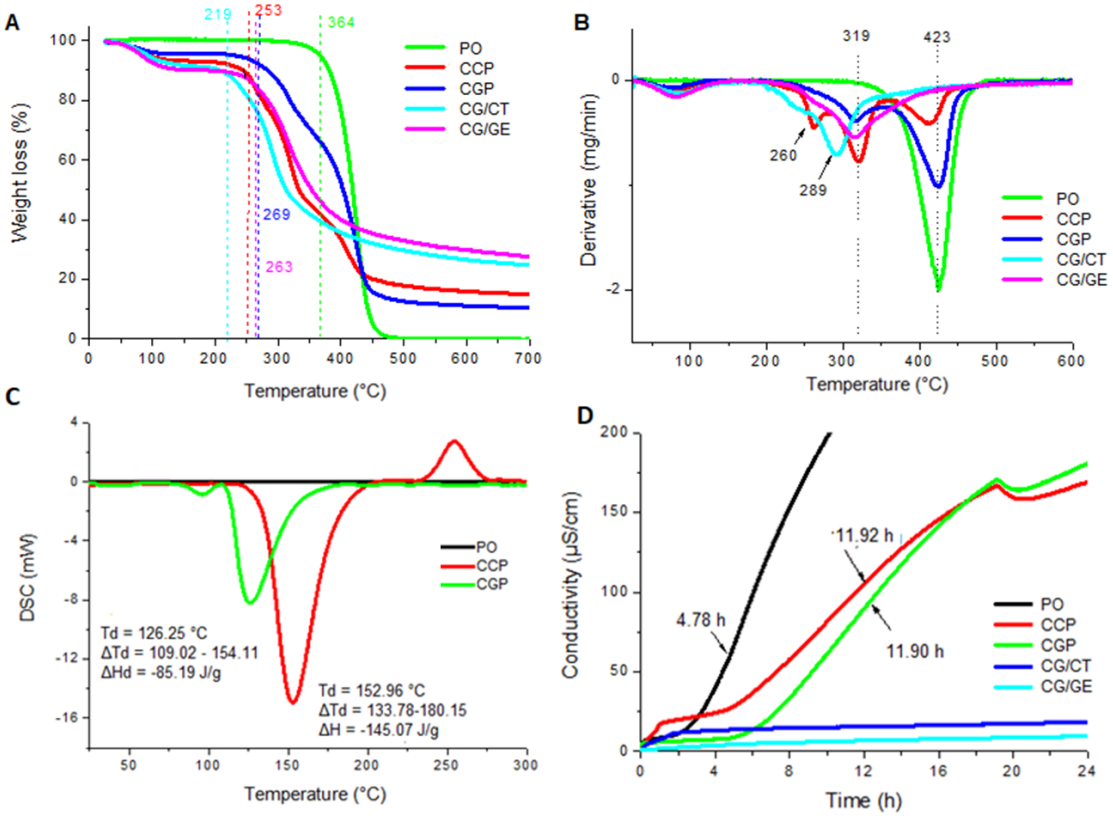

Figure 4: A) Weight loss (%) over time (°C); B) derivative weight loss (mg/min) over time (°C), for microcapsules of cashew gum/chitosan (CG/CT) and cashew gum/gelatin (CG/GE), and pequi oil (PO); C) Thermograms of cashew gum/chitosan-pequi oil (CCP) and cashew gum/gelatin-pequi oil (CGP) microcapsules, and PO where degradation temperature ranges (ΔTd), degradation temperature peaks (Td), and degradation enthalpies (ΔHd) were calculated; D) Oxidation curves (conductivity versus time) were obtained from Rancimat accelerated test. Arrows point out the mean induction period determined for each sample.

Fig. 4 C shows the DSC thermograms of the CCP and CGP microcapsules and PO. The range of degradation temperature (ΔTd), degradation temperature peak (Td), and degradation enthalpy (ΔHd) of the CCP and CGP microcapsules were determined from the thermograms. The peak degradation temperature and enthalpy of degradation of CGP (Td = 126.25 °C and ΔHd = -85.10 J/g) were lower than the CCP microcapsules (Td = 152.96 °C and ΔHd = -145.07 J/g). The higher peak degradation temperature and the degradation enthalpy of the CCP microcapsules indicated greater thermal stability than the CGP microcapsules. Both CCP and CGP microcapsules achieved better results than free biopolymers when compared with the data

in the literature, where Td can vary from 80 to 110 °C, proving the increase in thermal stability due to the complexation of polymers (Soares et al., 2019; Huang et al., 2012).

One of the aims of microencapsulation is to delay the onset of oxidation. Under the condition of accelerated oxidation at high temperatures, the induction time of PO and microcapsules with and without oil can be seen in Figure 4 D. For the PO the mean induction time found was 4.78 h, for the CCP and CGP microcapsules (with oil) the mean induction time was 11.92 h and 11.90 h, respectively. The microencapsulation of PO by the CCP and CGP matrices delayed oxidation by approximately 2.5 times the heating time compared to the pure oil, whereas no difference in the mean induction time was found for the two encapsulating matrices. Encapsulation studies by complex coacervation have previously been shown to protect oils when subjected to the condition of accelerated oxidation. Thus, Oliveira et al. (2020) and Comunian et al. (2016a) verified protection against oxidation of up to 6 and 2 times, respectively, a longer time than pure oil, using cashew gum and gelatin to microencapsulate green coffee oil and echium oil, respectively.

3.3. Pequi oil-controlled release study

The release profile of active material encapsulated isolated is important for an understanding of its behavior and the mechanism by which the release occurs. Tests carried out under specific conditions can provide useful information, especially to determinate the type of product that can be incorporated. In the accelerated release simulated *in vitro* (Figure 5 A), it was observed that the “burst” effect occurred, where there is a rapid release of the oil up to 120 min, followed by a steady release between 120 to 360 min. Both microcapsules showed this behavior, differentiating only in the percentage of oil released.

CCP microcapsules reached the release point in the range of 30%, whereas the CGP microcapsules reached > 60% (Figure 5 A). The lower release results obtained by the CCP microcapsules may be related to the lower load capacity which provided a better packaging of the active material, making its release more difficult. Jain et al. (2016) carried out the accelerated *in vitro* release of beta-carotene microcapsules obtained by complex coacervation and reported an initial “burst” up to 30%, followed by a slower release. They attributed the initial explosive effect of release to the oil adsorbed on the surface and the slow release to the mixed mechanism of penetration, solubilization, and diffusion of oil into the matrix.

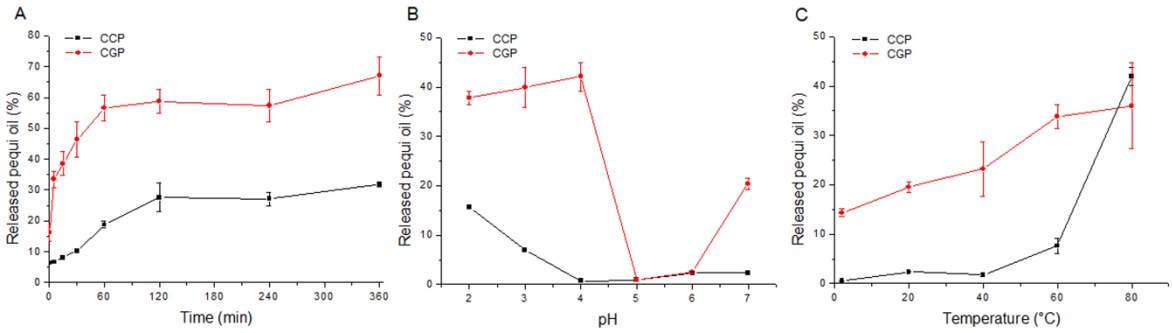

Figure 5: The pequi oil (PO) release profile of cashew gum/chitosan-pequi oil (CCP) and cashew gum/gelatin-pequi oil (CGP) microcapsules performed by: A) *in vitro* accelerated over time (min); B) as a function of pH (2 to 7); C) as a function of temperature (2 - 80 °C)

At the acidic pH range, both matrices had similar behaviors and showed the highest release values at the most extreme pH values (2 and 3). A decrease was observed at the pH near the isoelectric point of the formed complexes (3 to 5) and a slight increase close to neutrality (Figure 5 B). The release values of the CGP were higher than the CCP microcapsules. The low values found for the released oil in the pH used for the formation of the complexes was lower than the oil from particles; this can be explained by the greater agglomeration and precipitation of the microcapsules in the medium (increasing the oil retention), a behavior also noted by Comunian et al. (2016a) and Cruz et al. (2019).

The CCP microcapsules exhibited better resistance to the temperature increase up to 60 °C and then showed a “burst” increase in the transition up to 80 °C (Figure 5 C). The CGP microcapsules manifested a more gradual increase, ending in a result similar to that of CCP at 80 °C. Comunian et al. (2016a) evaluated the release at different temperatures of echium oil capsules obtained by complex coacervation, where, the microcapsules without crosslinking had the wall destroyed at 40 °C and the disruption of crosslinking started at 80 °C. Heating of the microcapsules may cause destabilization of the complex formed (Comunian et al., 2016b).

3.3.1. Release kinetics

For a better understanding of the kinetic profile of oil release from microcapsules, a study of accelerated *in vitro* release was performed. The results obtained up to 120 min were analyzed using mathematical models to simulate the release mechanism of the active material from the microcapsules. The values of the kinetic parameters used for the applied models are shown in Table 1.

Table 1: Kinetic parameters for the release of microcapsules CCP (cashew gum/chitosan-pequi oil) and CGP (cashew gum/gelatin-pequi oil) using mathematical models of zero order, first order, Higuchi and Korsmeyer-Peppas. Where, K is the release constant, R^2 is the correlation coefficient and n is the diffusion exponent.

Mathematical model	**CCP**	**CGP**
Zero order		
K_0	0.1857	0.2907
R^2	0.9615	0.6923
First order		
K_1	0.0128	0.0077
R^2	0.9494	0.5375
Higuchi		
K_H	2.0418	3.8702
R^2	0.8887	0.9160
Korsmeyer-Peppas		
K_{kp}	4.7512	18.5005
R^2	0.7896	0.9477
n	0.3039	0.2645

For the CCP microcapsules, the zero-order model gave the highest R^2, followed by the first-order model, indicating a linear trend during release; a similar result was also found by Cruz et al. (2019). The microcapsules that follow this type of profile do not disintegrate but release the active substance slowly and steadily (Fortunato et al., 2007). In the case of CGP microcapsules, a simple linear release was not the best model, and models such as Higuchi and Korsmeyer-Peppas were more preferred as shown in Table 2. The Korsmeyer-Peppas model had the highest value of R^2, followed by the Higuchi model; a similar result was also reported by Budinčić et al. (2021). This model is generally used to analyze the release of polymeric systems, when the release mechanism is not well known or when more than one type of release may be involved (Costa, 2002).

The diffusion exponent n varied from 0.3039 to 0.2645 for the CCP and CGP microcapsules, respectively. According to the literature, the value of n = 0.43 corresponds to the release of the active substance by diffusion according to Fick's law, while values less than 0.43 indicate pseudo-Fickian diffusion, where the diffusion shows similarities to a Fickian process. In this case, the release is quicker than Fickian (Ferreira, Pradela Filho, Santos, Takeuchi, & Assunção, 2019). Thus, it can be assumed that the release of pequi oil was based on a combination of removal of the superficial oil from the microcapsules and diffusion through the capsule wall; similar results were presented by Liu et al. (2016) and Budinčić et al. (2021)

3.4. Release of oil in yogurt and fatty acids profile

The oil release behavior of superficial oil from microcapsules introduced into different stages of yogurt production was evaluated in this study. As shown in the previous results of the release, the microcapsules showed better resistance to disintegration in an acid pH range from 4 to 5 and at a temperature below 80 °C. For this reason, the application in yogurt was chosen since the production conditions for yogurt are favorable to maintain the integrity of the microcapsule. The amount of superficial oil in the microcapsules introduced now of adding the microorganism was 20.78% and 31.29% for CCP and CGP respectively. The values of superficial oil were higher than those added after the cooling step at 0.37% and 0.36%, respectively. Under the third condition tested, a similar result for microcapsules added after 24 hours, the release was 0.34% and 0.54% for CCP and CGP respectively.

The fatty acids profiles of the PO, microcapsules, and the yogurt added with the microcapsules were also evaluated (Table 2). Among the results of the oil and the microcapsules, no differences were found for the percentages of saturated fatty acids (SFA), unsaturated (MUFA), and polyunsaturated (PUFA); thus, there is no interference from the microencapsulation process in the fatty acid profiles. On the other hand, for microcapsules introduced at the moment of adding the microorganism, variations were found with increased SFA and decreased MUFA, when compared with the PO values, showing that in the production process of the yogurt there is already oxidation of the oil resulting in differences in the percentage of SFA and MUFA.

The fatty acid profile of milk and yogurt, compared to formulations added with encapsulated and non-encapsulated oil, obtained higher values of SFA. This addition caused an increase in the percentage of oleic acid, between 25 and 33%. The yoghurt formulations that obtained better results in relation to amount of oleic acid were Y2-CCP and Y3-CCP.

Pereira et al. (2016) evaluated the variation of fatty acids exposed to radiation and observed some variations in the profile of SFA and MUFA due to material oxidation. Estrada, Boeneke, Bechtel, & Sathivel (2011) fortified the strawberry yogurt and analyzed the fatty acid profile, and no variation in the PUFA (predominant fatty acid) was observed during the production of the yogurt, but oxidation happened during storage.

Table 2: Fatty acid profile (%) of pequi oil (PO), cashew gum/chitosan-pequi oil (CCP) and cashew gum/gelatin-pequi oil (CGP) microcapsules and yogurt added from microcapsules in stages Y1 (the PO microcapsules were added along with the microorganism culture), Y2 (the PO microcapsules were added after being kept cooled overnight) and Y3 (the PO microcapsules was added after 2 hours of cooling and kept cooled overnight).

Fatty acid	PO	CCP	CGP	Milk	Yogurt	Y1-CCP	Y1-CGP	Y2-CCP	Y2-CGP	Y3-CCP	Y3-CGP
Decanoic (C10:0)	---*	---	---	0.78	1.38	---	---	---	---	---	---
Lauric (C12:0)	---	0.03	0.02	1.95	2.20	0.45	0.09	---	---	---	---
Myristic (C14:0)	0.06	0.06	0.05	9.56	10.03	2.08	0.29	---	---	---	1.61
Palmitic (C16:0)	32.40	32.55	32.62	29.71	29.26	32.63	33.69	32.49	32.35	34.67	34.30
Palmitoleic (C16:1)	0.41	0.44	0.44	2.35	2.16	0.62	0.43	0.42	---	---	---
Stearic (C18:0)	1.74	1.73	1.73	13.95	13.40	4.71	4.02	2.02	2.07	2.08	3.53
cis-9-Oleic (C18:1 w9)	61.25	61.22	61.06	30.38	30.06	55.20	57.14	61.64	60.28	63.24	58.56
Linolelaid (C18:2 trans-w6)	---	---	---	0.28	0.34	---	---	---	---	---	---
gamma-linoleic (C18:2 w6)	2.23	2.16	2.19	0.31	0.24	2.07	2.24	2.02	---	---	2.00
cis-11-eicosenoic (C20:1 w9)	0.73	0.71	0.72	0.41	0.25	0.66	0.72	0.94	---	---	---
Linolenic (C18:3 w3)	0.39	0.37	0.37	1.08	1.20	0.43	0.42	0.47	---	---	---
Saturated	34.20	34.37	34.42	54.95	56.27	39.87	38.09	34.51	34.42	36.75	39.44
Unsaturated	62.39	62.37	62.22	33.14	32.47	56.48	58.29	63.00	60.28	63.25	58.56
Polyunsaturated	2.62	2.53	2.56	1.67	1.78	2.50	3.00	2.49	----	----	2.00
Other/unidentified	0.79	0.73	0.80	10.24	9.48	1.15	0.62	0.00	5.30	0.00	0.00

***** Fatty acid values not identified

4. CONCLUSIONS

The microcapsules of pequi oil produced by complex coacervation using CCP and CGP as wall materials showed a good potential for producing oil-enriched yogurts. The combined results of oil release from the particles under the different conditions studied were important to determine their application in the yogurt food matrix, given that other matrices can promote an immediate capsule release due to pH (very acidic of basic) for example, or during the manufacturing process due to high temperature. The microcapsules showed better resistance to disintegration at pH range from 4 to 5 and at temperature below 80 °C. The study of the best moment to add the microcapsules during the manufacture of the yogurt was equally important to ensure greater capsule integrity and yogurt quality. The result of our work is encouraging, and it is possible that the microencapsulation methodology used for CCP and CGP may be adapted to other pertinent food systems in the future. Efficiency, stability, and fatty acid profile analyzes indicated that the CCP microcapsules provided higher protection to pequi oil than CGP.

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REFERENCES

Alcântara, M. A., Lima, A. E. A., Braga, A. L. M., Tonon, R. V., Galdeano, M. C., Mattos, M. C., Brígida, A. I. S., Rosenhaim, R., Santos, N. A., & Cordeiro, A. M. T. de M. (2019). Influence of the emulsion homogenization method on the stability of chia oil microencapsulated by spray drying. *Powder Technology, 354*, 877–885. https://doi.org/10.1016/j.powtec.2019.06.026

Alexandre, J. B., Barroso, T. L. C. T., Oliveira, M. A., Mendes, F. R. S., Costa, J. M. C., Moreira, R. A., & Furtado, R. F. (2019). Cross-linked coacervates of cashew gum and

gelatin in the encapsulation of pequi oil. *Ciencia Rural*, *49*(12). https://doi.org/10.1590/0103-8478cr20190079

Baba, W. N., Jan, K., Punoo, H. A., Wani, T. A., Dar, M. M., & Masoodi, F. A. (2018). Techno-functional properties of yoghurts forti fi ed with walnut and fl axseed oil emulsions in guar gum. *LWT - Food Science and Technology*, *92*(October 2017), 242–249. https://doi.org/10.1016/j.lwt.2018.02.007

Bello, B. D., Torri, L., Piochi, M., & Zeppa, G. (2015). Healthy yogurt fortified with n-3 fatty acids from vegetable sources. *Journal of Dairy Science*, *98*, 8375–8385.

Bligh E.G, & Dyer W.J. (1959). A Rapid Method Of Total Lipid Extraction And Purification. *Canadian Journal of Biochemistry and Physiology*, *37*(8), 911–917.

