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FACULDADE DE FARMÁCIA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA DE ALIMENTOS

LARISSA SANTOS ASSUNÇÃO

NANOENCAPSULAMENTO DO AZEITE DE DENDÊ HÍBRIDO UNAUÉ HIE OxG COM COPRODUTOS VEGETAIS: OTIMIZAÇÃO, CARACTERIZAÇÃO, AVALIAÇÃO DA ATIVIDADE ANTIOXIDANTE CELULAR E CITOTOXICIDADE

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Tese apresentada ao Programa de Pós-Graduação em Ciência de Alimentos (PGAli) da Universidade Federal da Bahia, como requisito parcial para a obtenção do título de Doutor em Ciência de Alimentos.

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"*A persistência é o caminho do êxito*." (Charles Chaplin)

RESUMO

A busca por alternativas inovadoras na preservação de compostos bioativos, como os encontrados no azeite de dendê híbrido ou óleo de palma bruto híbrido Unaué HIE OxG, destaca-se pela rica composição em carotenoides, tocoferóis e tocotrienóis. A atividade antioxidante celular é um indicador mais preciso do comportamento destes fitoquímicos em sistemas biológicos. Contudo, a degradação desses compostos em presença de oxigênio e luz desafia a estabilidade, impulsionando o nanoencapsulamento como uma alternativa. Além disso, há um crescente interesse em biopolímeros e farinhas de coprodutos de vegetais como encapsulantes, devido a sua composição nutricional e bioativa. Ademais, a segurança biológica das nanopartículas em células epiteliais e a sua citotoxicidade são áreas ainda pouco exploradas. Nesse contexto, o presente estudo teve como objetivo nanoencapsular o azeite de dendê híbrido Unaué HIE OxG com coprodutos vegetais. As nanopartículas de azeite de dendê híbrido com farinha da semente de jaca e nanopartículas de azeite de dendê híbrido com farinha do eixo da jaca foram otimizadas através de planejamento experimental 2^2. A quantidade de azeite de dendê híbrido e encapsulantes foram as variáveis independentes testadas, e o tamanho de partícula e índice de polidispersibilidade as respostas experimentais. As formulações 7 selecionadas no planejamento experimental (razão de massa 0,8:1; farinha da semente de jaca ou farinha do eixo central da jaca : azeite de dendê híbrido) foram caracterizadas em relação ao potencial zeta, viscosidade aparente, pH, parâmetros de cor e carotenoides totais. Células diferenciadas de adenocarcinoma colorretal humano (Caco-2) foram utilizadas para avaliar a atividade antioxidante celular em diferentes concentrações (2,5; 50; 100; 150; 200; 250 µg/mL) para as nanopartículas e o óleo livre, e os resultados foram expressos em unidades de atividade antioxidante celular (CAA unit). A citotoxicidade foi avaliada pelo ensaio colorimétrico de brometo de 3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazólio (MTT) nas mesmas concentrações, e a viabilidade celular relativa (%) foi calculada. As nanopartículas apresentaram diâmetros em nanoescala (< 250 nm), distribuição monodispersa, boa uniformidade e estabilidade (índice de polidispersibilidade < 0,25; potencial zeta entre +30 mV e -30 mV), alta eficiência do encapsulamento (%) (86,44 ± 0,01 e 90,43±1,34), para nanopartículas com a farinha da semente de jaca e do eixo central da jaca, respectivamente, e ótima retenção de carotenoides (>85%). A viscosidade aparente média foi de 21,89 ± 1,20 cP, e ambas as formulações apresentaram um pH mais ácido do que básico. As nanopartículas apresentaram uma tendência maior ao amarelo (maiores valores de b*) do que ao vermelho, e um valor médio para L* de 40,52 ± 2,97. A atividade antioxidante celular das nanopartículas superou a do óleo livre em todas as concentrações estudadas para células Caco-2, e nenhuma das nanopartículas exibiu efeitos tóxicos nas células. As concentrações de 150, 200 e 250 µg/mL para nanopartículas com a farinha da semente de jaca e do eixo central da jaca, respectivamente, aumentaram a viabilidade celular, o que indica um efeito proliferativo. Diante dos resultados expostos, conclui-se que as nanopartículas de azeite de dendê híbrido com coprodutos vegetais como encapsulantes é uma alternativa sustentável, *clean label* e inovadora no encapsulamento de óleos para a preservação dos compostos bioativos e aplicação do óleo na indústria de alimentos. Além disso, os achados enfatizam a importância de estudar a atividade antioxidante celular de nanopartículas de óleos comestíveis em diferentes linhagens celulares, e a natureza não citotóxica das nanopartículas indica uma segurança biológica e a capacidade de evitar danos às células epiteliais intestinais nas concentrações estudadas.

Palavras-chave: *Elaeis guineensis. Elaeis oleifera. Óleo de palma de alto oleico. Nanopartículas. Potencial antioxidante.*

ABSTRACT

The search for innovative alternatives in preserving bioactive compounds, as found in *azeite de dendê híbrido* or hybrid crude palm oil Unaué HIE OxG, stands out due to its rich composition of carotenoids, tocopherols, and tocotrienols. Cellular antioxidant activity proves to be a more precise indicator of the behavior of these phytochemicals in biological systems. However, the degradation of these compounds in the presence of oxygen and light challenges stability, driving nanoencapsulation as an alternative. Additionally, there is a growing interest in biopolymers and vegetable by-product flours as encapsulants, owing to their nutritional and bioactive composition. Furthermore, the biological safety of nanoparticles in epithelial cells and their cytotoxicity remains an underexplored area. In this context, this study aimed to nanoencapsulate hybrid crude palm oil Unaué HIE OxG with vegetable by-products as encapsulant. Nanoparticles of hybrid crude palm oil with jackfruit seed flour and nanoparticles of hybrid crude palm oil with jackfruit axis flour as encapsulant were optimized using a 2^2 experimental design. The amount of hybrid crude palm oil and encapsulants were the independent variables tested, and particle size and polydispersity index were the experimental responses. The formulations 7 selected in the experimental design (mass ratio 0.8:1; jackfruit seed flour or jackfruit axis flour: hybrid crude palm oil) were characterized for zeta potential, apparent viscosity, pH, color parameters, and total carotenoids. Differentiated human colorectal adenocarcinoma cells (Caco-2) were employed to evaluate cellular antioxidant activity at different concentrations (2.5; 50; 100; 150; 200; 250 µg/mL) for both nanoparticles and free oil, with results expressed in cellular antioxidant activity units (CAA units). Cytotoxicity was evaluated through the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay at the same concentrations, and relative cell viability (%) was calculated. The nanoparticles exhibited nano-scale diameters (< 250 nm), monodisperse distribution, good uniformity, and stability (polydispersity index < 0.25; zeta potential between +30 mV and -30 mV), high encapsulation efficiency (%) (86.44 ± 0.01 and 90.43 ± 1.34) for nanoparticles with jackfruit seed flour and jackfruit axis flour, respectively, and excellent carotenoid retention (>85%). The average apparent viscosity was 21.89 ± 1.20 cP, and both formulations showed an acidic pH. The nanoparticles displayed a tendency towards more yellow (higher b* values) than red and had an average L* value of 40.52 ± 2.97. The cellular antioxidant activity of the nanoparticles surpassed that of the free oil at all concentrations studied for Caco-2 cells, and none of the nanoparticles exhibited toxic effects on the cells. Concentrations of 150, 200, and 250 µg/mL for nanoparticles with jackfruit seed flour and jackfruit axis flour, respectively, increased cell viability, indicating a proliferative effect. Based on the results presented, it is concluded that hybrid crude palm oil nanoparticles with vegetable by-products as encapsulants are a sustainable, clean label, and innovative alternative for encapsulating oils to preserve bioactive compounds for application in the food industry. Additionally, the findings underscore the importance of studying the cellular antioxidant activity of edible oil nanoparticles in different cell lines, and the non-cytotoxic nature of the nanoparticles indicates biological safety and the ability to prevent damage to intestinal epithelial cells at the studied concentrations.