Budinčić, J. M., Petrović, L., Đekić, L., Fraj, J., Bučko, S., Katona, J., & Spasojević, L. (2021). Study of vitamin E microencapsulation and controlled release from chitosan/sodium lauryl ether sulfate microcapsules. *Carbohydrate Polymers*, *251*, 116988. https://doi.org/10.1016/j.carbpol.2020.116988

Castelo, R. M., Silva, L. C., Sousa, J. R., Magalhães, H. C. R., & Furtado, R. F. (2020). Development and Characterization of Pequi Oil (Caryocar coriaceum wittm.) Microparticles by Vibration Nozzle Encapsulation. *Macromolecular Symposia*, *394*(1), 1–6. https://doi.org/10.1002/masy.202000061

Chang, C., Varankovich, N., & Nickerson, M. T. (2016). Microencapsulation of canola oil by lentil protein isolate-based wall materials. *Food Chemistry*, *212*, 264–273. https://doi.org/10.1016/j.foodchem.2016.05.136

Comunian, T. A., Boillon, M. R. G., Thomazini, M., Nogueira, M. S., Castro, I. A., & Favaro-Trindade, C. S. (2016b). Protection of echium oil by microencapsulation with phenolic compounds. *Food Research International*, *88*, 114–121. https://doi.org/10.1016/j.foodres.2016.03.008

Comunian, T. A., Gomez-Estaca, J., Ferro-Furtado, R., Conceição, G. J. A., Moraes, I. C. F., De Castro, I. A., & Favaro-Trindade, C. S. (2016a). Effect of different polysaccharides and crosslinkers on echium oil microcapsules. *Carbohydrate Polymers*, *150*, 319–329. https://doi.org/10.1016/j.carbpol.2016.05.044

Comunian, T. A., Nogueira, M., Scolaro, B., Thomazini, M., Ferro-Furtado, R., Castro, I. A.,

& Favaro-Trindade, C. S. (2018). Enhancing stability of echium seed oil and beta-sitosterol by their coencapsulation by complex coacervation using different combinations of wall materials and crosslinkers. *Food Chemistry*, *252*(January), 277–284. https://doi.org/10.1016/j.foodchem.2018.01.121

Costa, P. J. C. da. (2002). Avaliação in vitro da lioequivalência de formulações farmacêuticas. *Brazilian Journal of Pharmaceutical Sciences*, *38*(2), 141–153.

Devi, N., Hazarika, D., Deka, C., & Kakati, D. K. (2012). Study of complex coacervation of gelatin a and sodium alginate for microencapsulation of olive oil. *Journal of Macromolecular Science, Part A: Pure and Applied Chemistry*, *49*(11), 936–945. https://doi.org/10.1080/10601325.2012.722854

Eghbal, N., & Choudhary, R. (2018). Complex coacervation: Encapsulation and controlled release of active agents in food systems. *LWT - Food Science and Technology*, *90*, 254–264. https://doi.org/10.1016/j.lwt.2017.12.036

Estrada, J. D., Boeneke, C., Bechtel, P., & Sathivel, S. (2011). Developing a strawberry yogurt fortified with marine fish oil. *Journal of Dairy Science*, *94*(12), 5760–5769. https://doi.org/10.3168/jds.2011-4226

Ferreira, M. V., Pradela Filho, L. A., Santos, A. L., Takeuchi, R. M., & Assunção, R. M. N. (2019). Avaliação do perfil de liberação do fármaco ibuprofeno em membranas simétricas e assimétricas de acetato de celulose: efeito da morfologia. *Química Nova*, *42*(8), 823–830.

Fortunato, K. A., Doile, M. M., Schucker, I. C., Schucko, S. K., Silva, M. A. S., & Rodrigues, P. O. (2007). Influência da Complexação com β -ciclodextrina sobre a Liberação do Acetato de Dexametasona a partir de Matrizes Hidrofílicas de Hidroxipropilmetilcelulose (HPMC) e Polioxetileno (PEO). *Latin American Journal of Pharmacy*, *26*(4), 513–521.

Ghafarloo, M. H., Jouki, M., & Tabari, M. (2020). Production and characterization of synbiotic Doogh, a yogurt-based Iranian drink by gum arabic, ginger extract and B. bifidum. *Journal of Food Science and Technology*, *57*(3), 1158–1166. https://doi.org/10.1007/s13197-019-04151-4

Huang, G. Q., Sun, Y. T., Xiao, J. X., & Yang, J. (2012). Complex coacervation of soybean

protein isolate and chitosan. *Food Chemistry*, *135*(2), 534–539. https://doi.org/10.1016/j.foodchem.2012.04.140

Jain, A., Thakur, D., Ghoshal, G., Katare, O. P., & Shivhare, U. S. (2016). Characterization of microcapsulated β-carotene formed by complex coacervation using casein and gum tragacanth. *International Journal of Biological Macromolecules*, *87*, 101–113. https://doi.org/10.1016/j.ijbiomac.2016.01.117

Jeon, S., Yoo, C. Y., & Park, S. N. (2015). Improved stability and skin permeability of sodium hyaluronate-chitosan multilayered liposomes by Layer-by-Layer electrostatic deposition for quercetin delivery. *Colloids and Surfaces B: Biointerfaces*, *129*, 7–14. https://doi.org/10.1016/j.colsurfb.2015.03.018

Jouki, M., & Khazaei, N. (2021). Effects of active batter coatings enriched by quince seed gum and carvacrol microcapsules on oil uptake and quality loss of nugget during frying. *Journal of Food Science and Technology*. https://doi.org/10.1007/s13197-021-05114-4

Jouki, M., Khazaei, N., Rashidi-alavijeh, S., & Ahmadi, S. (2021). Encapsulation of Lactobacillus casei in quince seed gum-alginate beads to produce a functional synbiotic drink powder by agro-industrial by-products and freeze-drying. *Food Hydrocolloids*, *120*(March), 106895. https://doi.org/10.1016/j.foodhyd.2021.106895

Jouki, M., Khazaei, N., Rezaei, F., & Taghavian-saeid, R. (2021). Production of synbiotic freeze-dried yoghurt powder using microencapsulation and cryopreservation of L . plantarum in alginate- skim milk microcapsules. *International Dairy Journal*, *122*, 105133. https://doi.org/10.1016/j.idairyj.2021.105133

Khodashenas, M., & Jouki, M. (2020). Optimization of stabilized probiotic Doogh formulation by edible gums and response surface methodology : assessment of stability , viability and organoleptic attributes. *Journal of Food Science and Technology*. https://doi.org/10.1007/s13197-020-04351-3

Lemos, Y. P., Marfil, P. H. M., & Nicoletti, V. R. (2017). Particle size characteristics of buriti oil microcapsules produced by gelatin-sodium alginate complex coacervation: Effect of stirring speed. *International Journal of Food Properties*, *20*(July), 1438–1447. https://doi.org/10.1080/10942912.2017.1349139

Lima, A. C., Barros, M. E. S., Souza, A. C. R., Araújo, Í. M. S., Magalhães, H. C. R., &

Pacheco, G. M. (2019). Obtenção do Óleo da Polpa de Pequi por Separação Física Utilizando-se Centrifugação. *Embrapa Comunicado Técnico*, *254*, 1–6.

Liu, B., Wang, Y., Yang, F., Wang, X., Shen, H., Cui, H., & Wu, D. (2016). Construction of a controlled-release delivery system for pesticides using biodegradable PLA-based microcapsules. *Colloids and Surfaces B: Biointerfaces*, *144*, 38–45. https://doi.org/10.1016/j.colsurfb.2016.03.084

Mezzomo, N., Mileo, B. R., Friedrich, M. T., Martínez, J., & Ferreira, S. R. S. (2010). Supercritical fluid extraction of peach (*Prunus persica*) almond oil: Process yield and extract composition. *Bioresource Technology*, *101*(14), 5622–5632. https://doi.org/10.1016/j.biortech.2010.02.020

Nascimento, M. A., Silva, L. C., Mendes, L. G., Furtado, R. F., Costa, J. M. C., Biswas, A., Cheng, H. N., & Alves, C. R. (2020). Pequi oil microencapsulation by complex coacervation using gelatin-cashew gum. *International Journal of Food Studies*, *9*(2020), SI97–SI109. https://doi.org/10.7455/ijfs/9.SI.2020.a8

Nieuwenhove, C. P. Van, Moyano, A., Castro-gómez, P., Fontecha, J., Sáez, G., Zárate, G., & Luna, P. (2019). Comparative study of pomegranate and jacaranda seeds as functional components for the conjugated linolenic acid enrichment of yogurt. *LWT - Food Science and Technology*, *111*(May), 401–407. https://doi.org/10.1016/j.lwt.2019.05.045

Oliveira, E. F., Paula, H. C. B., & Paula, R. C. M. d. (2014). Alginate/cashew gum nanoparticles for essential oil encapsulation. Colloids and Surfaces B: Biointerfaces, 113, 146–151. https://doi.org/10.1016/j.colsurfb.2013.08.038

Oliveira, M. A., Furtado, R. F., Bastos, M. S. R., Leitão, R. C., Benevides, S. D., Muniz, C. R., Cheng, H. N., & Biswas, A. (2018). Performance evaluation of cashew gum and gelatin blend for food packaging. *Food Packaging and Shelf Life*, *17*(October 2017), 57–64. https://doi.org/10.1016/j.fpsl.2018.05.003

Oliveira, W. Q., Wurlitzer, N. J., Araújo, A. W. O., Comunian, T. A., Bastos, M. S. R., Oliveira, A. L., Magalhães, H. C. R., Ribeiro, H. L., Figueiredo, R. W., & Sousa, P. H. M. (2020). Complex coacervates of cashew gum and gelatin as carriers of green coffee oil: The effect of microcapsule application on the rheological and sensorial quality of a fruit juice. *Food Research International*, *131*, 109047. https://doi.org/10.1016/j.foodres.2020.109047

Ombredane, A. S., Araujo, V. H. S., Borges, C. O., Costa, P. L., Landim, M. G., Pinheiro, A. C., Szlachetka, Í. O., Benedito, L. E. C., Espindola, L. S., Dias, D. J. S., Oliveira, D. M., Chaker, J. A., Silva, S. W., Azevedo, R. B., & Joanitti, G. A. (2020). Nanoemulsion-based systems as a promising approach for enhancing the antitumoral activity of pequi oil (*Caryocar brasilense* Cambess.) in breast cancer cells. *Journal of Drug Delivery Science and Technology*, *58*(January), 101819. https://doi.org/10.1016/j.jddst.2020.101819

Pereira, E., Barros, L., Barreira, J. C. M., Carvalho, A. M., Antonio, A. L., & Ferreira, I. C. F. R. (2016). Electron beam and gamma irradiation as feasible conservation technologies for wild Arenaria montana L.: Effects on chemical and antioxidant parameters. *Innovative Food Science and Emerging Technologies*, *36*, 269–276. https://doi.org/10.1016/j.ifset.2016.07.012

Pinto, M. R. M. R., Paula, D. de A., Alves, A. I., Rodrigues, M. Z., Vieira, É. N. R., Fontes, E. A. F., & Ramos, A. M. (2018). Encapsulation of carotenoid extracts from pequi (*Caryocar brasiliense* Camb) by emulsification (O/W) and foam-mat drying. *Powder Technology*, *339*, 939–946. https://doi.org/10.1016/j.powtec.2018.08.076

Ribeiro, B., & Bonif, T. (2021). Incorporation of olive pomace ingredients into yoghurts as a source of fibre and hydroxytyrosol : Antioxidant activity and stability throughout gastrointestinal digestion. *Jourl of Food Engineering*, *297*(December 2020), 110476. https://doi.org/10.1016/j.jfoodeng.2021.110476

Cruz, M. C. R., Dagostin, J. L. A., Perussello, C. A., & Masson, M. L. (2019). Assessment of physicochemical characteristics, thermal stability and release profile of ascorbic acid microcapsules obtained by complex coacervation. *Food Hydrocolloids*, *87*(November 2017), 71–82. https://doi.org/10.1016/j.foodhyd.2018.07.043

Rutz, J. K., Borges, C. D., Zambiazi, R. C., Crizel-Cardozo, M. M., Kuck, L. S., & Noreña, C. P. Z. (2017). Microencapsulation of palm oil by complex coacervation for application in food systems. *Food Chemistry*, *220*, 59–66. https://doi.org/10.1016/j.foodchem.2016.09.194

Shariati, Z., Jouki, M., & Rafiei, F. (2020). Flavored functional drinking yogurt (Doogh) formulated with Lactobacillus plantarum LS5 , cress seed gum , and coriander leaves extract. *Food Science & Nutrition*, *8*(2), 894–902. https://doi.org/10.1002/fsn3.1367

Silva, D. A., Feitosa, J. P. A., Paula, H. C. B., & Paula, R. C. M. (2009). Synthesis and characterization of cashew gum/acrylic acid nanoparticles. Materials Science and Engineering C, 29(2), 437–441. https://doi.org/10.1016/j.msec.2008.08.029

Silva, L. C., Nascimento, M. A., Mendes, L. G., Furtado, R. F., Costa, J. M. C., & Cardoso, A. L. H. (2018). Optimization of cashew gum and chitosan for microencapsulation of pequi oil by complex coacervation. *Journal of Food Processing and Preservation*, *42*(3), 1–8. https://doi.org/10.1111/jfpp.13538

Soares, B. S., Siqueira, R. P., 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*(June), 125045. https://doi.org/10.1016/j.foodchem.2019.125045

Timilsena, Y. P., Adhikari, R., Barrow, C. J., & Adhikari, B. (2016). Microencapsulation of chia seed oil using chia seed protein isolate-chia seed gum complex coacervates. *International Journal of Biological Macromolecules*, *91*, 347–357. https://doi.org/10.1016/j.ijbiomac.2016.05.058

Timilsena, Y. P., Vongsvivut, J., Tobin, M. J., Adhikari, R., Barrow, C., & Adhikari, B. (2019b). Investigation of oil distribution in spray-dried chia seed oil microcapsules using synchrotron-FTIR microspectroscopy. *Food Chemistry*, *275*, 457–466. https://doi.org/10.1016/j.foodchem.2018.09.043

Timilsena, Y. P., Wang, B., Adhikari, R., & Adhikari, B. (2017). Advances in microencapsulation of polyunsaturated fatty acids (PUFAs)-rich plant oils using complex coacervation: A review. *Food Hydrocolloids*, *69*, 369–381. https://doi.org/10.1016/j.foodhyd.2017.03.007

Yu, D., Kwon, G., An, J., Lim, Y., Jhoo, J., & Chung, D. (2021). Influence of prebiotic biopolymers on physicochemical and sensory characteristics of yoghurt. *International Dairy Journal*, *115*, 104915. https://doi.org/10.1016/j.idairyj.2020.104915

Yu, F., Xue, C., & Zhang, Z. (2021). Mechanical characterization of fish oil microcapsules by a micromanipulation technique. *LWT - Food Science and Technology*, *144*, 111194. https://doi.org/10.1016/j.lwt.2021.111194

4.3 CAPÍTULO III - Yogurts supplemented with pequi oil microcapsules: Effects on physicochemical properties, stability, in vitro digestion and bioaccessibility

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Yogurts supplemented with pequi oil microcapsules: Effects on physicochemical properties, stability, in vitro digestion and bioaccessibility

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Abstract: Microcapsules can improve the protection of bioactive compounds in pequi oil and mask the smell and taste inherent for application in yogurt. This work was evaluated the influence of pequi oil microcapsules on the physicochemical characteristic of yogurt and other aspects of stability, digestion, and bioaccessibility by analysis of beta-carotene present in the oil. In the 28 days of storage, pH values decreased from 4.5 to 4.32 for all formulations, but total solids values increased (>11%) and syneresis decreased (<50%) due to retention of water caused by the biopolymers of the microcapsules. The stability, in vitro digestion, and bioaccessibility of beta-carotene, obtained better results in the formulations containing the microencapsulated oil. The microcapsules influenced positively the physicochemical properties of the yogurt. Finally, the importance of microencapsulation technology in the protection of beta-carotene of the pequi oil was verified, as well as the controlled release, which contributes to increasing its bioaccessibility.

Keywords: Complex coacervation; Pequi oil; Beta-carotene; Biopolymers; Yogurt

1. Introduction

Yogurts are the most popular dairy products resulting from chemical acidification or slow lactic fermentation of milk lactose by starters cultures strains of *Streptococcus thermophilus* e *Lactobacillus delbrueckii* subsp. *bulgaricus* (Gharibzahedi & Chronakis, 2018; Ladjevardi, Gharibzahedi, & Mousavi, 2015). Due to its high consumption, it can be a good carrier to ensure and improve the daily intake of nutrients and consequently to cause positive impacts on the health of consumers (Silva et al., 2019). However, yogurt is not considered a rich source of bioactive compounds (Ozturkoglu-Budak, Akal, & Yetisemiyen, 2016), being the addition of these compounds very interesting. Yogurts that offer more health benefits due to the presence of bioactive compounds have gained market share, increasing the consumer acceptance (Mohammadi-gouraji, Soleimanian-zad, & Ghiaci, 2019).

Pequi oil has stood out as a natural material from plant for the food and pharmaceutical industry due to its excellent quality associated with high levels of unsaturated fatty acids, predominantly oleic (60.6%) (Pessoa et al., 2015), and carotenoids (Pinto et al., 2018). Due to the presence of these bioactive, it is widely used in popular medicine to treat various health disorders such as respiratory diseases, edema, eye problems related to low vitamin A , burns, wound healing, bruises, swelling and menstrual disorders (Ombredane et al., 2020). Pequi has great potential to be explored as a source of nutrients (Magalhães, Sá, Cardoso, & Reis, 2019), even with a strong aroma and smell. For this reason, the use of microencapsulation methods can mask the strong flavor of the pequi oil, in addition to protecting the bioactive compounds and promoting their controlled release.

Beta-carotene is found in pequi oil (about 270 μL g^{-1} of oil) and is an important vitamin A source. It has a crucial role in human health, such as improving immunity, decreasing the risk of heart disease and increasing gastrointestinal functions (Geng et al., 2022; Xie et al., 2021). Several carotenoids, including beta-carotene, are produced on an industrial scale and are available in fortified foods or as supplements. However, carotenoids have a non-polar structure and highly unsaturated molecules making them susceptible to oxidation and isomerization during processing or storage (Šeregelj et al., 2021), therefore, several recent studies use microencapsulation technology to promote its stability and bioaccessibility (Girón-Hernández, Gentile, & Benlloch-Tinoco, 2021; Lin, Wu, Singh, & Ye, 2021; Niu, Shao, Feng, Qiu, & Sun, 2020).

Complex coacervation microencapsulation is used to encapsulate bioactive compounds and sustain their release under various conditions. Animal proteins, plant proteins and

polysaccharides are widely used during complex coacervation process (Muhoza et al., 2021). As it is a highly recommended technique for the microencapsulation of lipophilic substances, several studies use it to encapsulate vegetable oils (Silva et al., 2018; Nascimento et al., 2020; Timilsena, Akanbi, Khalid, Adhikari, & Barrow, 2019). In complex coacervation, the associative electrostatic interactions of two oppositely charged polyelectrolytes, initially soluble in water, undergo reciprocal and reversible complexation upon changes in temperature and/or pH, producing a complex of insoluble hydrophilic macromolecules (González-Monje, García, Ruiz-Molina, & Roscini, 2021).

The composition of microcapsules wall material make a crucial role in the physicochemical, rheological, morphological, microbial and sensory properties of the final product (Gumus & Gharibzahedi, 2021), as well as the chosen food matrix influences the stability and bioaccessibility of the bioactive compound of interest (Donhowe, Flores, Kerr, Wicker, & Kong, 2014). Therefore, this work evaluated the influence of the addition of pequi oil microcapsules on the physicochemical and morphological properties of yogurt. Subsequently, the stability of the encapsulated and non-encapsulated bioactive compounds in the yogurt was verified during the storage time. Finally, the release profile during *in vitro* gastrointestinal digestion and the bioaccessibility of beta-carotene was determinate of the microcapsules into the product. Microencapsulated pequi oil was obtained by complex coacervation using two polysaccharides' combinations of wall materials, cashew gum/chitosan and cashew gum/gelatin.

2. Materials and methods

2.1. Materials

Cashew gum (CG) polysaccharides were obtained from the exudate of *Anacardium occidentale* L., collected from cashew trees in the Pacajus experimental field of Embrapa Agroindústria Tropical. Polysaccharide isolation was performed using the methodology previously described by Silva et al. (2018). Chitosan (CT), of low molecular weight and 75-85% deacetylated was purchased from Sigma-Aldrich and bovine gelatine 225H type B was provided by Rousselot® (The Netherlands). The culture of the microorganism was purchased commercially by Bio Rich ® (*Lactobacillus acidophilus* LA-5 $1x10^6$ UFC/g, *Bifidobacterium* BB-12 $1x10^6$ UFC/g e *Streptococcus thermophilus*. The beta-carotene (beta-carotene; ≥93%, UV), Porcine gastric mucosa pepsin (P7000), porcine bile extracts (B8631) and porcine pancreatin (P7545, 8 × USP specifications) were purchased from Sigma-Aldrich.

2.2. Extraction of oil from pequi pulp

The pequi fruits (*Caryocar coriaceum* Wittm.) were washed, sanitized (Chlorine 200 ppm for 30 min), peeled and submitted to a hammer pulper (capacity 300 kg h^{-1}). The pulp was stored in plastic containers and kept in a freezer until oil extraction. For the extraction of pequi oil (PO), the pulps were heated to 45 °C and centrifuged at 4,500 rpm for 15 min to separate the oil (Lima et al., 2019).

2.3. Microcapsules formation

The microcapsules were obtained by complex coacervation, using CG/CT and CG/GE as wall materials.

The CG/CT microcapsules with the PO (CCP) were prepared according to the methodology described by Silva et al. (2018). For each homogenization step an ultra turrax (IKA T-25 digital) was used for 3 min at 10,000 rpm. Initially, the emulsions were prepared from 100 mL of CT (0.5% w/v) in deionized water (acetic acid 2% w/v) with 2 g of PO and homogenization. Then was added 100 mL of solution of CG (11% w/v) in water and homogenization. Finally, 400 mL of water were added and homogenized.

For the preparation of CG/GE microcapsules with the PO (CGP), initially a solution of 100 mL of GE (2% w/v) and a solution of 100 mL of CG (4% w/v) in deionized water were prepared according to methodology described by Nascimento et al. (2020). In the GE solution, 2 g of PO was added and homogenized, then it was added to the CG solution and homogenized again, and finally, 400 mL of deionized water was added and homogenized.

After preparing the CCP and CGP emulsions, the pH was adjusted to 4.5 and cooled (5 °C) overnight. The solutions containing the microcapsules were centrifuged (Heraeus Multifuge X3R) at 15,303*xg* for 10 min at 25 °C, removing excess water, and frozen in an ultra-freezer (-80 °C) for 24 hours. The frozen materials were taken to the freezer-drying (Liotop LP 820) until complete drying and was realized dispersion in an impact mill (IKA A11 basic).

2.4. Yogurt preparation

Ultra-high temperature (UHT) liquid whole milk was used according to the methodology applied by Yu et al. (2021) with modification. The milk was heated to 43 °C and added to the microorganism culture at a concentration of 0.4 g L^{-1} (w/v). The milk was kept in an oven (43 °C) until clot formation at pH 4.5 (approximately 4h). After the coagulation time, the yogurt

was cooled (4 °C) overnight for later addition of pequi oil microcapsules and pure oil. Four yogurt formulations were obtained, where Y-C is the control yogurt without additions; Y-PO is yogurt added with 0.8% (w/w) pure oil; Y-CCP is yogurt added with 2.4% (w/w) of CCP microcapsules; Y-CGP is yogurt added with 1.7% CGP microcapsules (Figure 1). The calculations for additions of microcapsules and pure oil to yogurt were in accordance with the roles of the National Health Surveillance Agency (ANVISA) that recommends daily intake of vitamin A of 600 µg for adults and 375–500 µg for children (Brazil, 2005), where 1 µg of beta-carotene is equivalent to 0.167 µg of retinol (vitamin A). Yogurt samples were stored in refrigeration (4 °C) and analyzed at storage times of 1, 7, 14, 21, 28 days.

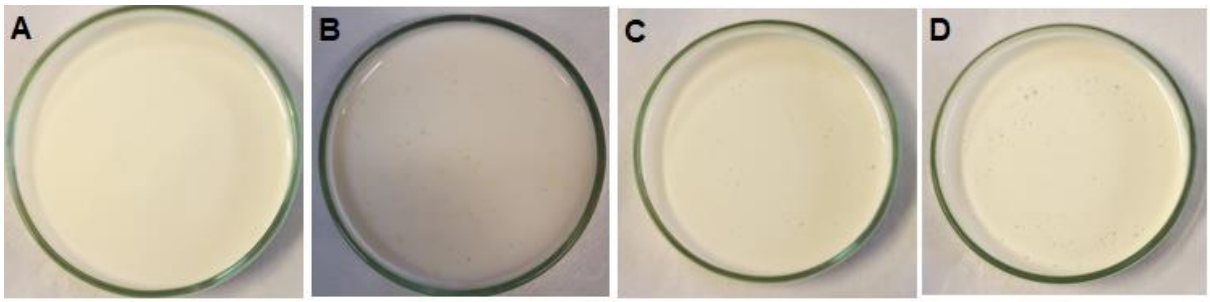

Figure 1: A) Y-C is the control yogurt without additions; B) Y-PO is yogurt added with 0.8% (w/w) pure oil; C) Y-CCP is yogurt added with 2.4% (w/w) of CCP microcapsules; D) Y-CGP is yogurt added with 1.7% CGP microcapsules.

2.5. Characterization of yogurt

2.5.1. pH

The pH of the samples was analyzed using a pH meter (mPA210, MS Tecnopon®) and a viscous liquid electrode (Analyser® 2A09E). In a beaker, 50 g of yogurt samples were weighed and analyzed during the storage period.

2.5.2. Total solids

The total solids amount of all yogurts was measured according to the AOAC 990.20 method (AOAC, 2000). Yogurt samples (2 g) were dried in a hot air oven at 105 °C for 24 h, and the residues were weighed. The total solid (%) was calculated as the percentage weight of residual dry matter over the initial weight of the yogurt sample.

2.5.3. Syneresis

The amount of syneresis was realized according to method described by Akgün et al. (2019), with modification. 20 g yogurt was centrifuged for 10 min at 4000 rpm at 20 °C. The supernatant was removed and weighed. Syneresis (%) was expressed as the percentage weight of the supernatant over the initial weight of the yogurt sample.

2.5.4. Colorimetry

For colorimetric analysis, all yogurts during storage were monitored. For color measurement, yogurt samples (20 g) were placed in a petri dish, and the values of L* (light), a* (red/green) and b* (yellow/blue) were obtained using a colorimeter ChromaMeter CR-400 (Konica Minolta, Sensing, INC, Japan). The total color difference (ΔE), at 28 days of storage, was obtained by equation 1.

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

2.5.5. Morphology

A drop of yogurt was placed on the slides and covered with coverslips. Optical micrographs were performed using a Zeiss optical microscope coupled to a digital image acquisition system using a CCD camera. Yogurt micrographs were also obtained by transmission electron microscopy (TEM) (Model Vega 3, Tescan). An aliquot of yogurt was placed on a carbon-coated copper grid and allowed to stand for 3 min. Then, the sample was stained with phosphotungstic acid (0.4% w/v) for 3 min and the excess removed with filter paper. The TEM analysis was performed at an accelerating voltage of 30 kV.

2.5.6. Texture profile analysis

Texture profile analysis (TPA) was performed using a TA-TX2i texturometer (Stable Micro Systems Ltd., Surrey, England), equipped with a 30 kg load cell. 20 g of each yogurt sample was placed in three different bottles, forming an analytical triplicate. These samples were analyzed at a temperature of 7 ± 2 °C, using a P0.5 probe in two cycles of 40% penetration. The test speed was 1 mm s^{-1}, with a 2 g trigger. The parameters of hardness, springiness, cohesiveness, gumminess, and chewiness were calculated.

2.5.7. Rheology

The viscosity of yogurts was analyzed using a HAAKE MARS III rheometer (Thermo Scientific). The yogurt sample was loaded onto the cone plate geometry (C35/2° Ti L) and 0.105 mm gap with the temperature maintained at 4 °C. The viscosity flow curve was determined logarithmically increasing the frequency from 0.1 s^{-1} to 100 s^{-1} and descents from 100 s^{-1} to 0.1 s^{-1} controlled by the Haake Rheowin Job Manager software. The samples were conditioned for 5 min at 4 °C to recover the yogurt structure and the readings were carried out in a time of 5 min each. Apparent viscosity was measured at a shear rate of 1 s^{-1}. The pseudoplasticity of the yogurt samples was determined using the following power law model (Ostwald-de Waele) (Eq. 2):

$$\tau = k(\gamma)^n \quad \text{(Eq. 2)}$$

2.6 Beta-carotene stability

The beta-carotene (BC) stability present in the Y-PO, Y-CCP and Y-CGP formulations was analyzed during the 28-day storage period. In 50 g of each yogurt formulation, 100 mL of 4M HCl was added to extract the oil (during 4h). After, 10 mL of hexane was added and centrifuged (15,303xg for 10 min at 25 °C) to capture the supernatant oil. The collected organic phase was dried under a stream of nitrogen, where 50 mg of the oil was redissolved in 5 mL of hexane to quantify BC. The stability of BC was expressed as a percentage (%), by the ratio of the BC amount calculated over the storage time over the initial BC amount.

2.6.1 Beta-carotene quantification

BC quantification was evaluated in a spectrophotometer (Varian Carry 50) at 450 nm. A standard curve was obtained y = 0.1536x - 0.0351 with R^2 of 0.99 from 1 mg of BC in 100 mL of hexane (10 μg mL^{-1}). Results were expressed as μg BC g^{-1} oil sample.

2.7 Beta-carotene release profile in simulated in vitro gastrointestinal digestion

The digestion behavior of BC present in pequi oil (encapsulated and non-encapsulated) applied in yogurt were evaluated using a two-stage *in vitro* static digestion model (Chen et al. (2020), Rutz et al. (2017) with modification). In 10 g of yogurt were added 10 mL (1:1 w/v) of simulated gastric fluid (SGF) (pH 2.0, NaCl 0.15 M) containing 3.2 mg mL^{-1} of pepsin and kept in a shaker incubator (Solab SL222) at 150 rpm, 37 °C for 1 h. After SGF incubation, the mixture was immediately adjusted to pH 7.0 using 2.5 M NaOH and 20 mL (1:1 v/v) of

simulated intestinal fluid (SIF) (pH 7.0, $CaCl_2$ 5 mM), containing 8 mg mL^{-1} bile salts and 1 mg mL^{-1} pancreatin. The mixture was incubated and shaken at 150 rpm for 2 h at 37 °C.

In the SIF step, beta-carotene release was analyzed at 5 different time intervals (0, 30, 60, 90 and 120 min). Hexane was added and the mixture was vortexed for 1 min to extract from BC. Subsequently, the samples were centrifuged at 1057 g for 3 min. The hexane-containing phase was collected and dried (the volume of hexane was adjusted to 5 mL) under a stream of nitrogen. BC release during SIF digestion was expressed as a percentage (%), by the ratio of the BC amount calculated over time to the initial BC amount.

2.8 Bioaccessibility of beta-carotene after digestion

Bioaccessibility, also known as micellization rate of BC after digestion was calculated according to the method described by Chen et al. (2020), with modifications. The material submitted to complete digestion in two stages was collected and centrifuged at 15,303xg, 4 °C, for 30 min. It was separated into three phases after centrifugation, the oil phase, the aqueous phase (micellar phase) and the sediment phase. The aqueous phase was collected and filtered. The BC in the micellar phase was extracted with methanol and hexane in a 2:3 (v/v) ratio, vortexed for 30 sec and centrifuged (15,303xg for 4 min). The upper yellow organic phase was then removed and transferred to a glass tube. The extraction process was repeated 3 times to ensure complete extraction of BC from the aqueous phase. The collected organic phase was dried under a stream of nitrogen and redissolved in 5 mL of hexane. Bioaccessibility was calculated according to the following equation 3:

$$\text{Bioaccessibility (BC)}\% = \frac{\text{BC concentration in micelles}}{\text{BC concentration in digesta}} x\ 100 \qquad \text{(Eq. 3)}$$

2.9. Statistical analysis

Yogurt analyzes were performed in triplicate and the results are expressed as mean ± standard deviation (SD). The analyzed samples were submitted to a two-way ANOVA with a significance level of p = 0.05, using Tukey test for comparison of means. Statistical analyzes were performed using STATISTICA software version 13.0

3. Results and discussion

The supplementation of yogurts with vegetable oil microcapsules, which have a bioactive composition of interest, has been widely implemented, offering greater health benefits and

7better acceptance by consumers. In this work, the production of yoghurt supplemented with pequi oil microcapsules was carried out, using two different combinations of wall materials (CCP and CGP). The influence of microcapsules on the yoghurt matrix was verified by pH, total solid, syneresis, color, texture, and rheology analyses. Subsequently, the stability, in vitro digestion and bioaccessibility of beta-carotene present in pequi oil was evaluated.

3.1. pH, total solids and syneresis

The effect of the incorporation of CCP and CGP microcapsules into yogurt during the storage period was evaluated by pH, total solids, and syneresis analysis (Table 1).

The pH values ranged from 4.5 to 4.32 during the 28-day storage period. In all yogurt formulations studied, the pH values decreased significantly ($p>0.05$) in the first 14 days of storage, maintaining these values until 28 days. According to Estrada, Boeneke, Bechtel, & Sathivel (2011) the decrease in pH may be related to the persistent metabolic activity of microorganisms, also called post-acidification. Post-acidification or post-fermentation acidification is an unwanted process in fermented dairy products that refers to continuous acidification beyond its ideal range due to the persistent metabolic activity of the microflora into product during its shelf life (Deshwal, Tiwari, Kumar, Raman, & Kadyan, 2021).

The addition of encapsulated and non-encapsulated pequi oil not changed the pH in relation to the control, but the greater initial decrease can be considered by the presence of elements that made the metabolic activity of microorganisms more favorable (Ladjevardi et al., 2015). Fat content in yogurt formulation facilitates substrate inhibition for microorganism growth and activity and therefore lowers the pH value. Baba et al. (2018) also observed a decrease in pH, especially during the first 15 days of storage, of yogurts fortified with walnut oil emulsions and linseed with guar gum.

Comparing the syneresis values of the yogurt formulations, the yogurts added with microcapsules obtained lower values than the Y-C and Y-PO, showing the interference of the addition of biopolymers in the yogurt matrix. Syneresis values noted changed during the storage time considering each treatment of yogurt. The presence of biopolymers causes a stabilization of the yogurt's three-dimensional network, reducing the diffusion or separation rate of whey in yogurts (Gharibzahedi & Chronakis, 2018). Akgün et al. (2019) and Yu et al. (2021) obtained similar results and reported that the lower syneresis results are due to water retention caused by the biopolymers used.

Table 1: Effect of the incorporation of pequi oil and pequi oil microcapsules on the pH, total solids and syneresis of yogurts, where: Y-C is the control yogurt, Y-PO yogurt added with pequi oil; Y-CCP yogurt added with pequi oil microcapsules using cashew/chitosan gum and Y-CGP yogurt added with pequi oil microcapsules using cashew gum/gelatin.

Properties	Yogurts	Day 1	Day 7	Day 14	Day 21	Day 28
pH	Y-C	4.50 ± 0.01^{aA}	4.40 ± 0.01^{aB}	4.34 ± 0.01^{abC}	4.34 ± 0.01^{aC}	4.34 ± 0.01^{aC}
	Y-PO	4.44 ± 0.02^{bA}	4.36 ± 0.01^{bB}	4.36 ± 0.01^{bB}	4.31 ± 0.03^{aC}	4.33 ± 0.01^{aBC}
	Y-CCP	4.47 ± 0.01^{abA}	4.39 ± 0.01^{aB}	4.34 ± 0.01^{abCD}	4.31 ± 0.02^{aC}	4.36 ± 0.01^{bD}
	Y-CGP	4.46 ± 0.01^{bA}	4.36 ± 0.01^{bB}	4.32 ± 0.01^{aC}	4.30 ± 0.01^{aC}	4.32 ± 0.01^{aC}
Total solids (%)	Y-C	11.82 ± 0.02^{aA}	10.71 ± 0.11^{aA}	9.66 ± 0.09^{aB}	8.63 ± 0.09^{aB}	10.55 ± 0.05^{abAB}
	Y-PO	10.20 ± 0.26^{bA}	9.80 ± 0.13^{bA}	11.28 ± 0.15^{bB}	10.81 ± 0.15^{bC}	10.53 ± 0.25^{abC}
	Y-CCP	10.36 ± 0.41^{b}	10.85 ± 0.18^{a}	11.87 ± 0.19^{b}	11.67 ± 0.37^{c}	11.61 ± 0.31^{a}
	Y-CGP	11.41 ± 0.21^{aA}	10.64 ± 0.10^{aAB}	11.62 ± 0.30^{bA}	11.44 ± 0.01^{cA}	9.39 ± 0.14^{bB}
Syneresis (%)	Y-C	59.33 ± 0.08^{aA}	55.93 ± 0.23^{aB}	56.25 ± 0.41^{aB}	56.52 ± 1.20^{aB}	57.53 ± 1.91^{aAB}
	Y-PO	57.63 ± 0.06^{bA}	56.22 ± 0.03^{aA}	57.38 ± 0.12^{aA}	57.63 ± 0.12^{aA}	57.83 ± 1.49^{aA}
	Y-CCP	44.28 ± 0.43^{cA}	44.67 ± 0.48^{bA}	46.90 ± 0.61^{bB}	47.63 ± 1.44^{bB}	47.73 ± 0.76^{bB}
	Y-CGP	45.30 ± 0.43^{dA}	44.47 ± 1.85^{bA}	44.35 ± 1.73^{cA}	44.33 ± 0.66^{cA}	45.30 ± 2.08^{bA}

Different lowercase letters show a significant difference ($p < 0.05$) between the columns of each property. Different capital letters show a significant difference ($p < 0.05$) between lines for each property.