Keywords: *Elaeis guineensis. Elaeis oleifera. High-oleic palm oil. Nanoparticles. Antioxidant potential.*

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AAC	Atividade antioxidante celular
ABAP	2,2′-azobis (2-amidi-nopropane) dihydrochloride
AD	Azeite de dendê
ADH	Azeite de dendê híbrido
CAA	Cellular antioxidant activity
Caco-2	Células diferenciais de adenocarcinoma colorretal humano
CPO	Crude palm oil
DCFH-DA	2′,7′-dichlorodihydro-fluorescein diacetate
EE	Eficiência do nanoencapsulamento
FEJ	Farinha do eixo central da jaca
FSJ	Farinha da semente de jaca
HCPO	Hybrid crude palm oil
HepG2	Derivadas de carcinoma hepatocelular humano
HIE OxG	Híbrido interespecífico entre o dendezeiro de origem africana (*Elaeis guineensis*) e o Caiaué, de procedência americana (*Elaeis oleífera*)
JAF	Jackfruit axis flour
JAF-NP	Nanoparticles with jackfruit axis flour
JSF	Jackfruit seed flour
JSF-NP	Nanoparticles with jackfruit seed flour
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
N-FEJ	Nanopartículas com a farinha do eixo de jaca como encapsulantes
N-FSJ	Nanopartículas com a farinha de semente de jaca como encapsulantes
NP-ADH	Nanopartículas de azeite de dendê híbrido
OP	Óleo de palma
OPB	Óleo de palma bruto
OPBH	Óleo de palma bruto híbrido

PBS	Phosphate-buffered saline
TEM	Transmission electron microscopy

SUMÁRIO

Capítulo I

Nanoencapsulamento do azeite de dendê híbrido Unaué HIE OxG com coprodutos de vegetais: otimização, caracterização, avaliação da atividade antioxidante celular e citotoxicidade

1 INTRODUÇÃO

O óleo de palma bruto (OPB) ou azeite de dendê (AD) é extraído do mesocarpo dos frutos da palmeira da espécie *Elaeis guineensis*. Com 78,9 milhões de toneladas em 2022, ele é o óleo mais produzido no mundo, sendo os maiores produtores a Indonésia (44,76 milhões de toneladas), Malásia (20,14 milhões) e Tailândia (2,69 milhões), respectivamente. O Brasil ocupa o 9° lugar no *ranking* dos principais países produtores desse óleo (FAOSTAT, 2023).

No entanto, devido à sua menor resistência a pragas e doenças que estão associadas às práticas de cultivo das palmeiras e que podem comprometer o seu crescimento, como por exemplo a doença chamada de amarelecimento fatal, tem-se pensado em alternativas ao uso do óleo advindo da palmeira da espécie *Elaeis guineensis*. Então, experimentos realizados pela Comissão Executiva do Plano da Lavoura Cacaueira (CEPLAC) com uma variedade híbrida de dendê desenvolvida pela Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Amazônia Ocidental (Manaus-AM), resultaram na produção de um novo tipo de azeite. Esta nova variedade é cultivada apenas no Brasil, no município de Una-Bahia e batizada de Unaué HIE OxG. Ela é obtida do cruzamento entre o dendezeiro africado (*Elaeis guineensis*) e o Caiaué, de procedência americana (*Elaeis oleifera*), e contribui com a sustentabilidade da dendeicultura da região, principalmente de pequenos produtores, por aliar alta produtividade em óleo, baixo custo de produção, além de uma maior resistência a pragas e doenças, em comparação ao híbrido produzido em outros países (Rodríguez *et al*., 2016).

A Colômbia é um dos cinco países líderes na produção de óleo de palma bruto, e a forma híbrida representa 12% da área total de cultivo deste óleo. A produção média é de 38 toneladas ha^{-1} ano^{-1}, com algumas plantações com cerca de 45 toneladas ha^{-1} ano^{-1} (Romero *et al*., 2021). No Brasil, a área plantada para produção de óleo de palma bruto HIE OxG no Pará é estimada em, aproximadamente, 11.500 ha, com potencial de produção de mais de 40.000 toneladas de óleo por ano (Antoniassi *et al*., 2018). Uma área de cerca de 30 mil ha de dendezeiros para produção do azeite de dendê híbrido está localizada na Bahia (Pinto *et al.*, 2019).

Em relação a outras variedades, o óleo de palma bruto híbrido (OPBH) fornece um óleo bruto com melhores propriedades nutricionais, tais como maiores quantidades de vitamina E, carotenoides e ácidos graxos insaturados, além de um menor teor de ácidos graxos saturados (Pinto *et al*., 2019; Choon *et al*., 2021; Sambanthamurthi, Sundram e Tan, 2000). Segundo o Codex (2023), por conter pelo menos 48% de ácido oleico (em % do total de ácidos graxos), esse óleo é reconhecido como “óleo de palma de alto oleico”. Este óleo se destaca em relação

ao teor de carotenoides, com teores variando entre 500 e 10.000 µg/g, sendo os principais componentes o β-caroteno (52–60%) e α- caroteno (33–36%) (Mozzon, Foligni, Tylewicz, 2018). Quanto ao teor de tocoferóis e tocotrienóis, os valores variam de 562 a 1417 mg/kg, tendo o γ-tocotrienol como o componente principal (406 a 887 µg/g) (Codex, 2023).

O azeite de dendê híbrido (ADH) tem sido explorado na literatura científica em relação a suas propriedades antioxidantes (Rodríguez *et al*., 2016; Ojeda *et al*., 2016), por meio de ensaios que envolvem métodos químicos, como capacidade de absorção de oxigênio radical (ORAC), 2,2-difenil-1-picrilhidrazil (DPPH) e eliminação radical de 2,2'-azino-bis (3-etilbenzotiazolina-6-sulfônico) (ABTS) (Liu *et al*., 2019; Mozzon, Foligni, Tylewicz, 2018). Porém, a atividade biológica de antioxidantes naturais a nível celular também deve ser considerada. Nesse contexto, a atividade antioxidante celular (AAC) avalia fatores importantes como a absorção celular, metabolismo, localização e distribuição de compostos bioativos nas células. Portanto, podendo prever melhor o comportamento antioxidante em sistemas biológicos (Chen *et al*., 2015; Lu *et al*., 2020).

Entretanto, apesar do conteúdo interessante de bioativos do ADH, estes são susceptíveis a processos oxidativos durante o processamento e armazenamento, podendo levar a alterações em suas propriedades antioxidante, antimicrobiana e corante. Portanto, com o objetivo de preservar esses fitoquímicos da degradação, mascarar ou minimizar sabores indesejáveis, assim como prolongar a vida útil e liberar componentes de maneira controlada, muitos ingredientes, incluindo óleos, têm sido encapsulados (Ahn *et al*., 2008; Domian *et al*., 2014).

A nanoencapsulação é uma tecnologia em expansão que protege substâncias em nanoescala (<1000nm) (Silva *et al*., 2021). O encapsulamento de óleos é uma abordagem eficaz, proporcionando soluções para os desafios enfrentados em suas aplicações, ao aumentar a biodisponibilidade e solubilidade, reduzir a volatilidade, melhorar a estabilidade química e térmica, e aprimorar o sistema de entrega de compostos bioativos (Oliveira *et al*., 2022).

Entretanto, a formação de nanopartículas estáveis, deve-se considerar a escolha do material de parede/agente encapsulante, além do solvente orgânico e do emulsificante. Neste contexto, a literatura tem explorado estudos que investigam o desenvolvimento, otimização e caracterização de nanopartículas de OPBH, assim como o uso de diferentes materiais encapsulantes (Hernández-Carrión, Moyano e Quintanilla-Carvajal, 2020). As principais variáveis independentes examinadas nos estudos compreendem o tipo e as concentrações dos encapsulantes e do composto a ser encapsulado. Por outro lado, as principais variáveis de resposta analisadas incluem tamanho de partícula, índice de polidispersibilidade (PDI) e potencial zeta (Ricaurte *et al*., 2016; Ricaurte *et al*., 2018).

Diversos são os encapsulantes que podem ser empregados na produção de nanopartículas. Porém, há um interesse crescente na utilização de materiais de parede mais sustentáveis e alternativos aos sintéticos para encapsular compostos bioativos e óleos. Biopolímeros como quitosana (Raeisi *et al*., 2019; Upadhyay *et al*., 2021), goma arábica e caseína (Ferreira-Ribeiro *et al*., 2022), albedo de maracujá (Bezerra *et al*., 2019) e poli (ácido lático) (PLA)/ poli (ácido lático-co-ácido glicólico e poli-β-hidroxibutirato) (Assunção *et al*., 2021) são mencionados na literatura. No entanto, não foram identificados estudos que utilizaram coprodutos vegetais, como farinhas da semente e eixo central de jaca e do tegumento do feijão caupi, como materiais encapsulantes em óleos comestíveis.

Ademais, é essencial investigar se as nanopartículas exibem efeitos tóxicos, sendo essa área de estudo ainda carente na literatura para nanopartículas de óleos comestíveis (Handford *et al*., 2014). Uma abordagem para avaliar a toxicidade das nanopartículas é conduzir estudos em células (*in vitro*), nos quais alterações celulares são observadas, indicando possíveis efeitos tóxicos ou protetores às células (Rogero *et al*., 2003).

A investigação do processo do nanoencapsulamento do azeite de dendê híbrido Unaué HIE OxG com coprodutos vegetais, assim como a otimização das formulações, caracterização das nanopartículas e a avaliação conjunta da atividade antioxidante celular e da citotoxicidade, pode possibilitar o aprimoramento e a compreensão dos aspectos fundamentais associados a essa tecnologia. Além disso, vem crescendo o interesse pelo uso de biopolímeros como encapsulantes, e ainda são escassos os estudos sobre AAC e citotoxicidade de nanopartículas de óleos comestíveis. Assim, este estudo pode contribuir para o desenvolvimento de estratégias sustentáveis, eficazes e seguras na preservação dos compostos bioativos presentes no azeite de dendê híbrido, visando possíveis aplicações na indústria alimentícia.

2 OBJETIVOS

2.1 Objetivo geral

- ✓ Nanoencapsular o azeite de dendê híbrido Unaué HIE OxG com coprodutos vegetais.

2.2 Objetivos específicos

- ✓ Obter as farinhas de coprodutos vegetais para uso como encapsulantes no desenvolvimento das nanopartículas;
- ✓ Desenvolver nanopartículas de azeite de dendê híbrido Unaué HIE OxG (NP-ADH) utilizando diferentes coprodutos de vegetais como encapsulantes;
- ✓ Otimizar as melhores formulações das nanopartículas através da análise de tamanho de partícula e índice de polidispersibilidade (PDI);
- ✓ Analisar a eficiência do nanoencapsulamento das nanopartículas obtidas nas melhores condições;
- ✓ Caracterizar as nanopartículas otimizadas em relação ao potencial zeta, morfologia, viscosidade aparente, pH, parâmetros de cor e carotenoides totais;
- ✓ Avaliar a atividade antioxidante celular das NP-ADH otimizadas e do óleo livre em células Caco-2;
- ✓ Estudar a citotoxicidade das NP-ADH otimizadas e do óleo livre em células Caco-2 através de ensaios de viabilidade celular.

3 FUNDAMENTAÇÃO TEÓRICA

3.1. Azeite de dendê híbrido: histórico, características gerais e aspectos nutricionais

Há um aumento na procura por óleo vegetais para uso na alimentação humana, animal e combustível, e o óleo de palma bruto (OPB) ou azeite de dendê (AD) é considerado versátil e atraente devido ao seu preço, textura, sabor, odor e ao prazo de validade (European Palm Oil Alliance, 2022; Ritchie e Roser, 2020; WWF, 2022). Neste contexto, OPB é extraído do mesocarpo dos frutos da palmeira da espécie *Elaeis guineenses*, originalmente encontrada na África Ocidental, e é o óleo mais produzido no mundo (FAOSTAT, 2023).