3.2. Colorimetry

The color parameters L*, a* and b* obtained can vary from white (100) to black (0), from green (-) to red (+) and from blue (-) to yellow (+), respectively, according to presented in Table 2. Comparing the results of L* of the Y-C and Y-PO formulations, it was observed a significant decrease ($p>0.05$) during the storage time, different from the formulations with microcapsules, which obtained better stability of the L* values. No relationship apparent between the presence and/or absence of microcapsules and non-encapsulated bioactive compounds and brightness values was found in this work, a similar result obtained by Comunian et al. (2017).

Table 2: Color parameters, where: Y-C is the control yogurt, Y-PO yogurt added with pequi oil; Y-CCP yogurt added with pequi oil microcapsules using cashew/chitosan gum and Y-CGP yogurt added with pequi oil microcapsules using cashew gum/gelatin.

Color	Yogurts	Day 1	Day 7	Day 14	Day 21	Day 28
L*	Y-C	92.19 ± 0.77^{aA}	89.5 ± 0.94^{aB}	96.8 ± 0.60^{aC}	88.63 ± 0.66^{abB}	88.66 ± 0.36^{aB}
	Y-PO	91.37 ± 0.59^{abA}	100.34 ± 0.25^{bB}	95.96 ± 0.66^{aC}	91.96 ± 0.81^{cA}	88.57 ± 0.44^{aD}
	Y-CCP	90.47 ± 0.23^{bA}	98.86 ± 0.59^{cB}	93.99 ± 0.96^{bC}	89.4 ± 0.50^{bA}	90.33 ± 0.43^{bA}
	Y-CGP	90.42 ± 0.31^{bA}	96.75 ± 0.62^{dB}	95.25 ± 0.98^{abB}	87.85 ± 0.82^{aC}	90.32 ± 0.79^{bA}
a*	Y-C	-1.24 ± 0.05^{aA}	-1.39 ± 0.17^{aA}	-1.36 ± 0.43^{abA}	-1.12 ± 0.08^{aA}	-1.03 ± 0.32^{aA}
	Y-PO	-2.2 ± 0.14^{bA}	-1.96 ± 0.25^{bA}	-1.71 ± 0.36^{aAB}	-1.45 ± 0.17^{aB}	-1.22 ± 0.14^{abB}
	Y-CCP	-0.95 ± 0.15^{acAB}	-0.71 ± 0.13^{cA}	-0.81 ± 0.18^{bAB}	-1.17 ± 0.3^{aB}	-1.68 ± 0.1^{bC}
	Y-CGP	-0.95 ± 0.18^{cAB}	-0.59 ± 0.3^{cA}	-0.94 ± 0.27^{bAB}	-1.24 ± 0.34^{aB}	-1.48 ± 0.25^{abB}
b*	Y-C	13.3 ± 0.52^{aA}	14.61 ± 0.2^{aB}	12.47 ± 0.40^{aA}	15.12 ± 0.07^{aB}	15.45 ± 0.52^{abB}
	Y-PO	15.8 ± 0.94^{bAB}	15.59 ± 0.99^{aAB}	14.55 ± 0.69^{bA}	15.27 ± 0.36^{aAB}	16.7 ± 0.67^{aB}
	Y-CCP	14.34 ± 0.47^{acA}	15.29 ± 0.33^{aA}	15.04 ± 0.28^{bA}	14.45 ± 0.75^{aA}	12.08 ± 0.63^{cB}
	Y-CGP	14.73 ± 0.29^{bcA}	15.56 ± 0.85^{aA}	14.39 ± 0.94^{bA}	12.46 ± 0.66^{bB}	14.23 ± 0.98^{bA}

Different lowercase letters show a significant difference ($p < 0.05$) between the columns of each property. Different capital letters show a significant difference ($p < 0.05$) between lines for each property.

The a* parameter had negative results, showing a slight tendency to greenish color and the b* had positive results, showing the yellowish hue, with greater results for the formulation with the non-encapsulated oil. Thus suggesting that the addition of microcapsules offers improves, in terms of maintaining the original color appearance of the yogurt, as also was shown by Tan et al. (2017).

Regarding the total color difference (ΔE), values of 4.14, 3.10, 2.38 and 0.74 were obtained for the formulations Y-C, Y-PO, Y-CCP and Y-CGP, respectively. The highest values obtained may be related to higher whey separation in the formulations without microcapsules (Y-C and Y-PO).

3.3. Morphological characterization

Micrographs of samples yogurt were obtained by optical microscopy (Figure 2 A, B and C) and transmission electronics microscopy (TEM) (Figure 2 D, E and F).

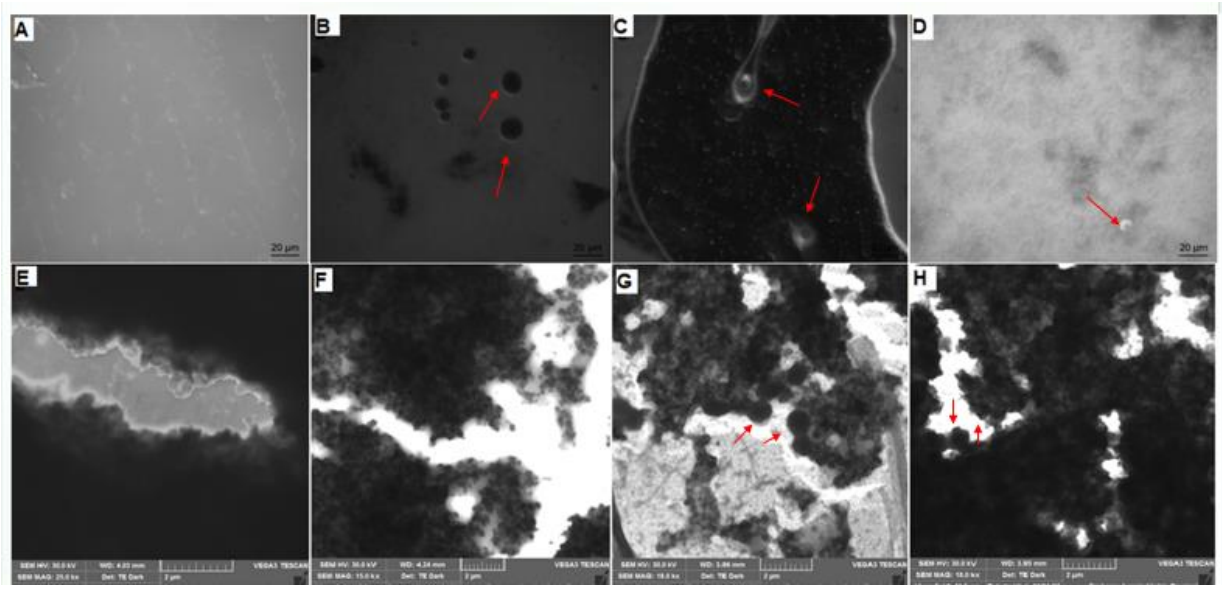

Figure 2: Optical microscopy micrographs of yogurt formulations, where A is control yogurt (Y-C), B is yogurt added with pequi oil (Y-PO), C yogurt added with pequi oil microcapsules, using cashew gum/ chitosan (Y-CCP) and D is yogurt added to pequi oil microcapsules using cashew gum/gelatin (Y-CGP). Transmission electron microscopy micrographs (TEM) of yogurts, where E is Y-C, F is Y-PO, G is Y-CCP, and H is Y-CGP.

Observing Figures 2 A and D (control yogurt formulation Y-C) a dense structure was visualized, since that these formulations were not submitted to mechanical stress for homogenization of the added materials. The dark areas observed correspond to the protein structure of the yogurt and the small size structures observed in the yogurt samples are related to the fat globules, which are characteristic of dairy products such as yogurt (Campo et al., 2019)

In figure 2 B, it is possible to observe the presence of microparticles of non-encapsulated oil from the Y-PO formulation. The denser and more compact microstructures in the yogurt formulations added with microcapsules prove that there was a dispersion and not agglomeration of the microcapsules, as also was observed in the works of Comunian et al. (2017) and Yu et al. (2021).

3.4 Texture profile analysis

Texture profile analysis (TPA) of yogurt formulations during storage time are showed in Figure 3. Elasticity and cohesiveness were the characteristics that had not significant difference (p<0.05) between the formulations and the storage time.

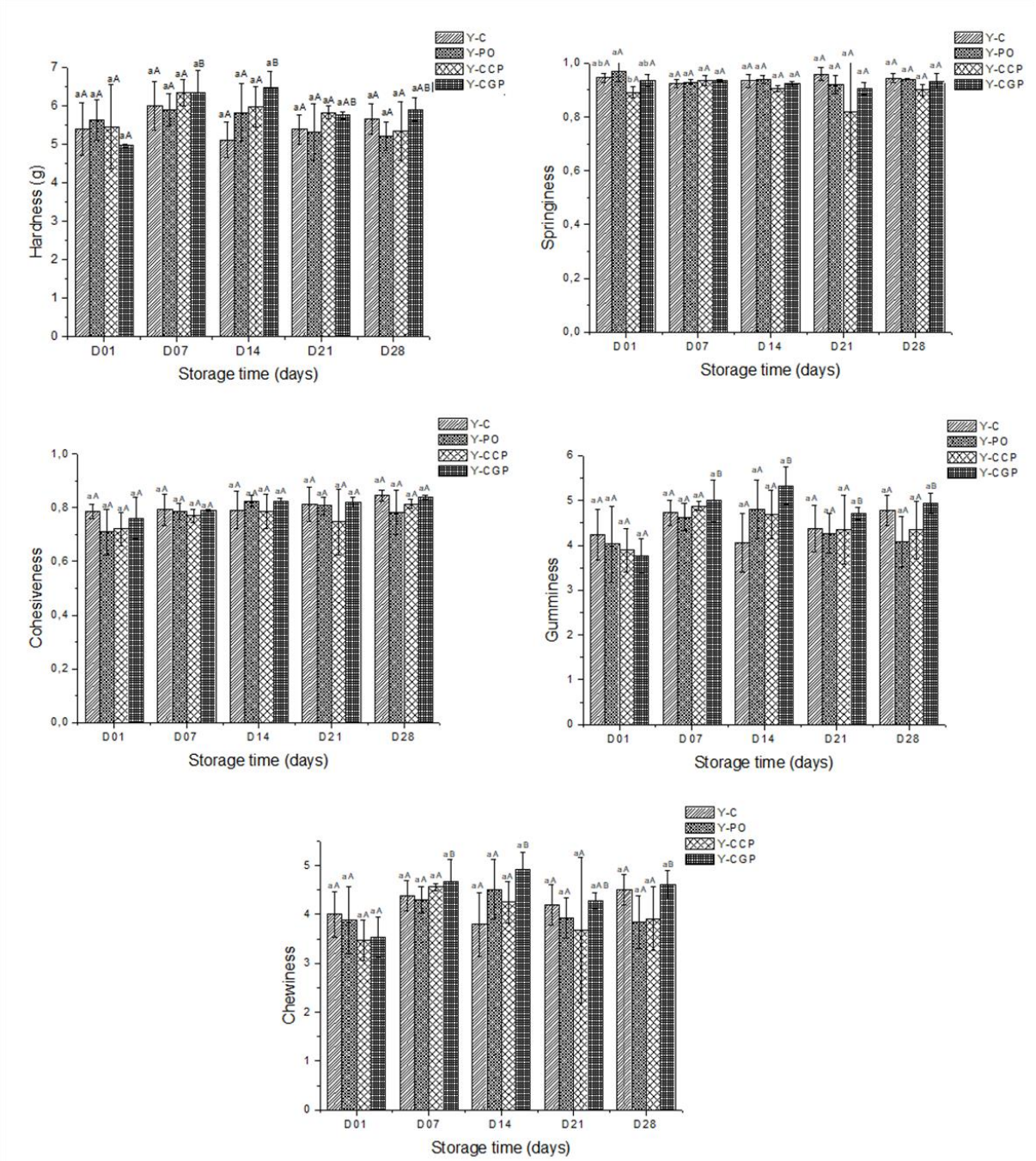

Figure 3: Texture profile analysis (TPA) of yogurt formulations, where Y-C is the control yogurt, Y-PO is the yogurt added with pequi oil, Y-CCP is the yogurt added with pequi oil microcapsules, using the gum cashew/chitosan and Y-CGP is yogurt added with pequi oil microcapsules, using cashew gum/gelatin. Different lowercase and capital letters show a significant difference ($p < 0.05$).

The parameters of hardness, gumminess and chewability had a significant increase on days 7 and 14 of storage, followed by a decrease, which is related to the decrease of the pH. Lower pH influence the increase in whey production, both are decisive factors to the product quality during storage time. Ladjevardi et al. (2015) reported that an increase in the total amount of yogurt solids can improve texture properties and therefore decrease the rate of syneresis. Studies carried out by Comunian et al. (2017), Ghorbanzade, Jafari, Akhavan, & Hadavi (2017) and Tan et al, (2017) obtained similar results to this study, where the encapsulates allowed more yogurt proteins to interact with each other, forming a three-dimensional structure with greater resistance to deformation. Thus, the texture profile results were maintained over the time of storage.

3.5 Reology aspects

Yogurts are commonly classified as pseudo-plastic liquids (n<1), so one of the mathematical models that describe this type of liquid (non-Newtonian) is the Ostwald-de Waele. As can be seen in table 3, the R^2 values were above 0.9, thus obtaining a correlation coefficient value within the standards for applying this model. All yogurt formulations, regardless of the storage day, had fluid behavior index values (n) lower than 1, and could therefore be classified as non-Newtonian pseudo-plastic liquids.

The rheological analyzes allow to evaluate the deformations and fluxes of matter, when subjected to a certain shear rate. The apparent viscosity of yogurts decreased with increasing rate of deformation during shear. They results are agree with works realized by (Comunian et al., 2017; Tan et al., 2017) (Figure 4). In the case of the Y-C, the apparent viscosity remained stable throughout storage, it was measured at a shear rate of 1 s^{-1}. However, there was a greater difference between the Y-PO formulation on the first day of storage, compared to the rest of the days, for samples stored for 28 days, showing a negative influence of the formulation containing the non-encapsulated pequi oil. The Y-CCP and Y-CGP formulations showed higher values, comparing the 1st and 28th day of storage, due to the addition of microcapsules, which increases the solids content of the final product. In contrast to the microcapsule added to yogurt, the gel structure of the pequi oil added yogurt was weak and this eventually reduced the viscosity, increasing the yogurt's tendency to flow, shrink and expel the whey (Dai, Corke, & Shah, 2016).

Table 3: Parameters of the Ostwald-de Waele model (K, n and R^2) and apparent viscosity (η) calculated for a shear rate of 1 s^{-1} during storage time at 4 °C, where Y-C is the control yogurt, Y-PO is pequi oil added yogurt, Y-CCP is pequi oil microcapsule added yogurt, using cashew gum/chitosan gum and Y-CGP is pequi oil microcapsule added yogurt, using cashew gum/gelatin.

Parameters	Yogurts	Day 1	Day 7	Day 14	Day 21	Day 28
K	Y-C	2.84	2.86	4.87	3.91	3.24
	Y-PO	2.45	1.19	1.16	1.47	1.26
	Y-CCP	1.26	1.74	1.51	2.21	1.80
	Y-CGP	43.97	1.27	5.09	6.14	5.22
n	Y-C	0.12	0.23	0.22	0.20	0.15
	Y-PO	0.37	0.37	0.31	0.37	0.30
	Y-CCP	0.30	0.40	0.34	0.36	0.42
	Y-CGP	0.52	0.53	0.26	0.28	0.20
R^2	Y-C	0.98	0.99	0.99	0.97	0.99
	Y-PO	0.85	0.96	0.95	0.95	0.99
	Y-CCP	0.99	0.96	0.95	0.98	0.92
	Y-CGP	0.96	0.83	0.98	0.77	0.98
η (mPa.s)	Y-C	3268	2710	5237	3820	3355
	Y-PO	7512	881,8	1240	1559	1252
	Y-CCP	1252	1970	1620	2336	2051
	YCGP	4380	1834	5387	7166	9613

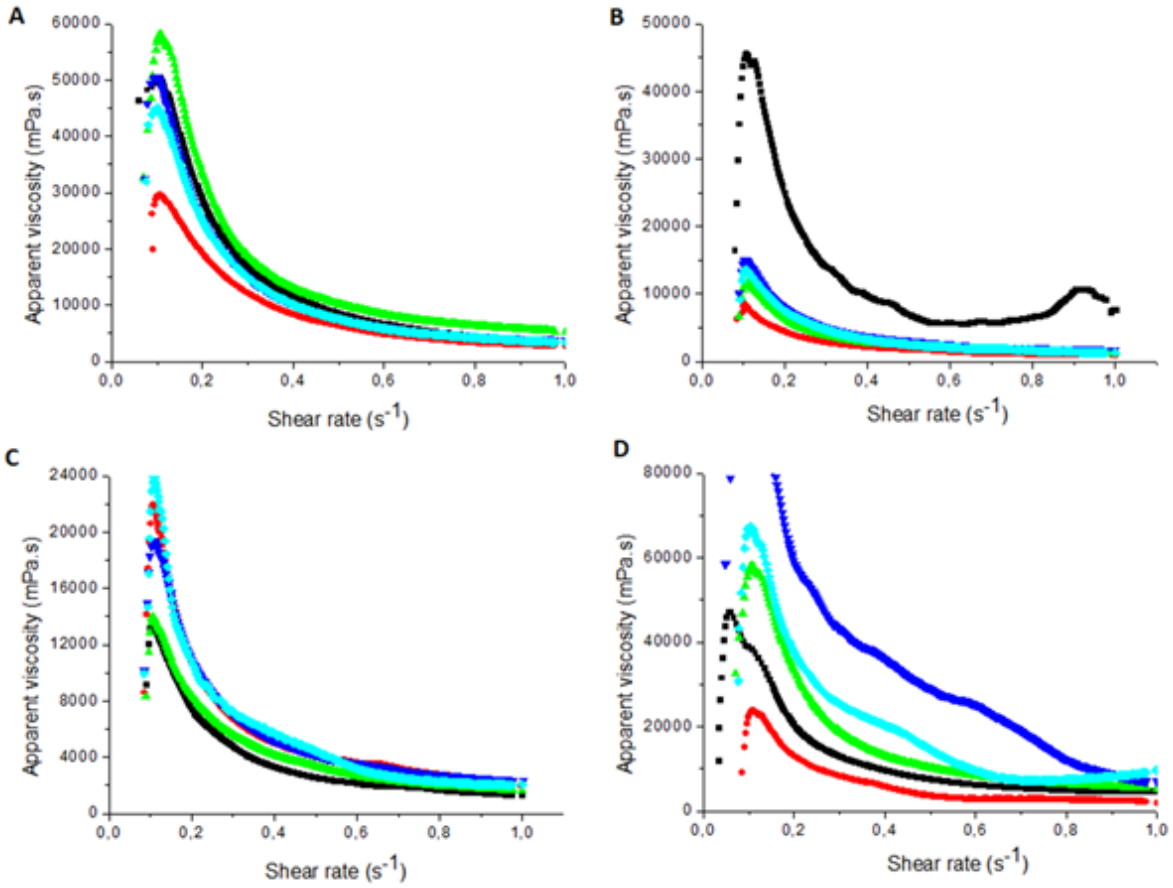

Figure 4: Shear rate x apparent viscosity ratio of yogurts made during storage time at 4 °C, where A is the control yogurt, B is the yogurt added with pequi oil, C is the yogurt added with pequi oil microcapsules, using cashew gum/chitosan and D is yogurt added with pequi oil microcapsules using cashew gum/gelatin. Where black, red, green, blue, and cyan lines represent the storage time of 1, 7, 14, 21 and 28 days, respectively.