Em 2021, o valor de mercado do setor do óleo de palma ultrapassou 50 milhões de dólares e, sua produção foi de 74,7 milhões de toneladas em 2020, provenientes do cultivo de 29 milhões de hectares de dendê (VOORA *et al*., 2023; FAOSTAT, 2023). Somado a isso, mais de 7 milhões de pequenos produtores cultivam o dendezeiro como a principal forma de subsistência (RSPO, 2022). A distribuição da produção de OPB por países, é de 44,76 milhões de toneladas na Indonésia, 20,14 milhões na Malásia e 2,69 milhões na Tailândia. Além disso, este óleo é cultivado em países da África, outros países da Ásia e na América do Sul (FAOSTAT, 2023).

A busca por alternativas ao uso do OPB vem crescendo, principalmente relacionado à vulnerabilidade da palmeira da espécie *Elaeis guineensis* a pragas e doenças relacionadas às práticas de cultivo, o que pode comprometer o crescimento da planta. Neste contexto, o híbrido interespecífico HIE OxG obtido do cruzamento entre o dendezeiro africano (*Elaeis guineensis*) e o Caiaué, de procedência americana (*Elaeis oleifera*), tem sido explorado em programas de melhoramento genético com o objetivo de associar a alta produtividade em óleo do dendezeiro, com resistência ou tolerância a pragas e doenças, comparado ao OPB, além de porte baixo e a qualidade nutricional do Caiaué (Pinto *et al.* 2019).

A Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) possui estudos acerca do cultivo do HIE OxG no Brasil desde a década de 1990, mas apenas em 2010, foi lançado pela EMBRAPA a primeira cultivar do híbrido interespecífico, denominada BRS Manicoré (Cunha e Lopes, 2010; EMBRAPA, 2021). No Brasil, no estado do Pará, a área destinada para o cultivo do Manicoré é estimada em, aproximadamente, 11.500 hectares, apresentando uma capacidade de produção acima de 40 mil toneladas de óleo por ano (Antoniassi *et al*., 2018). Sabe-se que o estado da Bahia possui um alto consumo do óleo de palma bruto, como ingrediente em diversos pratos típicos da culinária local. Por isso, principalmente a região do baixo sul deste estado, também destina uma área expressiva, cerca de 30 mil hectares (Pinto *et al.*, 2019).

O HIE OxG híbrido interespecífico é batizado de Unaué, pois foi desenvolvido na Estação Experimental Lemos Maia (Esmai/CEPLAC), no município de Una-Bahia (Figura 1). Esta variedade apresenta como diferencial a produção de um óleo com menor acidez e sabor mais suave, contribuindo com a culinária local. Além disso, quando comparado a produção do híbrido de outros países, o cultivo do Unaué oferece maior resistência a pragas, principalmente ao Anel vermelho, doença comum e altamente letal, além de ter uma alta produtividade e baixo custo de produção (Rodríguez *et al*., 2016; EMBRAPA, 2021; Pinto *et al.*, 2019).

Figura 1 – Azeite de dendê híbrido Unaué HIE OxG desenvolvido na Estação Experimental Lemos Maia (Esmai/CEPLAC), no município de Una-BA.

Fonte: *EMBRAPA, 2021.*

O azeite de dendê híbrido HIE OxG se destaca em relação à sua composição nutricional, com menor teor de ácidos graxos saturados (23 – 38% de ácido palmítico, 1,5 – 4,5% de ácido esteárico, até 0,8% de ácido mirístico e palmitoléico) e um maior teor de ácidos graxos insaturados (52-57% de ácido oleico e 9 – 17% de ácido linoleico) quando comparado ao óleo de palma bruto. Além disso, por conter no mínimo 48% de ácido oleico, esse óleo é reconhecido como "óleo de palma com alto conteúdo de ácido oleico" (CODEX, 2023). Vale destacar que o consumo de ácidos graxos insaturados tem sido associado com a melhora do perfil lipídico, através da redução do colesterol, e, consequentemente, o risco de doenças cardiovasculares (Romero *et al.*, 2021; Rodríguez *et al.*, 2016).

Algumas evidências apoiam o conceito de que o azeite de dendê híbrido (ADH) pode ser visto como o "equivalente tropical do azeite". O impacto da suplementação diária de ADH (25 mL/dia por 3 meses) foi investigado quanto a capacidade antioxidante no plasma/soro e no

conteúdo fenólico total em adultos (50 a 77 anos) e esses resultados foram comparados a uma quantidade equivalente de azeite extravirgem, amplamente reconhecido pelos seus benefícios à saúde. Nesse estudo, foi demonstrado que o OPBH exerce um efeito bioquímico benéfico adicional, como a melhora do status antioxidante, que pode servir como um óleo comestível tropical promissor e/ou ingrediente para preparações de alimentos funcionais (Ojeda *et al*., 2016).

O teor de carotenoides do ADH também se destaca (500 e 10.000 µg/g). Onze pigmentos diferentes foram identificados no ADH, entre eles α-, β-, ζ- e γ-caroteno, sendo que o β-caroteno (52–60%) e α-caroteno (33–36%) são os principais componentes (Mozzon, Foligni, Tylewicz, 2018; de Almeida *et al*., 2021). O teor total de tocoferóis e tocotrienóis variam de 562 a 1417 µg/g, sendo o α-tocoferol e o γ-tocotrienol como componentes principais (49 a 188 µg/g e 406 a 887 µg/g, respectivamente) (CODEX, 2023).

Ademais, a relevância do ADH também se relaciona com o seu potencial funcional e sua capacidade antioxidante, através da presença de compostos bioativos, como os tocotrienóis, tocoferóis e os carotenoides, que estão associados a benefícios na saúde, como a proteção contra doenças cardiovasculares e a redução do estresse oxidativo (de Almeida *et al*., 2021; Rodríguez *et al*., 2016; Lucci *et al.,* 2016). Diante destas características, o ADH tem potencial para proporcionar produtos com melhor sabor e qualidade nutricional e funcional, além de contribuir para a sustentabilidade da dendeicultura local (Pinto *et al*., 2019).

O ADH tem sido explorado na literatura científica em relação à sua atividade antioxidante, seu potencial como óleo funcional na prevenção de doenças e possíveis benefícios à saúde, principalmente atribuído ao seu elevado percentagem em ácido oleico (de Almeida *et al*, 2021; Rodríguez *et al*., 2016; Lucci *et al*. 2016). De acordo com Rodríguez *et al*. (2016) o consumo de 25 mL/dia de ADH por um período de 3 meses teve um efeito favorável no padrão lipídico plasmático relacionado a fatores de risco cardiovascular, como colesterol total, lipoproteína de baixa densidade (LDL-c) e lipoproteína de alta densidade (HDL-c), e que este efeito não foi estatisticamente diferente daquele para o azeite extravirgem. Além disso, os autores reportam que, além de seu alto percentual de ácido oleico (54,6 ± 1,0%) e baixo teor de ácidos graxos saturados (33,5 ± 0,5%).

3.2. Nanoencapsulamento de óleos comestíveis

A tecnologia de encapsulamento em escala nanométrica envolve a síntese, caracterização e aplicação de partículas com dimensões inferiores a 1000 nm, as quais,

dependendo do método de obtenção, podem se manifestar tanto como nanocápsulas quanto nanoesferas (Konan *et al*., 2002; Sanguansri e Augustin, 2006; Zhang *et al*., 2016).

Assim, a nanotecnologia refere-se à utilização de materiais na escala nanométrica e o processo de nanoencapsulamento relaciona-se com o envolvimento destas partículas menores, seja na forma líquida, sólida ou gasosa, por um encapsulante, comumente chamado como "material de parede", formando pequenas cápsulas (He; Deng; Hwang, 2019). Quando comparada ao microencapsulamento, o nanoencapsulamento tem como vantagens o tamanho subcelular, o que leva a um aumento da área superficial em relação ao volume, e com isso essas partículas menores podem apresentar maior biodisponibilidade e estabilidade físico-química, melhora da solubilidade, entre outras modificações nas propriedades dos compostos (Silva *et al.*, 2021; Granata *et al.*, 2018).

Nos últimos anos, a utilização da nanotecnologia tem ganhado destaque em diversos setores, como na agricultura, medicina, cosméticos e na área de alimentos, em que esta tecnologia pode ser utilizada para produção de aditivos, conservantes alimentares, além de embalagens para alimentos (Bajpai *et al*., 2018; Kumar, Kaur, Gautam, 2020).

Os óleos comestíveis têm sido aplicados em alimentos com o objetivo de melhorar a qualidade e as características organolépticas, além de proporcionar controle microbiológico e a fortificação, como por exemplo com Ácido graxo eicosapentaenoico (EPA) e Ácido graxo docosahexaenoico (DHA) (Sales *et al.*, 2023). Neste contexto, uma forma de incorporar os óleos comestíveis em produtos alimentícios é utilizando o nanoencapsulamento, tendo em vista que este processo visa preservá-los e facilita a sua incorporação em alimentos processados, melhorando a sua biodisponibilidade, mascarando sabor, além de proteger seus compostos bioativos da degradação, mantendo assim seus efeitos benéficos (Singh *et al*. 2017; Fang e Bhandari, 2010).

Nos óleos comestíveis, os compostos bioativos enfrentam desafios de susceptibilidade à oxidação durante o processamento e armazenamento de alimentos, levando a alterações em suas propriedades. Assim, o desenvolvimento de nanopartículas tem despertado interesse como uma estratégia para melhorar a estabilidade química, controlar a liberação desses compostos e aprimorar sua bioacessibilidade e biodisponibilidade. Além disso, as nanopartículas podem ter benefícios como mascarar sabores, reduzir volatilidade e retardar alterações que resultam em perda de cor e aroma (Ferreira; Nunes, 2019; Silva *et al.*, 2021; Granata *et al.*, 2018).

Os óleos comestíveis usualmente utilizados na nanoencapsulação são o óleo de peixe (Ilyasoglu e El, 2014; Ghorbanzade *et al*., 2017; Walia *et al*., 2017), óleo de semente de chia (Campo *et al*., 2017), super oleína de palma (Ricaurte *et al*., 2016), óleo de palma bruto (Donato

et al. 2020; Ferreira-Ribeiro *et al*., 2022), óleo de coco (Santos *et al*., 2014), óleo de farelo de arroz roxo (Jang e Xu, 2009) e óleo de gérmen de trigo (Ghafoor *et al*., 2017). O ADH também vem sendo explorado na literatura no preparo de nanoemulsões, e dentre os objetivos destaca-se o uso do azeite híbrido para aplicação em embalagens, assim como a avaliação das propriedades físicas, térmicas e termoninâmicas das nanoemulsões (Ricaurte *et al*., 2022; Ricaurte *et al.* 2020; Beltrán *et al.* 2020; Ricaurte *et al*. 2018; Hernández-Carrión, Moyano e Quintanilla-Carvajal, 2020).

3.2.1 Principais técnicas de nanoencapsulamento de óleos

Diferentes técnicas podem ser empregadas no nanoencapsulamento de óleos, como a nanoprecipitação, homogeneização, emulsão-difusão, emulsificação de alto cisalhamento (microfluidização), emulsificação espontânea, *spray drying*, extração de emulsão por fluido supercrítico e gelificação iônica (Ferreira e Nunes, 2019; Sridhar, Inbaraj, Chen, 2021; Ferreira-Ribeiro *et al*., 2022). A escola do método é influenciada pelo tipo de polímero e pela substância a ser encapsulada. Contudo, independente do método adotado, o resultado é uma solução coloidal contendo as nanopartículas (Schaffazick *et al*., 2003).

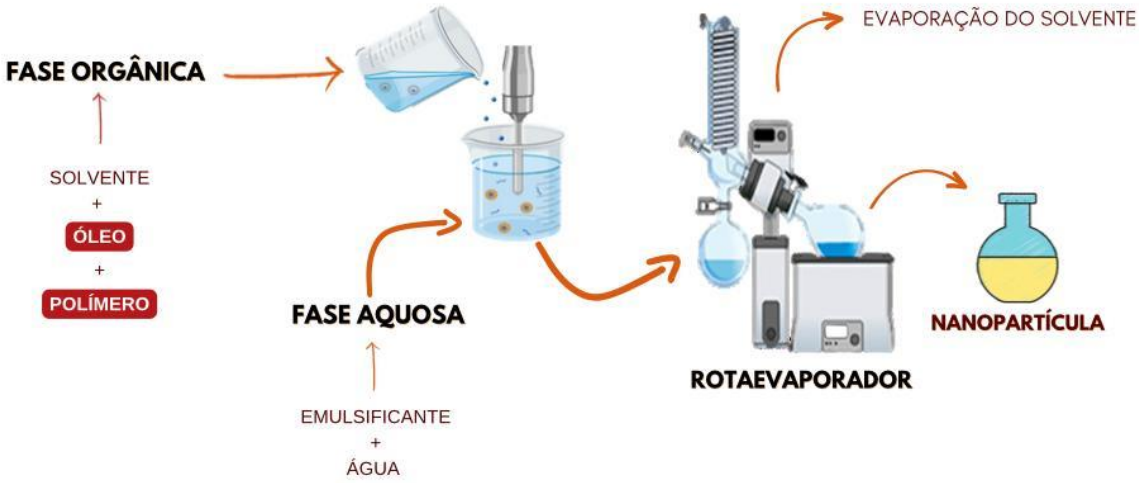
O método de emulsão-difusão é um método de baixa energia, em que ocorre uma diluição seguida da deposição do encapsulante ao redor da partícula e posterior evaporação do solvente utilizado, formando a emulsão. A gelificação iônica se baseia na interação eletrostática entre um polímero com carga oposta e um polieletrólito (poliânion tripolifosfato de sódio), e pode ser considerada mais sustentável do que os outros métodos visto que evita uso de surfactantes e de solventes orgânicos tóxicos (Ferreira e Nunes, 2019; Sridhar, Inbaraj, Chen, 2021), porém a sua utilização ocorre principalmente em escala de laboratório, pois as cápsulas em geral possuem alta porosidade e são facilmente rompidas (Mahdavi *et al*., 2014).