3.6 Beta-carotene stability

The stability analysis of beta-carotene (BC) of pequi oil incorporated to yogurt formulations with encapsulated and non-encapsulated oil was performed to verify the oil protection during the 28 days of storage, as shown in Figure 5 A. In all formulations, the decrease in BC concentration was more accentuated in the first 7 days of storage, followed by a more gradual decrease up to 28 days. This BC degradation in the first days of storage occurred during the decrease in pH in the yogurt matrix, where there was a environment more favorable to for BC oxidation. Šeregelj et al. (2021) attributed that the apolar structure and highly unsaturated molecules become the carotenoids susceptible to oxidation and isomerization

during processing or storage. In this sense, the use of encapsulation technology to improve their stability is especially attractive for application in food.

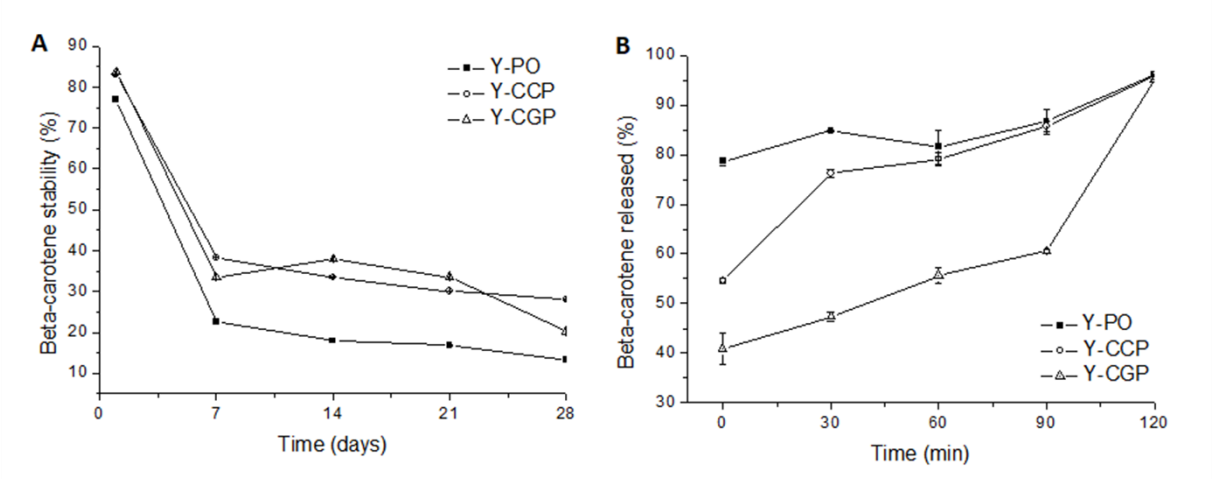

Figure 5: Concentration of beta-carotene (%) of formulations Y-PO (yogurt added with pequi oil), Y-CCP (yogurt added with microcapsules of pequi oil, using cashew/chitosan gum) and Y-CGP (yogurt added of pequi oil microcapsules, using cashew gum/gelatin), where A is the stability of beta-carotene over the 28 days of storage, and B is the release profile of beta-carotene during *in vitro* digestion.

The influence of microencapsulation on the BC protection can be proven by the higher BC concentrations at all storage times, when compared to Y-CCP and Y-CGP formulations with Y-PO formulation. At 28-day storage, beta-carotene retention was 13.26 ± 0.02, 28.03 ± 0.08 and 20.16 ± 0.12 for the Y-PO, Y-CCP and Y-CGP formulations, respectively. Studies carried out by Campo et al. (2019) evaluated the stability of zeaxanthin nanoparticles applied in yogurts and also observed a decrease in retention during storage time, in the range of 16 to 22%, corroborating the results obtained. Šeregelj et al. (2021) observed that beta-carotene content in fortified yogurt was slightly changed during the storage period, observing decreasing trends for the tested yogurts.

3.7 Beta-carotene release profile by in vitro digestion

The BC release profile in a fluid that simulates gastrointestinal conditions can be seen in Figure 4 B. As expected, the formulations with microcapsules had a more gradual release, different of the formulation with only pequi oil. The formulation with CGP microcapsules had lower levels of release during up to 90 min in contact with SIF but was like Y-CCP during a

time of 120 min, when was released 96.11 ± 0.02, 95.99 ± 0.04 and 95.16 ± 0.01, for the Y-PO, Y-CCP and Y-CGP formulations, respectively. The non-full release is due to the fraction of beta-carotene that may have been absorbed in the digesta, which will be considered in the bioaccessibility step. Keršienė et al. (2020) verified the release kinetics of bioactive molecules during the *in vitro* digestion of the product and compared as the incorporation of the bioactive (loaded in double emulsion or added directly to the product). At the end time of the simulated intestinal condition, a complete release (approximately 100%) of the vitamins was recorded independent of the incorporation method. The fact that the Y-PO formulation not showed maximum release since the first contact with SIF can be explained by the influence of the yogurt matrix, which makes digestion difficult. During the digestion time, the matrix is desestabilized and there was an increase in oil release. Rutz et al. (2017) observed that after application in food, the release of microencapsulated carotenoids from palm oil was lower and the released compounds were not degraded, indicating that food matrices can interact with bioactive compounds in different forms and protect them, even after the release of the microcapsules.

3.8. Beta-carotene bioaccessibility

Bioaccessibility was based on BC concentrations solubilized in the micellar fraction. The values obtained were 4.05 ± 0.03, 4.18 ± 0.04 and 5.08 ± 0.01 for the Y-PO, Y-CCP and Y-CGP yogurt formulations, respectively. The low bioaccessibility values can be explained by the fact that the oil could barely be digested in the intestinal phase, due to the large droplet size and also by the highly acidic environment, which can cause BC isomerization and/or oxidation (Guo et al., 2022). The gradual released of the oil favors the formation of lower droplet and facilitates the digestion in the intestinal phase as it was verified in the Y-CGP formulation.

Several factors can influence the effectiveness of micellarization of carotenoids in the intestinal phase, including physicochemical properties, composition and interaction with other compounds in the food matrix, such as proteins, lipids and fibers (Xavier, Carvajal-Lérida, Garrido-Fernández, & Pérez-Gálvez 2018), which can affect digestive behavior and reduce the release of carotenoids, consequently, their bioaccessibility (Campo et al., 2019). Donhowe et al. (2014) evaluated the bioaccessibility of microencapsulated and free beta-carotene in yogurt and pudding. The authors reported that the incorporation of microencapsulated and free beta-carotene in micelles was lower in yogurt (0.8 and 5.5%) than in pudding (13.1 and 17%), which implies that the food matrix influenced significantly in the decrease of the carotenoids content of in the micellar phase.

5. Conclusion

The CCP and CGP microcapsules influenced the physicochemical properties (pH, total solid, syneresis, color, texture, and rheology) of the yogurt. In this work was possible to verify that wall material and oil interact with the other ingredients of the product. Sometimes, this interaction was positive bringing desirable characteristics to the product such as less syneresis. The stability of BC was considerably higher in all formulations with microcapsules during the yogurt storage, proving the importance of using microencapsulation technology in the protection of bioactive compounds. In the release profile by *in vitro* digestion, there was a gradual release of the oil, fact that increased the beta-carotene bioaccessibility, mainly observed in the Y-CGP formulation.

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References

Akgün, D., Gültekin-özgüven, M., Yücetepe, A., Altin, G., Gibis, M., Weiss, J., & Özçelik, B. (2019). Stirred-type yoghurt incorporated with sour cherry extract in chitosan-coated liposomes. *Food Hydrocolloids*, *101*, 105532. https://doi.org/10.1016/j.foodhyd.2019.105532

AOAC. (2000). Official methods of analysis of AOAC (17th ed.). Rockville, MD, USA: AOAC International.

Baba, W. N., Jan, K., Punoo, H. A., Wani, T. A., Dar, M. M., & Masoodi, F. A. (2018). Techno-functional properties of yoghurts forti fi ed with walnut and fl axseed oil emulsions in guar gum. *LWT - Food Science and Technology*, *92*, 242–249. https://doi.org/10.1016/j.lwt.2018.02.007

Brazil, 2005 Brazil, Ministério da Saúde, Agência Nacional de Vigilância Sanitária. Resolução n° 269, de 22 de setembro de 2005. Aprova o regulamento técnico sobre a ingestão diária recomendada (IDR) de proteína, vitaminas e minerais Diário Oficial da República Federativa do Brasil, Brasília, DF, Brazil (2005)

Campo, C., Assis, R. Q., Silva, M. M., Costa, T. M. H., Paese, K., Guterres, S. S., Rios, A. O., & Flôres, S. H. (2019). Incorporation of zeaxanthin nanoparticles in yogurt: Influence on physicochemical properties, carotenoid stability and sensory analysis. *Food Chemistry*,

301, 125230. https://doi.org/10.1016/j.foodchem.2019.125230

Chen, L., Liang, R., Yokoyama, W., Alves, P., Pan, J., & Zhong, F. (2020). Effect of the co-existing and excipient oil on the bioaccessibility of β-carotene loaded oil-free nanoparticles. *Food Hydrocolloids*, *106*, 105847. https://doi.org/10.1016/j.foodhyd.2020.105847

Comunian, T. A., Chaves, I. E., Thomazini, M., Moraes, I. C. F., Ferro-Furtado, R., de Castro, I. A., & Favaro-Trindade, C. S. (2017). Development of functional yogurt containing free and encapsulated echium oil, phytosterol and sinapic acid. *Food Chemistry*, *237*, 948–956. https://doi.org/10.1016/j.foodchem.2017.06.071

Dai, S., Corke, H., & Shah, N. P. (2016). Utilization of konjac glucomannan as a fat replacer in low-fat and skimmed yogurt. *Journal of Dairy Science*, *99*(9), 7063–7074. https://doi.org/10.3168/jds.2016-11131

Deshwal, G. K., Tiwari, S., Kumar, A., Raman, R. K., & Kadyan, S. (2021). Review on factors affecting and control of post-acidification in yoghurt and related products. *Trends in Food Science and Technology*, *109*, 499–512. https://doi.org/10.1016/j.tifs.2021.01.057

Donhowe, E. G., Flores, F. P., Kerr, W. L., Wicker, L., & Kong, F. (2014). Characterization and invitro bioavailability of β-carotene: Effects of microencapsulation method and food matrix. *LWT - Food Science and Technology*, *57*(1), 42–48. https://doi.org/10.1016/j.lwt.2013.12.037

Estrada, J. D., Boeneke, C., Bechtel, P., & Sathivel, S. (2011). Developing a strawberry yogurt fortified with marine fish oil. *Journal of Dairy Science*, *94*(12), 5760–5769. https://doi.org/10.3168/jds.2011-4226

Geng, M., Wang, Z., Qin, L., Taha, A., Du, L., Xu, X., Pan, S., & Hu, H. (2022). Effect of ultrasound and coagulant types on properties of β-carotene bulk emulsion gels stabilized by soy protein. *Food Hydrocolloids*, *123*, 107146. https://doi.org/10.1016/j.foodhyd.2021.107146

Gharibzahedi, S. M. T., & Chronakis, I. S. (2018). Crosslinking of milk proteins by microbial transglutaminase: Utilization in functional yogurt products. *Food Chemistry*, *245*, 620–632. https://doi.org/10.1016/j.foodchem.2017.10.138

Ghorbanzade, T., Jafari, S. M., Akhavan, S., & Hadavi, R. (2017). Nano-encapsulation of fish oil in nano-liposomes and its application in fortification of yogurt. *Food Chemistry*, *216*, 146–152. https://doi.org/10.1016/j.foodchem.2016.08.022

Girón-Hernández, J., Gentile, P., & Benlloch-Tinoco, M. (2021). Impact of heterogeneously crosslinked calcium alginate networks on the encapsulation of β-carotene-loaded beads. *Carbohydrate Polymers*, *271*(March). https://doi.org/10.1016/j.carbpol.2021.118429

González-Monje, P., García, A. A., Ruiz-Molina, D., & Roscini, C. (2021). Encapsulation and sedimentation of nanomaterials through complex coacervation. *Journal of Colloid and Interface Science*, *589*, 500–510. https://doi.org/10.1016/j.jcis.2020.12.067

Gumus, C. E., & Gharibzahedi, S. M. T. (2021). Yogurts supplemented with lipid emulsions rich in omega-3 fatty acids: New insights into the fortification, microencapsulation, quality properties, and health-promoting effects. *Trends in Food Science and Technology*, *110*, 267–279. https://doi.org/10.1016/j.tifs.2021.02.016

Guo, Q., Bayram, I., Shu, X., Su, J., Liao, W., Wang, Y., & Gao, Y. (2022). Improvement of stability and bioaccessibility of β-carotene by curcumin in pea protein isolate-based complexes-stabilized emulsions: Effect of protein complexation by pectin and small molecular surfactants. *Food Chemistry*, *367*(17), 130726. https://doi.org/10.1016/j.foodchem.2021.130726

Keršienė, M., Jasutienė, I., Eisinaitė, V., Pukalskienė, M., Venskutonis, P. R., Damulevičienė, G., Knašienė, J., Lesauskaitė, V., & Leskauskaitė, D. (2020). Development of a high-protein yoghurt-type product enriched with bioactive compounds for the elderly. *Lwt*, *131*(July), 1–8. https://doi.org/10.1016/j.lwt.2020.109820

Ladjevardi, Z. S., Gharibzahedi, S. M. T., & Mousavi, M. (2015). Development of a stable low-fat yogurt gel using functionality of psyllium (Plantago ovata Forsk) husk gum. *Carbohydrate Polymers*, *125*, 272–280. https://doi.org/10.1016/j.carbpol.2015.02.051

Lima, A. C., Barros, M. E. S., Souza, A. C. R., Araújo, Í. M. S., Magalhães, H. C. R., & Pacheco, G. M. (2019). Obtenção do Óleo da Polpa de Pequi por Separação Física Utilizando-se Centrifugação. *Embrapa Comunicado Técnico*, *254*, 1–6.

Lin, Q., Wu, D., Singh, H., & Ye, A. (2021). Improving solubility and stability of β-carotene by microencapsulation in soluble complexes formed with whey protein and OSA-modified starch. *Food Chemistry*, *352*, 1–9. https://doi.org/10.1016/j.foodchem.2021.129267

Magalhães, F. S., Sá, M. S. M., Cardoso, V. L., & Reis, M. H. M. (2019). Recovery of phenolic compounds from pequi (*Caryocar brasiliense* Camb.) fruit extract by membrane filtrations: Comparison of direct and sequential processes. *Journal of Food Engineering*, *257*, 26–33. https://doi.org/10.1016/j.jfoodeng.2019.03.025

Mohammadi-gouraji, E., Soleimanian-zad, S., & Ghiaci, M. (2019). Phycocyanin-enriched yogurt and its antibacterial and physicochemical properties during 21 days of storage. *LWT - Food Science and Technology*, *102*(September 2018), 230–236. https://doi.org/10.1016/j.lwt.2018.09.057

Muhoza, B., Qi, B., Harindintwali, J. D., Farag Koko, M. Y., Zhang, S., & Li, Y. (2021). Combined plant protein modification and complex coacervation as a sustainable strategy to produce coacervates encapsulating bioactives. *Food Hydrocolloids*, 107239. https://doi.org/10.1016/j.foodhyd.2021.107239

Nascimento, M. A., Silva, L. C., Mendes, L. G., Furtado, R. F., Costa, J. M. C., Biswas, A., Cheng, H. N., & Alves, C. R. (2020). Pequi oil microencapsulation by complex coacervation using gelatin-cashew gum. *International Journal of Food Studies*, *9*, SI97–SI109. https://doi.org/10.7455/ijfs/9.SI.2020.a8

Niu, B., Shao, P., Feng, S., Qiu, D., & Sun, P. (2020). Rheological aspects in fabricating pullulan-whey protein isolate emulsion suitable for electrospraying: Application in improving β-carotene stability. *LWT - Food Science and Technology*, *129*, 109581. https://doi.org/10.1016/j.lwt.2020.109581

Ombredane, A. S., Araujo, V. H. S., Borges, C. O., Costa, P. L., Landim, M. G., Pinheiro, A. C., Szlachetka, Í. O., Benedito, L. E. C., Espindola, L. S., Dias, D. J. S., Oliveira, D. M., Chaker, J. A., da Silva, S. W., de Azevedo, R. B., & Joanitti, G. A. (2020). Nanoemulsion-based systems as a promising approach for enhancing the antitumoral activity of pequi oil (*Caryocar brasilense* Cambess.) in breast cancer cells. *Journal of Drug Delivery Science and Technology*, *58*, 101819. https://doi.org/10.1016/j.jddst.2020.101819

Ozturkoglu-Budak, S., Akal, C., & Yetisemiyen, A. (2016). Effect of dried nut fortification on functional , physicochemical , textural , and microbiological properties of yogurt. *Journal of Dairy Science*, *99*(11), 8511–8523. https://doi.org/10.3168/jds.2016-11217

Pessoa, A. S., Podestá, R., Block, J. M., Franceschi, E., Dariva, C., & Lanza, M. (2015). Extraction of pequi (*Caryocar coriaceum*) pulp oil using subcritical propane: Determination of process yield and fatty acid profile. *Journal of Supercritical Fluids*, *101*, 95–103. https://doi.org/10.1016/j.supflu.2015.03.006

Pinto, M. R. M. R., Paula, D. de A., Alves, A. I., Rodrigues, M. Z., Vieira, É. N. R., Fontes, E. A. F., & Ramos, A. M. (2018). Encapsulation of carotenoid extracts from pequi (*Caryocar brasiliense* Camb) by emulsification (O/W) and foam-mat drying. *Powder*

Technology, *339*, 939–946. https://doi.org/10.1016/j.powtec.2018.08.076

Rutz, J. K., Borges, C. D., Zambiazi, R. C., Crizel-Cardozo, M. M., Kuck, L. S., & Noreña, C. P. Z. (2017). Microencapsulation of palm oil by complex coacervation for application in food systems. *Food Chemistry*, *220*, 59–66. https://doi.org/10.1016/j.foodchem.2016.09.194

Šeregelj, V., Pezo, L., Šovljanski, O., Lević, S., Nedović, V., Markov, S., Tomić, A., Čanadanović-Brunet, J., Vulić, J., Šaponjac, V. T., & Ćetković, G. (2021). New concept of fortified yogurt formulation with encapsulated carrot waste extract. *LWT - Food Science and Technology*, *138*, 110732. https://doi.org/10.1016/j.lwt.2020.110732

Silva, L. C., Nascimento, M. A., Mendes, L. G., Furtado, R. F., Costa, J. M. C., & Cardoso, A. L. H. (2018). Optimization of cashew gum and chitosan for microencapsulation of pequi oil by complex coacervation. *Journal of Food Processing and Preservation*, *42*(3), 1–8. https://doi.org/10.1111/jfpp.13538

Silva, S. C., Fernandes, I. P., Barros, L., Fernandes, Â., José, M., Calhelha, R. C., Pereira, C., Barreira, J. C. M., & Manrique, Y. (2019). Spray-dried Spirulina platensis as an e ff ective ingredient to improve yogurt formulations : Testing di ff erent encapsulating solutions. *Journal of Functional Foods*, *60*, 103427. https://doi.org/10.1016/j.jff.2019.103427

Tan, P. Y., Tan, T. B., Chang, H. W., Tey, T., Chan, E. S., Lai, O. M., Baharin, B. S., Nehdi, I. A., & Tan, C. P. (2017). Department of Food Technology , Faculty of Food Science and Technology , Universiti Putra Chemical Engineering Discipline , School of Engineering , Monash University Malaysia , Jalan Department of Bioprocess Technology , Faculty of Biotechnology and Biomo. *Food Chemistry*, *241*, 79–85. https://doi.org/10.1016/j.foodchem.2017.08.075

Timilsena, Y. P., Akanbi, T. O., Khalid, N., Adhikari, B., & Barrow, C. J. (2019). Complex coacervation: Principles, mechanisms and applications in microencapsulation. *International Journal of Biological Macromolecules*, *121*, 1276–1286. https://doi.org/10.1016/j.ijbiomac.2018.10.144

Xavier, A. A. O., Carvajal-Lérida, I., Garrido-Fernández, J., & Pérez-Gálvez, A. (2018). In vitro bioaccessibility of lutein from cupcakes fortified with a water-soluble lutein esters formulation. *Journal of Food Composition and Analysis*, *68*, 60–64. https://doi.org/10.1016/j.jfca.2017.01.015

Xie, H., Zhang, Y., Cao, M., Liu, C., Mao, Y., Ren, G., Wu, Z., Fang, S., Tian, S., & Wu, D.