Com relação às técnicas de alta energia, como a microfluidização, o processo consiste no uso de microfluidizadores para geração de nanoemulsões e, comumente, ocorre a redução do tamanho de emulsões já formadas, que possuem tamanhos maiores que 1 nm. O método por *spray drying* refere-se a uma dispersão do composto em uma solução que é atomizada para que o solvente possa ser removido, obtendo uma nanopartícula seca. A técnica de extração de emulsão por fluido supercrítico tem como base a dissolução do composto com seu encapsulante em um solvente e a posterior extração deste solvente através do uso de dióxido de carbono (Bayraktar *et al*., 2017; Ferreira e Nunes, 2019; Sridhar, Inbaraj, Chen, 2021).

As técnicas de nanoprecipitação e homogeneização são amplamente empregadas para a formação de nanopartículas, devido a sua simplicidade e baixo custo, e se destacam no nanoencapsulamento de óleos (Ferreira e Nunes, 2019).

O método de nanoprecipitação, ou deslocamento de solvente (Figura 2), proposto por Fessi *et al*., (1988), envolve a presença de duas fases, uma contendo solvente e outra sem. A fase de solvente, conhecida como fase orgânica, inclui uma substância formadora de filme, como um polímero (sintético ou natural), juntamente com uma substância ativa, como óleo, entre outros componentes. Por outro lado, a fase não solvente é predominantemente composta por água, sendo denominada fase não orgânica. Nesse método, as nanopartículas são formadas como uma suspensão coloidal quando a fase orgânica é adicionada lentamente e agitada moderadamente na fase aquosa (Mora-Huertas *et al*., 2010; Fessi *et al*.,1989). Este método é considerado vantajoso, por ser simples de ser reproduzido, evita o uso de grandes quantidades de solventes tóxicos e apresenta alta eficiência de encapsulação para substâncias lipofílicas (Bayraktar *et al*., 2017; Antonioli *et al*., 2020; Froiio *et al*., 2019).

Figura 2 – Desenvolvimento de nanopartículas pelo método de nanoprecipitacão.



Fonte: *autoria própria (2023).*

De acordo com Galindo-Rodriguez *et al*., (2004), o processo de formação de nanopartículas por nanoprecipitação envolve interações entre água-solvente, água-polímero e solvente-polímero. Portanto, as interações entre água e solvente, juntamente com o movimento de difusão do solvente, desempenham um papel crucial na explicação da variação no tamanho das partículas durante o processo de preparação. Contri *et al*., (2015) desenvolveram

nanopartículas de óleo de rosa mosqueira utilizando o método de nanoprecipitação e copolímero de acrilato e etila e de metacrilato de metila como encapsulantes e encontraram diâmetro de partícula de 158 nm. A mesma técnica foi utilizada por Santos *et al*., (2014) ao nanoencapsular óleo de coco também com copolímero de acrilato e etila e de metacrilato de metila como encapsulantes e obtiveram diâmetro médio de partícula < 200 nm.

A homogeneização é um método que utiliza como estratégia principal a formação de emulsões de óleo em água (o/a), aplicando uma homogeneização de alta velocidade, seguida de evaporação do solvente, seja por agitação magnética contínua à temperatura ambiente ou sob pressão reduzida (Nagavarma *et al*., 2012) (Figura 3). Esse método, embora semelhante à nanoprecipitação, apresenta algumas distinções: a fase orgânica é composta por surfactante, solvente orgânico e óleo, sendo a fase aquosa composta por água e o polímero. A fase orgânica é adicionada gota a gota em solução aquosa, sendo o solvente removido pelo processo de rotaevaporação. Campo *et al*., (2017) utilizaram a técnica de homogeneização para nanoencapsular óleo de chia com mucilagem de semente de chia como material de parede e encontraram diâmetro médio de 205 nm. Nanopartículas de óleo de palma bruto e frações de oleína e estearina de palma utilizando o método de homogeneização apresentaram um diâmetro médio < 300 nm (Ferreira-Ribeiro *et al*., 2022.

Figura 3 – Desenvolvimento de nanopartículas pelo método de homogeneização.

Fonte: *autoria própria (2023).*

É importante destacar que no processo de nanoencapsulamento os solventes empregados na fabricação de nanopartículas precisam ser solúveis em água, tendo a acetona como um dos

mais utilizados, embora seja considerada tóxica. Nesse contexto, o álcool de cereais surge como uma alternativa segura para uso em alimentos, tendo em vista a crescente demanda por alimentos mais naturais, saudáveis, sustentáveis e que apresentem o mínimo impacto ambiental. O emprego de tecnologias limpas, em conjunto com a nanotecnologia, visa reduzir o uso de produtos químicos e promover a produção de alimentos livres de resíduos de solventes, garantindo uma abordagem segura tanto para o meio ambiente quanto para o consumo humano (Dordevic *et al.*, 2014; Klettenhammer *et al*., 2020; Temelli, 2018; Yada *et al*., 2014).

3.2.2 Encapsulantes

No intuito de garantir a formação de nanopartículas mais estáveis, deve-se considerar o composto que será encapsulado (tipo de óleo), a escolha do material de parede/encapsulante, o solvente orgânico e o emulsificante para auxiliar na formação da nanoemulsão. No que se refere aos encapsulantes, a sua escolha varia de acordo com o tamanho esperado da nanopartícula, assim como depende da solubilidade e estabilidade do material de parede. Estes materiais podem ser sintéticos, como o Eudragit, poliprolactona, ou naturais, como o quitosana, o acetato de celulose e a proteína do soro do leite (Lammari, Louaer, Meniai, Elaissari, 2020).

De acordo com Ferreira e Nunes (2019), para o nanoencapsulamento de óleos, os encapsulantes mais utilizados são a poliprolactona, a proteína do soro do leite, o caseinato de sódio, a mucilagem da semente de chia e a maltodextrina/amido modificado. Dentre eles, foi observado uma maior utilização da poliprolactona, por ser solúvel em solventes inorgânicos e apresentar boa solubilidade, porosidade e baixo ponto de fusão, e da proteína do soro do leite, por ser metabolizável e biodegradável.

O crescente interesse por materiais de parede mais sustentáveis e alternativos aos sintéticos destaca o potencial promissor de encapsulantes derivados de coprodutos de vegetais, como farinhas da semente, eixo central de jaca e tegumento do feijão-caupi, ainda não explorados na literatura científica para o nanoencapsulamento de óleos comestíveis (Campo *et al*., 2017; Bezerra *et al*., 2019; Upadhyay *et al*., 2021). Em um estudo conduzido por Bezerra *et al*. (2019), nanopartículas foram desenvolvidas utilizando farinha do albedo do maracujá como encapsulante, um coproduto vegetal, oferecendo uma alternativa para a aplicação de compostos bioativos em alimentos.

É importante destacar que os resíduos de alimentos ou coprodutos são aquelas substâncias ricas em matéria orgânica que são geradas após o processamento de uma matéria prima. Estes coprodutos representam cerca de 40 a 50% das frutas e vegetais e costumam ser descartados e desvalorizados, ainda que muitas vezes se apresentem como fonte de nutrientes,

sendo ricos em proteínas, carboidratos, fibras e compostos bioativos que poderiam ser reaproveitados pelas indústrias farmacêutica, alimentícia e química (Saraiva *et al*., 2018) (dos Santos *et al*., 2021).

A geração de resíduos agroindustriais pode ocorrer nas etapas de colheita (10%), transporte e industrialização (50%) e na casa dos consumidores devido ao desperdício (10%) (Ricardino *et al*., 2020). Assim, a agroindústria é responsável pela produção de uma grande quantidade de resíduos, incluindo, principalmente, cascas, sementes e bagaço. Na indústria de alimentos, os resíduos podem ser utilizados para o enriquecimento de produtos, suplementação alimentar, ou também como substitutos de ingredientes emulsificantes e gelificantes (Pojić *et al*., 2018; Saraiva *et al.*, 2018).

3.2.2.1 Coprodutos de vegetais como encapsulantes

O consumo da jaca (*Artocarpus heterophyllus*), assim como o seu processamento, resulta em uma quantidade significativa de resíduos ou coprodutos, como a casca, o eixo central e a semente, os quais não têm potencial mercadológico efetivamente explorado. A parte comestível da jaca para o consumo humano é aproximadamente 25-35%, enquanto os coprodutos contribuem com 75-65% (Pathak *et al*., 2022).

A semente de jaca compreende aproximadamente de 18% a 25% do peso seco da fruta (Figura 4), e é considerada um coproduto vegetal pouco explorado na literatura científica, principalmente em relação às suas propriedades nutricionais e antioxidantes (Mahanta; Kalita, 2015). Quanto à composição nutricional, a farinha da semente de jaca (FSJ) é rica em amido, principalmente o amido resistente, e proteína, além de conter fibras solúveis e insolúveis, minerais, vitaminas e compostos bioativos, estando associada a propriedades antimicrobiana e em auxiliar a manutenção de um intestino saudável e da glicemia sanguínea (Maurya; Mogra, 2016; Suzihaque *et al.*, 2022; Waghmare *et al*., 2019).

O eixo central da jaca, porção mais interna e uma estrutura responsável pela sustentação da fruta (Figura 4), apesar de geralmente ser descartada ou usada na alimentação animal, também apresenta características organolépticas e antioxidantes. A farinha do eixo central da jaca (FEJ) foi analisada quanto a compostos fenólicos e flavonoides, evidenciando seu potencial antioxidante (Li *et al.*, 2021).

Figura 4 – Semente e eixo central da jaca.

Fonte: *Autoria própria (2023).*

Neste contexto, o nanoencapsulamento de óleos comestíveis com a FSJ ou a FEJ como agente encapsulante pode oferecer uma maior proteção para os compostos bioativos, assim como pode resultar no aumento do teor de fibras, compostos bioativos, solubilidade em água e estabilidade coloidal. Isto amplia as possibilidades de aplicação na indústria de alimentos, visto que atende a requisitos tecnológicos e contribui para benefícios à saúde (Arabpoor *et al*., 2021).

No Brasil, o maior consumo do maracujá amarelo *(Passiflora edulis Sims f. Flavicarpa*) é na forma *in natura* ou na produção de sucos, de modo que a parte não comestível, apesar de ter valor funcional e nutricional, representa cerca de 60% do descarte desta fruta (FAO, 2015). A farinha do albedo do maracujá (mesocarpo da fruta) contém aproximadamente 20% de pectina, oferecendo propriedades como boa capacidade ligante, gelificação e estabilização, além de teores significativos de fibras insolúveis, minerais e compostos fenólicos, representa assim uma alternativa como encapsulante (Gharehbeglou *et al*., 2019).

A literatura já explorou a produção de nanopartículas usando esta farinha como material de parede, visando a incorporação de compostos bioativos em alimentos. Bezerra *et al*. (2019), utilizaram esta matéria-prima no processo de nanoencapsulamento do β-caroteno extraído da microalga Spirulina sp. LEB 18, resultando em partículas com tamanhos na faixa de 82,29 ± 0,51 a 86,83 ± 0,50 nm e eficiência de encapsulamento variando entre 96,67% e 98,25%. A conclusão do estudo destaca o potencial promissor dessa farinha como encapsulante.

Figura 5 – Partes do maracujá amarelo.

Fonte: *Adaptado de Oliveira (2016).*

O feijão caupi (*Vigna unguiculata*) é uma leguminosa conhecida por seu alto teor de proteínas, fibras e baixo teor de lipídios, apresentando valores respectivos de 12,09, 63 e 0,86%, de acordo com Marinho (2019). Além disso, é capaz de desenvolver-se em regiões semiáridas, com pouca necessidade de insumos. É amplamente utilizado na culinária baiana para o preparo do acarajé e abará, em que sua elaboração envolve a remoção do tegumento do feijão, gerando resíduos que são descartados, embora pudessem ser considerados coprodutos valiosos e explorados em outros processos, como na produção de nanopartículas (Gonçalves *et al.*, 2016).

Figura 6 – Feijão caupi, tegumento do feijão caupi e sua farinha.

Fonte: *Autoria própria (2023).*

Há uma tendência cada vez maior na indústria de alimentos pela produção mais sustentável, através do uso de insumos geralmente destinados ao descarte, evitando assim a geração de resíduos, além de agregar valor nutricional. Neste sentido, estes coprodutos se

destacam como uma alternativa sustentável para uso como material de parede no nanoencapsulamento. Além disso, a maioria dos coprodutos possuem um teor nutricional importante, ricos em proteínas, carboidratos, fibras e compostos bioativos, o que pode enriquecer nutricionalmente o produto (Saraiva *et al*., 2018).