(2021). Fabrication of PGFE/CN-stabilized β-carotene-loaded peppermint oil nanoemulsions: Storage stability, rheological behavior and intelligent sensory analyses. *LWT- Food Science and Technology*, *138*, 110688. https://doi.org/10.1016/j.lwt.2020.110688

Yu, D., Kwon, G., An, J., Lim, Y., Jhoo, J., & Chung, D. (2021). Influence of prebiotic biopolymers on physicochemical and sensory characteristics of yoghurt. *International Dairy Journal*, *115*, 104915. https://doi.org/10.1016/j.idairyj.2020.104915

5 CONCLUSÃO

As microcápsulas de óleo de pequi produzidas por coacervação complexa usando CCP e CGP como materiais de parede mostraram um bom potencial para a produção de iogurtes enriquecidos com óleo. Ambos os materiais de parede apresentaram bons resultados de eficiência de encapsulação (>80%), rendimento (>50%) e capacidade de carga (<50%). O FT-IR comprovou a interação eletrostática dos coacervados, confirmando a formação das microcápsulas, e o TGA, DSC e Rancimat mostraram a boa estabilidade térmica devido a interação dos materiais de parede.

As microcápsulas apresentaram melhor resistência à desintegração na faixa de pH de 4 a 5 e em temperaturas abaixo de 80 °C, resultados importantes para determinar sua aplicação na matriz alimentar de iogurte. O estudo do melhor momento para adicionar as microcápsulas durante a fabricação do iogurte foi igualmente importante para garantir maior integridade da cápsula e qualidade do iogurte. As análises de eficiência, estabilidade e perfil de ácidos graxos indicaram que as microcápsulas CCP proporcionaram maior proteção ao óleo de pequi do que o CGP.

As microcápsulas CCP e CGP influenciaram as propriedades físico-químicas (pH, sólido total, sinérese, cor, textura e reologia) do iogurte. Neste trabalho foi possível verificar que o material da parede e o óleo interagem com os demais ingredientes do produto. Às vezes, essa interação era positiva trazendo características desejáveis ao produto, como menos sinérese. A estabilidade do BC foi consideravelmente maior em todas as formulações com microcápsulas durante o armazenamento do iogurte, comprovando a importância do uso da tecnologia de microencapsulação na proteção de compostos bioativos. No perfil de liberação pela digestão *in vitro*, houve uma liberação gradativa do óleo, fato que aumentou a bioacessibilidade do beta-caroteno, observada principalmente na formulação Y-CGP.

Com isso, conclui-se que as microcápsulas de goma de cajueiro/quitosana proporcionaram uma matriz mais densa, protegendo melhor o óleo, e ao aplicar em iogurte, melhorou o perfil de ácidos graxos e promoveu a adição de um importante composto bioativo, o beta-caroteno, sendo que as formulações com as microcápsulas obtiveram melhor estabilidade e bioacessibilidade do beta-caroteno. O resultado do nosso trabalho é encorajador e é possível que a metodologia de microencapsulação usada para CCP e CGP possa ser adaptada a outros sistemas alimentares pertinentes no futuro.

REFERÊNCIAS

ALI, A. M. M.; KISHIMURA, H.; BENJAKUL, S. Physicochemical and molecular properties of gelatin from skin of golden carp (Probarbus Jullieni) as influenced by acid pretreatment and prior-ultrasonication. **Food Hydrocolloids**, v. 82, p. 164–172, 2018.

ALMEIDA, H. H. S.; BARROS, L.; BARREIRA, J. C. M.; CALHELHA, R. C.; HELENO, S. A.; SAYER, C.; MIRANDA, C. G.; LEIMANN, F. V.; BARREIRO, M. F.; FERREIRA, I. C. F. R. Bioactive evaluation and application of different formulations of the natural colorant curcumin (E100) in a hydrophilic matrix (yogurt). **Food Chemistry**, v. 261, p. 224–232, 2018.

ANANDAN, R.; CHATTERJEE, N. S.; SIVAKUMAR, R.; MATHEW, S.; ASHA, K. K.; GANESAN, B. Dietary Chitosan Supplementation Ameliorates Isoproterenol-Induced Aberrations in Membrane-Bound ATPases and Mineral Status of Rat Myocardium. **Biological Trace Element Research**, v. 167, n. 1, p. 103–109, 2015.

ANSARIFAR, E.; MOHEBBI, M.; SHAHIDI, F.; KOOCHEKI, A.; RAMEZANIAN, N. Novel multilayer microcapsules based on soy protein isolate fibrils and high methoxyl pectin: Production, characterization and release modeling. **International Journal of Biological Macromolecules**, v. 97, p. 761–769, 2017.

ARAGÃO-NETO, A. C.; SOARES, P. A. G.; LIMA-RIBEIRO, M. H. M.; CARVALHO, E. J. A.; CORREIA, M. T. S.; CARNEIRO-DA-CUNHA, M. G. Combined therapy using low level laser and chitosan-policaju hydrogel for wound healing. **International Journal of Biological Macromolecules**, v. 95, p. 268–272, 2017.

ARSYANTI, L.; ERWANTO, Y.; ROHMAN, A.; PRANOTO, Y. Chemical composition and characterization of skin gelatin from buffalo (Bubalus bubalis). **International Food Research Journal**, v. 25, n. 3, p. 1095–1099, 2018.

ASSEGEHEGN, G.; BRITO-DE LA FUENTE, E.; FRANCO, J. M.; GALLEGOS, C. The Importance of Understanding the Freezing Step and Its Impact on Freeze-Drying Process Performance. **Journal of Pharmaceutical Sciences**, v. 108, n. 4, p. 1378–1395, 2019.

ASSEGEHEGN, G.; BRITO-DE LA FUENTE, E.; FRANCO, J. M.; GALLEGOS, C. Use of a temperature ramp approach (TRA) to design an optimum and robust freeze-drying process

for pharmaceutical formulations. **International Journal of Pharmaceutics**, v. 578, p. 119116, 2020.

AZEREDO, H. M. C. Encapsulação: aplicação à tecnologia de alimentos. **Alimentos e Nutrição Araraquara**, v.16, n. 1, p. 89–97, 2005.

AYKIN-DINÇER, E.; KOÇ, A.; ERBAS, M. Extraction and physicochemical characterization of broiler (Gallus gallus domesticus) skin gelatin compared to commercial bovine gelatin. **Poultry Science**, v. 96, n. 11, p. 4124–4131, 2017.

BABA, W. N.; JAN, K.; PUNOO, H. A.; WANI, T. A.; DAR, M. M.; MASOODI, F. A. LWT - Food Science and Technology Techno-functional properties of yoghurts forti fi ed with walnut and fl axseed oil emulsions in guar gum. **LWT - Food Science and Technology**, v. 92, p. 242–249, 2018.

BALLESTEROS, L. F.; RAMIREZ, M. J.; ORREGO, C. E.; TEIXEIRA, J. A.; MUSSATTO, S. I. Encapsulation of antioxidant phenolic compounds extracted from spent coffee grounds by freeze-drying and spray-drying using different coating materials. **Food Chemistry**, v. 237, p. 623–631, 2017.

BARROW, C. J.; NOLAN, C.; JIN, Y. Stabilization of highly unsaturated fatty acids and delivery into foods. **Lipid Technology**, v. 19, n. 5, p. 108–111, 2007.

BELLO, B. D.; TORRI, L.; PIOCHI, M.; ZEPPA, G. Healthy yogurt fortified with n-3 fatty acids from vegetable sources. **Journal of Dairy Science**, v. 98, p. 8375–8385, 2015.

BEN AMARA, C.; KIM, L.; OULAHAL, N.; DEGRAEVE, P.; GHARSALLAOUI, A. Using complexation for the microencapsulation of nisin in biopolymer matrices by spray-drying. **Food Chemistry**, v. 236, p. 32–40, 2017.

BENEVIDES, S. D., DOS SANTOS, K. O., DO EGITO, A. S., VIEIRA, A. D. S., LAGUNA, L. E., BURITI, F. C. A. Processamento de Queijo de Coalho de Leite de Cabra Adicionado de Óleo de Pequi. **Embrapa Comunicado Técnico**, v. 103, p. 1–6, 2009.

BITTENCOURT, C. R.; DE OLIVEIRA FARIAS, E. A.; BEZERRA, K. C.; VÉRAS, L. M. C.; SILVA, V. C.; COSTA, C. H. N.; BEMQUERER, M. P.; SILVA, L. P.; SOUZA DE ALMEIDA LEITE, J. R. De; EIRAS, C. Immobilization of cationic antimicrobial peptides and natural cashew gum in nanosheet systems for the investigation of anti-leishmanial activity. **Materials Science and Engineering C**, v. 59, p. 549–555, 2016.

BJELOŠEVIĆ, M.; ZVONAR POBIRK, A.; PLANINŠEK, O.; AHLIN GRABNAR, P. Excipients in freeze-dried biopharmaceuticals: Contributions toward formulation stability and lyophilisation cycle optimisation. **International Journal of Pharmaceutics**, v. 576, p. 119029, 2020.

BOTREL, D. A.; BORGES, S. V.; FERNANDES, R. V. de B.; ANTONIASSI, R.; DE FARIA-MACHADO, A. F.; FEITOSA, J. P. de A.; DE PAULA, R. C. M. Application of cashew tree gum on the production and stability of spray-dried fish oil. **Food Chemistry**, v. 221, p. 1522–1529, 2017.

BRITO CANGUSSU, L.; PEREIRA LEÃO, D.; OY, L. S.; FY, A. S. Profile of bioactive compounds in pequi (Caryocar brasilense Camb.) peel flours. **Food Chemistry**, v. 350, p. 129221, 2021.

BUDINČIĆ, J. M.; PETROVIĆ, L.; ĐEKIĆ, L.; FRAJ, J.; BUČKO, S.; KATONA, J.; SPASOJEVIĆ, L. Study of vitamin E microencapsulation and controlled release from chitosan/sodium lauryl ether sulfate microcapsules. **Carbohydrate Polymers**, v. 251, p. 116988, 2021.

CALDEIRA, A. S. P.; MBIAKOP, U. C.; PÁDUA, R. M.; VAN DE VENTER, M.; MATSABISA, M. G.; CAMPANA, P. R. V.; CORTES, S. F.; BRAGA, F. C. Bioguided chemical characterization of pequi (Caryocar brasiliense) fruit peels towards an anti-diabetic activity. **Food Chemistry**, v. 345, p. 1–10, 2021.

CALEJA, C.; BARROS, L.; ANTONIO, A. L.; CAROCHO, M.; OLIVEIRA, M. B. P. P.; FERREIRA, I. C. F. R. Fortification of yogurts with different antioxidant preservatives: A comparative study between natural and synthetic additives. **Food Chemistry**, v. 210, p. 262–268, 2016.

CAMPELO-FELIX, P. H.; SOUZA, H. J. B.; FIGUEIREDO, J. D. A.; DE BARROS FERNANDES, R. V.; BOTREL, D. A.; DE OLIVEIRA, C. R.; YOSHIDA, M. I.; BORGES, S. V. Prebiotic carbohydrates: Effect on reconstitution, storage, release, and antioxidant properties of lime essential oil microparticles. **Journal of Agricultural and Food Chemistry**, v. 65, n. 2, p. 445–453, 2017.

CAMPELO, P. H.; SANCHES, E. A.; FERNANDES, R. V. de B.; BOTREL, D. A.; BORGES, S. V. Stability of lime essential oil microparticles produced with protein-carbohydrate blends. **Food Research International**, v. 105, p. 936–944, 2018.

CAMPOS, D. A.; RIBEIRO, A. C.; COSTA, E. M.; FERNANDES, J. C.; TAVARIA, F. K.; ARARUNA, F. B.; EIRAS, C.; EATON, P.; LEITE, J. R. S. A.; MANUELA PINTADO, M. Study of antimicrobial activity and atomic force microscopy imaging of the action mechanism of cashew tree gum. **Carbohydrate Polymers**, v. 90, n. 1, p. 270–274, 2012.

CARVALHO, N. S.; SILVA, M. M.; SILVA, R. O.; NICOLAU, L. A. D.; SOUSA, F. B. M.; DAMASCENO, S. R. B.; SILVA, D. A.; BARBOSA, A. L. R.; LEITE, J. R. S. A.; MEDEIROS, J. V. R. Gastroprotective properties of cashew gum, a complex heteropolysaccharide of Anacardium occidentale, in naproxen-induced gastrointestinal damage in rats. **Drug Development Research**, v. 76, n. 3, p. 143–151, 2015.

CHANG, H. weng; BOON, T.; YEE, P.; ARBI, I.; MOHAMED, H.; PING, C. Microencapsulation of fish oil-in-water emulsion using thiol-modified β- lactoglobulin fibrils-chitosan complex. **Journal of Food Engineering**, v. 264, p. 109680, 2020.

COMUNIAN, T. A.; CHAVES, I. E.; THOMAZINI, M.; MORAES, I. C. F.; FERRO-FURTADO, R.; DE CASTRO, I. A.; FAVARO-TRINDADE, C. S. Development of functional yogurt containing free and encapsulated echium oil, phytosterol and sinapic acid. **Food Chemistry**, v. 237, p. 948–956, 2017.

COMUNIAN, T. A.; FAVARO-TRINDADE, C. S. Microencapsulation using biopolymers as an alternative to produce food enhanced with phytosterols and omega-3 fatty acids: A review. **Food Hydrocolloids**, v. 61, p. 442–457, 2016.

COMUNIAN, T. A.; GOMEZ-ESTACA, J.; FERRO-FURTADO, R.; CONCEIÇÃO, G. J. A.; MORAES, I. C. F.; DE CASTRO, I. A.; FAVARO-TRINDADE, C. S. Effect of different polysaccharides and crosslinkers on echium oil microcapsules. **Carbohydrate Polymers**, v. 150, p. 319–329, 2016.

CORDEIRO, M. S. F. *et al.* Biopolymers and pilocarpine interaction study for use in drug delivery systems (DDS). **Journal of Thermal Analysis and Calorimetry**, v. 127, n. 2, p. 1777–1785, 2017.

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

DHAKAL, S. P.; HE, J. Microencapsulation of vitamins in food applications to prevent losses in processing and storage: A review. **Food Research International**, v. 137, p. 109326, 2020.

DIAS, S. F. L. *et al.* Acetylated cashew gum-based nanoparticles for transdermal delivery of diclofenac diethyl amine. **Carbohydrate Polymers**, v. 143, p. 254–261, 2016.

EGHBAL, N.; CHOUDHARY, R. Complex coacervation: Encapsulation and controlled release of active agents in food systems. **LWT - Food Science and Technology**, v. 90, p. 254–264, 2018.

ESTEVINHO, B. N.; ROCHA, F.; SANTOS, L.; ALVES, A. Microencapsulation with chitosan by spray drying for industry applications - A review. **Trends in Food Science and Technology**, v. 31, n. 2, p. 138–155, 2013.

FERNANDES, R. V. de B.; BOTREL, D. A.; SILVA, E. K.; BORGES, S. V.; OLIVEIRA, C. R. de; YOSHIDA, M. I.; FEITOSA, J. P. de A.; DE PAULA, R. C. M. Cashew gum and inulin: New alternative for ginger essential oil microencapsulation. **Carbohydrate Polymers**, v. 153, p. 133–142, 2016.

FIGUEIREDO, P. R. L. *et al.* Caryocar coriaceum Wittm. (Pequi) fixed oil presents hypolipemic and anti-inflammatory effects in vivo and in vitro. **Journal of Ethnopharmacology**, v. 191, p. 87–94, 2016.

FIGUEIREDO, R. W.; SOUSA, P. H. M. Complex coacervates of cashew gum and gelatin as carriers of green coffee oil: The effect of microcapsule application on the rheological and sensorial quality of a fruit juice. **Food Research International**, v. 131, p. 109047, 2020.

FORATO, L. A.; DE BRITTO, D.; DE RIZZO, J. S.; GASTALDI, T. A.; ASSIS, O. B. G. Effect of cashew gum-carboxymethylcellulose edible coatings in extending the shelf-life of fresh and cut guavas. **Food Packaging and Shelf Life**, v. 5, p. 68–74, 2015.

GHARIBZAHEDI, S. M. T.; CHRONAKIS, I. S. Crosslinking of milk proteins by microbial transglutaminase: Utilization in functional yogurt products. **Food Chemistry**, v. 245, n. August 2017, p. 620–632, 2018.

GHORBANZADE, T.; JAFARI, S. M.; AKHAVAN, S.; HADAVI, R. Nano-encapsulation of fish oil in nano-liposomes and its application in fortification of yogurt. **Food Chemistry**, v. 216, p. 146–152, 2017.

GOMEZ-ESTACA, J.; COMUNIAN, T. A.; MONTERO, P.; FERRO-FURTADO, R.; FAVARO-TRINDADE, C. S. Encapsulation of an astaxanthin-containing lipid extract from shrimp waste by complex coacervation using a novel gelatin-cashew gum complex. **Food**

Hydrocolloids, v. 61, p. 155–162, 2016.

GENG, M., WANG, Z., QIN, L., TAHA, A., DU, L., XU, X., PAN, S., & HU, H. Effect of ultrasound and coagulant types on properties of β-carotene bulk emulsion gels stabilized by soy protein. **Food Hydrocolloids**, v. 123, p. 107146, 2022.

GÓMEZ-MASCARAQUE, L. G.; HERNÁNDEZ-ROJAS, M.; TARANCÓN, P.; TENON, M.; FEUILLÈRE, N.; VÉLEZ RUIZ, J. F.; FISZMAN, S.; LÓPEZ-RUBIO, A. Impact of microencapsulation within electrosprayed proteins on the formulation of green tea extract-enriched biscuits. **LWT - Food Science and Technology**, v. 81, p. 77–86, 2017.

GONÇALVES, A.; ESTEVINHO, B. N.; ROCHA, F. Microencapsulation of vitamin A: A review. **Trends in Food Science and Technology**, v. 51, p. 76–87, 2016.

GONZÁLEZ, A.; MARTÍNEZ, M. L.; PAREDES, A. J.; LEÓN, A. E.; RIBOTTA, P. D. Study of the preparation process and variation of wall components in chia (Salvia hispanica L.) oil microencapsulation. **Powder Technology**, v. 301, p. 868–875, 2016.

GOWTHAMARAJAN, K.; KUMAR, G. K. P.; GAIKWAD, N. B.; SURESH, B. Preliminary study of Anacardium occidentale gum as binder in formulation of paracetamol tablets. **Carbohydrate Polymers**, v. 83, n. 2, p. 506–511, 2011.

HASHEMI GAHRUIE, H.; ESKANDARI, M. H.; MESBAHI, G.; HANIFPOUR, M. A. Scientific and technical aspects of yogurt fortification: A review. **Food Science and Human Wellness**, v. 4, n. 1, p. 1–8, 2015.

HÄSSIG, A.; LIANG, W. X.; STAMPFLI, K. Bronchial asthma: Information on phytotherapy with essential fatty acids. Interactions between essential fatty acids and steroid hormones. **Medical Hypotheses**, v. 54, n. 1, p. 72–74, 2000.

HELAL, A.; TAGLIAZUCCHI, D. Impact of in-vitro gastro-pancreatic digestion on polyphenols and cinnamaldehyde bioaccessibility and antioxidant activity in stirred cinnamon-fortified yogurt. **LWT - Food Science and Technology**, v. 89, p. 164–170, 2018.

HERNÁNDEZ-BARRUETA, T.; MARTÍNEZ-BUSTOS, F.; CASTAÑO-TOSTADO, E.; LEE, Y.; MILLER, M. J.; AMAYA-LLANO, S. L. Encapsulation of probiotics in whey protein isolate and modified huauzontle's starch: An approach to avoid fermentation and stabilize polyphenol compounds in a ready-to-drink probiotic green tea. **LWT - Food Science and Technology**, v. 124, p. 109131, 2020.

HUANG, Q.; YU, H.; RU, Q. Bioavailability and delivery of nutraceuticals using nanotechnology. **Journal of Food Science**, v. 75, n. 1, p. 50–57, 2010.

HUO, X.; LI, W.; WANG, Y.; HAN, N.; WANG, J.; WANG, N.; ZHANG, X. Chitosan composite microencapsulated comb-like polymeric phase change material via coacervation microencapsulation. **Carbohydrate Polymers**, v. 200, p. 602–610, 2018.

ISHWARYA, S. P.; ANANDHARAMAKRISHNAN, C.; STAPLEY, A. G. F. Spray-freeze-drying: A novel process for the drying of foods and bioproducts. **Trends in Food Science and Technology**, v. 41, n. 2, p. 161–181, 2015.

JANNAT, B.; GHORBANI, K.; KOUCHAKI, S.; SADEGHI, N.; ESLAMIFARSANI, E.; RABBANI, F.; BEYRAMYSOLTAN, S. Distinguishing tissue origin of bovine gelatin in processed products using LC/MS technique in combination with chemometrics tools. **Food Chemistry**, v. 319, p. 126302, 2020.

JANTARATHIN, S.; BOROMPICHAICHARTKUL, C.; SANGUANDEEKUL, R. Microencapsulation of probiotic and prebiotic in alginate-chitosan capsules and its effect on viability under heat process in shrimp feeding. **Materials Today: Proceedings**, v. 4, n. 5, p. 6166–6172, 2017.

JIANG, G. L.; ZHU, M. J. Preparation of astaxanthin-encapsulated complex with zein and oligochitosan and its application in food processing. **LWT - Food Science and Technology**, v. 106, p. 179–185, 2019.

JOHNER, J. C. F.; HATAMI, T.; ZABOT, G. L.; MEIRELES, M. A. A. Kinetic behavior and economic evaluation of supercritical fluid extraction of oil from pequi (Caryocar brasiliense) for various grinding times and solvent flow rates. **Journal of Supercritical Fluids**, v. 140, p. 188–195, 2018.

JUSTI, P. N.; SANJINEZ-ARGANDOÑA, E. J.; MACEDO, M. L. R. Microencapsulation of pequi pulp oil by complex coacervation. **Revista Brasileira de Fruticultura**, v. 40, n. 2, 2018.

KAEWRUANG, P.; BENJAKUL, S.; PRODPRAN, T.; ENCARNACION, A. B.; NALINANON, S. Impact of divalent salts and bovine gelatin on gel properties of phosphorylated gelatin from the skin of unicorn leatherjacket. **LWT - Food Science and Technology**, v. 55, n. 2, p. 477–482, 2014.

KAUSHIK, P.; DOWLING, K.; MCKNIGHT, S.; BARROW, C. J.; ADHIKARI, B. Microencapsulation of flaxseed oil in flaxseed protein and flaxseed gum complex coacervates. **Food Research International**, v. 86, p. 1–8, 2016.

KLINKESORN, U.; SOPHANODORA, P.; CHINACHOTI, P.; DECKER, E. A.; MCCLEMENTS, D. J. Encapsulation of emulsified tuna oil in two-layered interfacial membranes prepared using electrostatic layer-by-layer deposition. **Food Hydrocolloids**, v. 19, n. 6, p. 1044–1053, 2005.

KUMAR, A.; MOIN, A.; R, S.; AHMED, A.; G. SHIVAKUMAR, H. Cashew Gum A Versatile Hydrophyllic Polymer: A Review. **Current Drug Therapy**, v. 7, n. 1, p. 2–12, 2012.

LADJEVARDI, Z. S.; GHARIBZAHEDI, S. M. T.; MOUSAVI, M. Development of a stable low-fat yogurt gel using functionality of psyllium (Plantago ovata Forsk) husk gum. **Carbohydrate Polymers**, v. 125, p. 272–280, 2015.

LEÃO, D. P.; BOTELHO, B. G.; OLIVEIRA, L. S.; FRANCA, A. S. Potential of pequi (Caryocar brasiliense Camb.) peels as sources of highly esterified pectins obtained by microwave assisted extraction. **LWT - Food Science and Technology**, v. 87, p. 575–580, 2018.

LEE, P. S.; YIM, S. G.; CHOI, Y.; VAN ANH HA, T.; KO, S. Physiochemical properties and prolonged release behaviours of chitosan-denatured β-lactoglobulin microcapsules for potential food applications. **Food Chemistry**, v. 134, n. 2, p. 992–998, 2012.

LEMOS, Y. P.; MARIANO MARFIL, P. H.; NICOLETTI, V. R. Particle size characteristics of buriti oil microcapsules produced by gelatin-sodium alginate complex coacervation: Effect of stirring speed. **International Journal of Food Properties**, v. 20, p. 1438–1447, 2017.

LIAPIS, A. I.; BRUTTINI, R. A mathematical model for the spray freeze drying process: The drying of frozen particles in trays and in vials on trays. **International Journal of Heat and Mass Transfer**, v. 52, n. 1–2, p. 100–111, 2009.

LIAPIS, A. I.; LITHEFIELD, R. J. Optimal control of a freeze dryer-I. **Chemical Engineering Science,** v. 34, p. 975-981, 1979.

LIM, C.; HWANG, D. S.; LEE, D. W. Intermolecular interactions of chitosan: Degree of acetylation and molecular weight. **Carbohydrate Polymers**, v. 259, p. 117782, 2021.

LIMA, A.; SILVA, A. M. D. O.; TRINDADE, R. A.; TORRES, R. P.; MANCINI-FILHO, J. Composição química e compostos bioativos presentes na polpa e na amêndoa do pequi (Caryocar brasiliense, Camb.). **Revista Brasileira de Fruticultura**, v. 29, n. 3, p. 695–698, 2007.

LIMA, A. C.; BARROS, M. E. S.; SOUZA, A. C. R. de; ARAÚJO, Í. M. da S.; MAGALHÃES, H. C. R.; PACHECO, G. M. Obtenção do Óleo da Polpa de Pequi por Separação Física Utilizando-se Centrifugação. **Embrapa Comunicado Técnico**, v. 254, p. 1–6, 2019.

MA, T.; ZHAO, H.; WANG, J.; SUN, B. Effect of processing conditions on the morphology and oxidative stability of lipid microcapsules during complex coacervation. **Food Hydrocolloids**, v. 87, p. 637–643, 2019.

MARQUES, L. G.; SILVEIRA, A. M.; FREIRE, J. T. Freeze-drying characteristics of tropical fruits. **Drying Technology,** v. 24, p. 457-463, 2006.

MARTINS, I. M.; BARREIRO, M. F.; COELHO, M.; RODRIGUES, A. E. Microencapsulation of essential oils with biodegradable polymeric carriers for cosmetic applications. **Chemical Engineering Journal**, v. 245, p. 191–200, 2014.

MATTA, L. M. **Retenção do óleo de pequi em micropartículas de concentrado protéico de soro de leite e maltodextrina.** 2013, 93f. Dissertação (Mestrado em Ciência e Tecnologia de Alimentos) - Universidade Federal do Ceará, Fortaleza, 2013.

MCCLEMENTS, D. J.; DECKER, E. A.; WEISS, J. Emulsion-based delivery systems for lipophilic bioactive components. **Journal of Food Science**, v. 72, n. 8, 2007.

MEZZOMO, N.; MILEO, B. R.; FRIEDRICH, M. T.; MARTÍNEZ, J.; FERREIRA, S. R. S. Supercritical fluid extraction of peach (Prunus persica) almond oil: Process yield and extract composition. **Bioresource Technology**, v. 101, n. 14, p. 5622–5632, 2010.

MILKOVA, V. Electrosteric stabilization of oil/water emulsions by adsorption of chitosan oligosaccharides—An electrokinetic study. **Carbohydrate Polymers**, v. 265, n. April, p. 118072, 2021.

MIRANDA-VILELA, A. L.; PEREIRA, L. C. S.; GONÇALVES, C. A.; GRISOLIA, C. K. Pequi fruit (Caryocar brasiliense Camb.) pulp oil reduces exercise-induced inflammatory markers and blood pressure of male and female runners. **Nutrition Research**, v. 29, n. 12, p.

850–858, 2009.

MONTEIRO, F. M. F.; DE MEDEIROS E SILVA, G. M.; DA SILVA, J. B. R.; PORTO, C. S.; DE CARVALHO, L. B.; DE LIMA FILHO, J. L.; CARNEIRO-LEÃO, A. M. dos A.; CARNEIRO-DA-CUNHA, M. das G.; PORTO, A. L. F. Immobilization of trypsin on polysaccharide film from Anacardium occidentale L. and its application as cutaneous dressing. **Process Biochemistry**, v. 42, n. 5, p. 884–888, 2007.

MOURA, S. C. S. R.; BERLING, C. L.; GARCIA, A. O.; QUEIROZ, M. B.; ALVIM, I. D.; HUBINGER, M. D. Release of anthocyanins from the hibiscus extract encapsulated by ionic gelation and application of microparticles in jelly candy. **Food Research International**, v. 121, p. 542–552, 2019.

NASCIMENTO, M. A.; SILVA, L. C.; MENDES, L. G.; FURTADO, R. F.; COSTA, J. M. C.; BISWAS, A.; CHENG, H. N.; ALVES, C. R. Pequi oil microencapsulation by complex coacervation using gelatin-cashew gum. **International Journal of Food Studies**, v. 9, p. SI97–SI109, 2020.

NASCIMENTO-SILVA, N. R. R. Do; NAVES, M. M. V. Potential of Whole Pequi (Caryocar spp.) Fruit-Pulp, Almond, Oil, and Shell-as a Medicinal Food. **Journal of Medicinal Food**, v. 22, n. 9, p. 952–962, 2019.

NAZZARO, F.; ORLANDO, P.; FRATIANNI, F.; COPPOLA, R. Microencapsulation in food science and biotechnology. **Current Opinion in Biotechnology**, v. 23, n. 2, p. 182–186, 2012.

NIEUWENHOVE, C. P. Van; MOYANO, A.; CASTRO-GÓMEZ, P.; FONTECHA, J.; SÁEZ, G.; ZÁRATE, G.; LUNA, P. LWT - Food Science and Technology Comparative study of pomegranate and jacaranda seeds as functional components for the conjugated linolenic acid enrichment of yogurt. **LWT - Food Science and Technology**, v. 111, p. 401–407, 2019.

OLIVEIRA, E. F.; PAULA, H. C. B.; PAULA, R. C. M. d. Alginate/cashew gum nanoparticles for essential oil encapsulation. **Colloids and Surfaces B: Biointerfaces**, v. 113, p. 146–151, 2014.

OLIVEIRA, E. N. A.; SANTOS, D. da C.; MARTINS, J. N.; BEZERRA, L. C. N. de M. Obtenção e caracterização de margarina convencional e light à base de óleo de pequi. **Boletim Centro de Pesquisa de Processamento de Alimentos**, v. 29, n. 2, p. 294–304, 2011.

OLIVEIRA, W. Q.; WURLITZER, N. J.; ARAÚJO, A. W. de O.; COMUNIAN, T. A.; BASTOS, M. do S. R.; DE OLIVEIRA, A. L.; MAGALHÃES, H. C. R.; RIBEIRO, H. L.; OZKAN, G.; FRANCO, P.; DE MARCO, I.; XIAO, J.; CAPANOGLU, E. A review of microencapsulation methods for food antioxidants: Principles, advantages, drawbacks and applications. **Food Chemistry**, v. 272, n. July 2018, p. 494–506, 2019. Disponível em: https://doi.org/10.1016/j.foodchem.2018.07.205

PAULA, D. de A.; MARTINS, E. M. F.; COSTA, N. de A.; DE OLIVEIRA, P. M.; DE OLIVEIRA, E. B.; RAMOS, A. M. Use of gelatin and gum arabic for microencapsulation of probiotic cells from Lactobacillus plantarum by a dual process combining double emulsification followed by complex coacervation. **International Journal of Biological Macromolecules**, v. 133, p. 722–731, 2019.

PAULA, H. C. B.; DE OLIVEIRA, E. F.; ABREU, F. O. M. S.; DE PAULA, R. C. M. Alginate/cashew gum floating bead as a matrix for larvicide release. **Materials Science and Engineering C**, v. 32, n. 6, p. 1421–1427, 2012.

PAULA, R. C. M.; RODRIGUES, J. F. Composition and rheological properties of cashew tree gum, the exudate polysaccharide from *Anacardiumoccidentale L.* **Carbohydrate Polymer,** v. 26, 177-181, 1995.

PAULA, R. C. M; HEATLEY,F.; BUDD, P. M. Composition of *Anacardium occidentale* exsudate polysaccharide. **Polymer International,** v. 45, p. 27-35, 1998.

PAULO, F.; SANTOS, L. Design of experiments for microencapsulation applications: A review. **Materials Science and Engineering C**, v. 77, p. 1327–1340, 2017.

PESSOA, A. S.; PODESTÁ, R.; BLOCK, J. M.; FRANCESCHI, E.; DARIVA, C.; LANZA, M. Extraction of pequi (Caryocar coriaceum) pulp oil using subcritical propane: Determination of process yield and fatty acid profile. **Journal of Supercritical Fluids**, v. 101, p. 95–103, 2015.

PINTO, M. R. M. R.; PAULA, D. de A.; ALVES, A. I.; RODRIGUES, M. Z.; VIEIRA, É. N. R.; FONTES, E. A. F.; RAMOS, A. M. Encapsulation of carotenoid extracts from pequi (Caryocar brasiliense Camb) by emulsification (O/W) and foam-mat drying. **Powder Technology**, v. 339, p. 939–946, 2018.

PORTO, B. C.; CRISTIANINI, M. Evaluation of cashew tree gum (Anacardium occidentale

L.) emulsifying properties. **LWT - Food Science and Technology**, v. 59, n. 2P2, p. 1325–1331, 2014.

PORTO, B. C.; CRISTIANINI, M. Effect of dynamic high pressure on emulsifying and encapsulant properties of cashew tree gum. **Carbohydrate Polymers**, v. 186, n. May 2017, p. 350–357, 2018.

PRAJAPATI, V. D.; JANI, G. K.; MORADIYA, N. G.; RANDERIA, N. P. Pharmaceutical applications of various natural gums, mucilages and their modified forms. **Carbohydrate Polymers**, v. 92, n. 2, p. 1685–1699, 2013.

RATTI, C. Hot air and freeze-drying of high-value foods: a review. **Journal of Food Engineering,** v. 49, p. 311-319, 2001.

RIBEIRO, A. J.; DE SOUZA, F. R. L.; BEZERRA, J. M. N. A.; OLIVEIRA, C.; NADVORNY, D.; DE LA ROCA SOARES, M. F.; NUNES, L. C. C.; SILVA-FILHO, E. C.; VEIGA, F.; SOARES SOBRINHO, J. L. Gums' based delivery systems: Review on cashew gum and its derivatives. **Carbohydrate Polymers**, v. 147, p. 188–200, 2016.

RIBEIRO, B.; BONIF, T. Incorporation of olive pomace ingredients into yoghurts as a source of fibre and hydroxytyrosol : Antioxidant activity and stability throughout gastrointestinal digestion. **Jourl of Food Engineering**, v. 297, p. 110476, 2021.

RIBEIRO, J. S.; VELOSO, C. M. Microencapsulation of natural dyes with biopolymers for application in food: A review. **Food Hydrocolloids**, v. 112, n. July 2020, 2021.

RIBEIRO, M. C.; VILAS BOAS, E. V. de B.; RIUL, T. R.; PANTOJA, L.; MARINHO, H. A.; SANTOS, A. S. dos. Influence of the extraction method and storage time on the physicochemical properties and carotenoid levels of pequi (Caryocar brasiliense Camb.) oil. **Food Science and Technology**, v. 32, n. 2, p. 386–392, 2012.

RIOS-MERA, J. D.; SALDAÑA, E.; RAMÍREZ, Y.; AUQUIÑIVÍN, E. A.; ALVIM, I. D.; CONTRERAS-CASTILLO, C. J. Encapsulation optimization and pH- and temperature-stability of the complex coacervation between soy protein isolate and inulin entrapping fish oil. **LWT - Food Science and Tecnhology**, v. 116, p. 108555, 2019.

ROCHA, M. A. M.; COIMBRA, M. A.; NUNES, C. Applications of chitosan and their derivatives in beverages: a critical review. **Current Opinion in Food Science**, v. 15, p. 61–69, 2017.

RODRIGUES, R. A. F.; GROSSO, C. R. F. Cashew gum microencapsulation protects the aroma of coffee extracts. **Journal of Microencapsulation**, v. 25, n. 1, p. 13–20, 2008.

RODRÍGUEZ, J.; MARTÍN, M. J.; RUIZ, M. A.; CLARES, B. Current encapsulation strategies for bioactive oils: From alimentary to pharmaceutical perspectives. **Food Research International**, v. 83, p. 41–59, 2016.

ROMÁN, G. C.; JACKSON, R. E.; GADHIA, R.; ROMÁN, A. N.; REIS, J. Mediterranean diet: The role of long-chain ω-3 fatty acids in fish; polyphenols in fruits, vegetables, cereals, coffee, tea, cacao and wine; probiotics and vitamins in prevention of stroke, age-related cognitive decline, and Alzheimer disease. **Revue Neurologique**, v. 175, n. 10, p. 724–741, 2019.

RUDKE, A. R.; HELENO, S. A.; FERNANDES, I. P.; PRIETO, M. A.; HESS, O.; RODRIGUES, A. E.; FERREIRA, I. C. F. R.; FILOMENA, M. Microencapsulation of ergosterol and Agaricus bisporus L . extracts by complex coacervation using whey protein and chitosan : Optimization study using response surface methodology. **LWT - Food Science and Technology**, v. 103, p. 228–237, 2019.

RUTZ, J. K.; BORGES, C. D.; ZAMBIAZI, R. C.; CRIZEL-CARDOZO, M. M.; KUCK, L. S.; NOREÑA, C. P. Z. Microencapsulation of palm oil by complex coacervation for application in food systems. **Food Chemistry**, v. 220, p. 59–66, 2017.

SAMBORSKA, K.; BOOSTANI, S.; GERANPOUR, M.; HOSSEINI, H.; DIMA, C.; KHOSHNOUDI-NIA, S.; ROSTAMABADI, H.; FALSAFI, S. R.; SHADDEL, R.; AKBARI-ALAVIJEH, S.; JAFARI, S. M. Green biopolymers from by-products as wall materials for spray drying microencapsulation of phytochemicals. **Trends in Food Science and Technology**, v. 108, p. 297–325, 2021.

SANTOS, F. S.; SANTOS, R. S.; DIAS, P. P.; ZANÃO JR, L. A.; TOMASSONI, F. A cultura do Pequi (Caryocar brasiliense Camb.). **Acta Iguazu**, v. 2, n. 3, p. 46–57, 2013.

SARAIVA, R. A.; ARARUNA, M. K. A.; OLIVEIRA, R. C.; MENEZES, K. D. P.; LEITE, G. O.; KERNTOPF, M. R.; COSTA, J. G. M.; ROCHA, J. B. T.; TOMÉ, A. R.; CAMPOS, A. R.; MENEZES, I. R. A. Topical anti-inflammatory effect of Caryocar coriaceum Wittm. (Caryocaraceae) fruit pulp fixed oil on mice ear edema induced by different irritant agents. **Journal of Ethnopharmacology**, v. 136, n. 3, p. 504–510, 2011.

SEARLES, J. A.; CARPENTER, J. F.; RANDOLPH, T. W. The ice nucleation temperature determines the primary drying rate of lyophilization for samples frozen on a temperature-controlled shelf. **Journal of Pharmaceutical Sciences**, v. 90, n. 7, p. 860–871, 2001.

SENA, D. M.; RODRIGUES, F. F. G.; FREIRE, P. T. C.; DE LIMA, S. G.; COUTINHO, H. D. M.; CARVAJAL, J. C. L.; DA COSTA, J. G. M. Physicochemical and spectroscopical investigation of Pequi (Caryocar coriaceum Wittm.) pulp oil. **Grasas y Aceites**, v. 61, n. 2, p. 191–196, 2010.

SENGUPTA, S.; GOSWAMI, R.; BASU, S.; BHOWAL, J. Hypolipidemic effects of soy yogurt fortified with antioxidant rich vegetable oil on albino mice fed high cholesterol diet. **Materials Today: Proceedings**, v. 3, n. 10, p. 3222–3237, 2016.

ŠEREGELJ, V., PEZO, L., ŠOVLJANSKI, O., LEVIĆ, S., NEDOVIĆ, V., MARKOV, S., TOMIĆ, A., ČANADANOVIĆ-BRUNET, J., VULIĆ, J., ŠAPONJAC, V. T., & ĆETKOVIĆ, G. New concept of fortified yogurt formulation with encapsulated carrot waste extract. **LWT - Food Science and Technology**, v.138, p. 110732, 2021.

SHEN, Z.; AUGUSTIN, M. A.; SANGUANSRI, L.; CHENG, L. J. Oxidative stability of microencapsulated fish oil powders stabilized by blends of chitosan, modified starch, and glucose. **Journal of Agricultural and Food Chemistry**, v. 58, n. 7, p. 4487–4493, 2010.

SILVA, F.; TORRES, L.; SILVA, L.; FIGUEIREDO, R.; GARRUTI, D.; ARAÚJO, T.; DUARTE, A.; BRITO, D.; RICARDO, N. Cashew gum and maltrodextrin particles for green tea (Camellia sinensis var Assamica) extract encapsulation. **Food Chemistry**, v. 261, p. 169–175, 2018.

SILVA, L. C.; NASCIMENTO, M. A.; MENDES, L. G.; FURTADO, R. F.; COSTA, J. M. C.; CARDOSO, A. L. H. 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.

SILVA, S. C.; FERNANDES, I. P.; BARROS, L.; FERNANDES, Â.; JOSÉ, M.; CALHELHA, R. C.; PEREIRA, C.; BARREIRA, J. C. M.; MANRIQUE, Y. Spray-dried Spirulina platensis as an e ff ective ingredient to improve yogurt formulations : Testing di ff erent encapsulating solutions. **Journal of Functional Foods**, v. 60, n. June, p. 103427, 2019.

SILVA, T. M.; SANTIAGO, P. O.; PURCENA, L. L. A.; FERNANDES, K. F. Study of the

cashew gum polysaccharide for the horseradish peroxidase immobilization - Structural characteristics, stability and recovery. **Materials Science and Engineering C**, v. 30, n. 4, p. 526–530, 2010.

SILVA, V. M.; VIEIRA, G. S.; HUBINGER, M. D. Influence of different combinations of wall materials and homogenisation pressure on the microencapsulation of green coffee oil by spray drying. **Food Research International**, v. 61, p. 132–143, 2014.

SILVEIRA SERRA, D.; MATIAS DE SOUSA, A.; COSTA DA SILVA ANDRADE, L.; DE LIMA GONDIM, F.; EVANGELISTA DE ÁVILA DOS SANTOS, J.; MOURA DE OLIVEIRA, M. L.; TORRES ÁVILA PIMENTA, A. Effects of fixed oil of Caryocar coriaceum Wittm. Seeds on the respiratory system of rats in a short-term secondhand-smoke exposure model. **Journal of Ethnopharmacology**, v. 252, n. September 2019, p. 112633, 2020.

SOARES, B. S.; SIQUEIRA, R. P.; CARVALHO, M. G.; VICENTE, J.; GARCIA-ROJAS, E. E. Microencapsulation of sacha inchi oil (Plukenetia volubilis L.) using complex coacervation: Formation and structural characterization. **Food Chemistry**, v. 298, n. June, p. 125045, 2019.

SOUSA JÚNIOR, José Ribamar de**. Conhecimento e manejo tradicional de Caryocar coriaceum Wittm (Pequi) na Chapada do Araripe**, Nordeste do Brasil. 2012. 96f. Dissertação (Mestrado em Botânica) – Departamento de Biologia, Universidade Federal Rural de Pernambuco, Recife-PE, 2012.

SOUZA, V. B.; THOMAZINI, M.; CHAVES, I. E.; FERRO-FURTADO, R.; FAVARO-TRINDADE, C. S. Microencapsulation by complex coacervation as a tool to protect bioactive compounds and to reduce astringency and strong flavor of vegetable extracts. **Food Hydrocolloids**, v. 98, p. 105244, 2020.

SPRECHER, H. Biochemistry of essential fatty acids. **Progress in Lipid Research**, v. 20, n. C, p. 13–22, 1981.

TEIXEIRA, P. R. S.; DO NASCIMENTO MARREIRO, A. S.; FARIAS, E. A. de O.; DIONISIO, N. A.; FILHO, E. C. S.; EIRAS, C. Layer-by-layer hybrid films of phosphate cellulose and electroactive polymer as chromium (VI) sensors. **Journal of Solid State Electrochemistry**, v. 19, n. 7, p. 2129–2139, 2015.

TIMILSENA, Y. P.; ADHIKARI, R.; BARROW, C. J.; ADHIKARI, B. Microencapsulation of chia seed oil using chia seed protein isolate-chia seed gum complex coacervates. **International Journal of Biological Macromolecules**, v. 91, p. 347–357, 2016.

TIMILSENA, Y. P.; AKANBI, T. O.; KHALID, N.; ADHIKARI, B.; BARROW, C. J. Complex coacervation: Principles, mechanisms and applications in microencapsulation. **International Journal of Biological Macromolecules**, v. 121, p. 1276–1286, 2019.

TIMILSENA, Y. P.; WANG, B.; ADHIKARI, R.; ADHIKARI, B. Advances in microencapsulation of polyunsaturated fatty acids (PUFAs)-rich plant oils using complex coacervation: A review. **Food Hydrocolloids**, v. 69, p. 369–381, 2017.

TORQUATO, D. S.; FERREIRA, M. L.; SÁ, G. C.; BRITO, E. S.; PINTO, G. A. S.; AZEVEDO, E. H. F. Evaluation of antimicrobial activity of cashew tree gum. **World Journal of Microbiology and Biotechnology**, v. 20, n. 5, p. 505–507, 2004.

TORRES, L. R. d. O.; SANTANA, F. C. d.; TORRES-LEAL, F. L.; MELO, I. L. P. d.; YOSHIME, L. T.; MATOS-NETO, E. M.; SEELAENDER, M. C. L.; ARAÚJO, C. M. M.; COGLIATI, B.; MANCINI-FILHO, J. Pequi (Caryocar brasiliense Camb.) almond oil attenuates carbon tetrachloride-induced acute hepatic injury in rats: Antioxidant and anti-inflammatory effects. **Food and Chemical Toxicology**, v. 97, p. 205–216, 2016.

TRAESEL, G. K.; MENEGATI, S. E. L. T.; DOS SANTOS, A. C.; CARVALHO SOUZA, R. I.; VILLAS BOAS, G. R.; JUSTI, P. N.; KASSUYA, C. A. L.; SANJINEZ ARGANDOÑA, E. J.; OESTERREICH, S. A. Oral acute and subchronic toxicity studies of the oil extracted from pequi (Caryocar brasiliense, Camb.) pulp in rats. **Food and Chemical Toxicology**, v. 97, p. 224–231, 2016.

TURGEON, S. L.; SCHMITT, C.; SANCHEZ, C. Protein-polysaccharide complexes and coacervates. **Current Opinion in Colloid and Interface Science**, v. 12, n. 4–5, p. 166–178, 2007.

VÁZQUEZ-GONZÁLEZ, Y.; PRIETO, C.; FILIZOGLU, M. F.; RAGAZZO-SÁNCHEZ, J. A.; CALDERÓN-SANTOYO, M.; FURTADO, R. F.; CHENG, H. N.; BISWAS, A.; LAGARON, J. M. Electrosprayed cashew gum microparticles for the encapsulation of highly sensitive bioactive materials. **Carbohydrate Polymers**, v. 264, p. 118060, 2021.

VISHNU, K. V.; CHATTERJEE, N. S.; AJEESHKUMAR, K. K.; LEKSHMI, R. G. K.;

TEJPAL, C. S.; MATHEW, S.; RAVISHANKAR, C. N. Microencapsulation of sardine oil: Application of vanillic acid grafted chitosan as a bio-functional wall material. **Carbohydrate Polymers**, v. 174, p. 540–548, 2017.

WANG, M.; BI, S.; QIN, D.; SU, C.; WANG, H.; CHEN, X. Quantitative Evaluation of the Antibacterial Effectiveness and Efficiency of Chitosan Considering the Effect of Neutralization. **Carbohydrate Polymers**, v. 265, p. 117918, 2021.

YAP, B. K.; GAM, L. H. Differentiation of bovine from porcine gelatin capsules using gel electrophoresis method. **Food Chemistry**, v. 274, p. 16–19, 2019.

YE, Q.; GEORGES, N.; SELOMULYA, C. Microencapsulation of active ingredients in functional foods: From research stage to commercial food products. **Trends in Food Science and Technology**, v. 78, p. 167–179, 2018.

YU, D.; KWON, G.; AN, J.; LIM, Y.; JHOO, J.; CHUNG, D. In fl uence of prebiotic biopolymers on physicochemical and sensory characteristics of yoghurt. **International Dairy Journal**, v. 115, p. 104915, 2021a.

YU, J.; WANG, D.; GEETHA, N.; KHAWAR, K. M.; JOGAIAH, S.; MUJTABA, M. Current trends and challenges in the synthesis and applications of chitosan-based nanocomposites for plants: A review. **Carbohydrate Polymers**, v. 261, p. 117904, 2021b.

XIE, H., ZHANG, Y., CAO, M., LIU, C., MAO, Y., REN, G., WU, Z., FANG, S., TIAN, S., & WU, D. Fabrication of PGFE/CN-stabilized β-carotene-loaded peppermint oil nanoemulsions: Storage stability, rheological behavior and intelligent sensory analyses. **LWT - Food Science and Tecnhology**, 138, 110688, 2021.

ZANDI, M.; DARDMEH, N.; PIRSA, S.; ALMASI, H. Identification of Cardamom Encapsulated Alginate–Whey Protein Concentrates Microcapsule Release Kinetics and Mechanism during Storage, Stew Process and Oral Consumption. **Journal of Food Process Engineering**, v. 40, n. 1, 2017.

ZHANG, G.; LIU, T.; WANG, Q.; CHEN, L.; LEI, J.; LUO, J.; MA, G.; SU, Z. Mass spectrometric detection of marker peptides in tryptic digests of gelatin: A new method to differentiate between bovine and porcine gelatin. **Food Hydrocolloids**, v. 23, n. 7, p. 2001–2007, 2009.

ZHANG, W.; ZHANG, J.; JIANG, Q.; XIA, W. The hypolipidemic activity of chitosan

nanopowder prepared by ultrafine milling. **Carbohydrate Polymers**, v. 95, n. 1, p. 487–491, 2013.

ZHOU, P.; MULVANEY, S. J.; REGENSTEIN, J. M. Properties of Alaska pollock skin gelatin: A comparison with tilapia and pork skin gelatins. **Journal of Food Science**, v. 71, n. 6, p. 313–321, 2006.

ANEXO I – ARTIGO PUBLICADO

Título do artigo: Optimization of cashew gum and chitosan for microencapsulation of pequi oil by complex coacervation.

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ORIGINAL ARTICLE

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Optimization of cashew gum and chitosan for microencapsulation of pequi oil by complex coacervation

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CAPES

Abstract

Optimized conditions of cashew gum (CG)/and chitosan (CT) were evaluated for the encapsulation of pequi oil by complex coacervation. A comparison was established with gum Arabic (GA)/CT. At first, the coacervation process without pequi oil was performed for determining the most appropriate proportion and pH for microparticle formation. The best conditions were of 22:1 at pH 4.5 for the CG/CT, and 6:1 at pH 3.5 for the GA/CT. Pequi oil release in a pH range was higher in pH 4.5 and 5 for GA/CT and CG/CT, respectively. Microparticle yield was about 60% for both complexes. The microparticle size was 4.8 and 2.7 μm for CG/CT and GA/CT, respectively. The encapsulation efficiency was 86 and 89% for CG/CT and GA/CT, respectively. CG forms an interesting complex with CT, 22:1 ratio at pH 4.5, for a suitable encapsulation of pequi oil.

Practical applications

Pequi oil has attractive anti-inflammatory and antioxidant properties that could be used in the formulation of new cosmetics and nutraceuticals. The microencapsulation of pequi oil assists in the conservation of these properties because it avoids the direct exposure of the actives molecules to environmental factors and to the reagents and procedures involved in the preparation of a product. Complex coacervation method is recommended for the encapsulation of lipophilic substances and has the great advantage of not using high temperatures and being carried out in aqueous medium.

ANEXO II – ARTIGO PUBLICADO

Título do artigo: Pequi oil microencapsulation by complex coacervation using gelatin-cashew gum.

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Pequi Oil Microencapsulation by Complex Coacervation using Gelatin-Cashew Gum

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Abstract

New functional foods and beverages can be developed using bioactive compounds present in pequi oil. Complex coacervation is an encapsulation method used for preserving bioactive molecules, especially those that are hydrophobic or sensitive to high temperatures. The objective of this work was to produce and characterize pequi oil microparticles using cashew gum/gelatin matrix (CG/GE) through complex coacervation. Gum Arabic (GA) was also studied in comparison with CG. The coacervation process was performed without pequi oil to determine the ideal proportions of the matrix components, followed by the embedding of the oil in the microparticles for evaluation. Satisfactory microparticles were produced at pH 4.5 in the weight ratios of CG/GE = 2:1 and GA/GE = 1:3. Pequi oil release was greater in acidic pH, especially at pH 2 for the CG/GE matrix. The encapsulation efficiency for CG/GE and GA/GE was 72.53% (±4.80) and 82.77% (±6.09), respectively. The results showed that the CG/GE combination seemed very promising as an encapsulation matrix, especially for food applications involving pH values higher than 3.

Keywords: *Anacardium occidentale*; Coacervate; Encapsulation; Gelatin; *Caryocar coriaceum*; Polysaccharides