3.2.3 Emulsificantes

De acordo com a Instrução Normativa nº 211 de 1° de março de 2023 da Agência Nacional de Vigilância Sanitária (ANVISA), que estabelece as funções tecnológicas, os limites máximos e as condições de uso para os aditivos alimentares e os coadjuvantes de tecnologia autorizados para uso em alimentos, emulsionante ou emulsificante é a substância que torna possível a formação ou manutenção de uma mistura uniforme de duas ou mais fases imiscíveis no alimento (BRASIL, 2023). Estes tensoativos auxiliam na redução da tensão interfacial entre as fases aquosa e oleosa e, após a homogeneização, contribuem para a estabilidade a longo prazo das gotas de óleo. Tensoativos não iônicos não têm carga na molécula, sendo predominantemente lipofílicos, compostos por ácidos graxos combinados a grupos hidrofílicos, e são comumente utilizados na formação de nanoemulsões devido à sua baixa toxicidade e facilidade de formação (Rao e Mcclements, 2012; Devarajan e Ravichandran, 2011; Oliveira *et al*., 2004).

Na produção de nanopartículas, os polissorbatos, líquidos oleosos derivados de sorbatos, que são esterificados com ácidos graxos, são amplamente empregados tanto de forma isolada quanto em combinação com outros emulsificantes. Algumas marcas comuns de polissorbatos incluem Scattics, Alkest, Canarcel e Tween. As concentrações adequadas destes compostos possuem a capacidade de reduzir a coalescência das gotículas de óleo e aumentar a estabilidade da nanoemulsão, além de não serem voláteis e serem ecologicamente viáveis para o meio ambiente (Kumar; Mandal, 2018; Ferreira-Ribeiro, 2018).

Em um estudo conduzido por Ricaurte *et al.*, (2016), que envolveu a nanoencapsulação de óleo de palma de alto oleico (OPBH) por homogeneização de alto cisalhamento (microfluidização), foi utilizado o Tween 20 como emulsificante. Outro estudo realizado por Ferreira-Ribeiro *et al*. (2022) que nanoencapusulou óleo de palmo bruto e frações (oleína eestearina de palma), empregou Tween 20 (1mg/mL). Campo *et al.* (2017) conduziram a nanoencapsulação de óleo de semente de chia com Tween 80 como emulsificante

Além disso, outro emulsificante empregado no desenvolvimento de nanopartículas é a lecitina, derivada de um conjunto de fosfolipídios, geralmente extraídos de fontes oleaginosas, como a soja. Reconhecida como um surfactante não tóxico, a lecitina é bem tolerada pelo

organismo, pois constitui parte integral das membranas celulares e pode ser completamente metabolizada, podendo atuar como tensoativo para facilitar núcleos lipofílicos em nanopartículas de natureza hidrofílica ou como componente integrante desses materiais. (Adorne *et al.*, 2013; Machado *et al.*, 2014). Em um estudo conduzido por Ricaurte *et al*., (2018), que envolveu a nanoencapsulação de super oleína de palma, 10% de lecitina de soja foi utilizada como emulsificante, juntamente com proteína do soro do leite (0-25%) e gelatina (0-1%) como materiais de parede.

3.2.4 Planejamento experimental no nanoencapsulamento de óleos comestíveis

O planejamento experimental é uma abordagem estatística que envolve a realização de uma série de experimentos em que são feitas alterações intencionais nas variáveis independentes de um processo ou produto. Isso permite observar, identificar, analisar e interpretar as causas das mudanças em uma variável de resposta específica (Montgomery, 2009). Nesse contexto, várias metodologias são empregadas para otimizar e modelar diversos processos, visando obter o máximo de informações, resultando na diminuição do total de experimentos necessários (Weissman; Anderson, 2015).

Na nanotecnologia, o planejamento experimental surge como uma estratégia importante para coletar e analisar dados experimentais, garantindo conclusões válidas e objetivas e otimizando o processo e/ou produto (Croarkin; Tobias, 2020). O uso do planejamento experimental nesta área é especialmente relevante na formulação de nanopartículas e na otimização de processos, a fim de se obter as características mais desejáveis, como por exemplo a quantidade do material de parede e do composto a ser encapsulado. Assim, essa ferramenta possibilita a obtenção de sistemas com diversas características físico-químicas e respostas biológicas (Luiz *et al*., 2021).

De acordo com o Quadro 1, no nanoencapsulamento de óleos, pode-se observar alguns estudos que utilizaram o planejamento experimental com objetivos diversos: (1) otimizar as melhores formulações e condições para o nanoencapsulamento; (2) caracterizar as nanopartículas obtidas (tamanho, índice de polidispersibilidade, potencial zeta, eficiência do encapsulamento, dentre outros); (3) comparar o efeito da técnica empregada na estabilidade física das nanopartículas; (4) estudar o efeito do tipo de material de parede utilizado.

Quadro 1. Estudos que utilizaram planejamento experimental no nanoencapsulamento de óleos comestíveis.

Óleo nanoencapsulado	Técnica de nanoencapsulamento	Tipo de planejamento	Variáveis dependentes	Variáveis independentes	Referência
Óleo de alho	Evaporação do solvente e homogeneização	*Design* estatístico Box–Behnken	Tamanho de partícula; Eficiência do nanoencapsulamento; Fluxo em estado estacionário de finasterida; Concentração inibitória mínima	Fosfatidilcolina de soja (PC); Finasterida (FI); Óleo de alho (GO)	Hosny *et al.*, 2022
Óleo de palma de alto oleico	*Spray-drying*	Delineamento experimental multifatorial categórico com dois fatores	Teor de umidade; Distância de quebra dos flocos	Concentração do material de parede (soro de leite, goma arábica, amido e maltodextrina)	Hernández-Carrión *et al*, 2020
Óleo de café	Miniemulsão/ Evaporação do solvente	Planejamento experimental completo do tipo 2^4	Tamanho de partícula; Recuperação de óleo de café na nanopartícula	Polímero; Tipo de mecanismo de dispersão; Razão de massa polímero:óleo; Surfactante	Freiberger *et al.*, 2015
Óleo de copaíba	Polimerização interfacial	Planejamento experimental completo do tipo 2^3	Tamanho de partícula; Potencial zeta	pH; Temperatura; Concentração de quitosana	Xavier-Júnior *et al.*, 2018
Óleo de palma de alto oleico	Homogeneização de alto cisalhamento	*Design* experimental de otimização de superfície de resposta	Tamanho médio da gota (ADS); Índice de polidispersibilidade (PDI); Potencial zeta; Parâmetros de cor;	Concentração de óleo e do material de parede (soro de leite); Pressão do equipamento; Número de ciclos	Ricaurte *et al.*, 2016

		(modelo quadrático e linear)	Viscosidade		
Óleo de uva	Emulsão-evaporação de solvente	*Design* estatístico Box–Behnken	Tamanho de partícula; Eficiência de encapsulamento; Índice de polidispersão (PDI) e potencial zeta	Tempo de sonicação para a formação da emulsão; concentração do óleo de uva e concentração do agente estabilizante	Fernández *et al*., (20216)

As principais variáveis dependentes analisadas nos estudos são o tamanho da partícula, PDI, potencial zeta e eficiência do encapsulamento.

O tamanho de partícula é um dos fatores mais importantes que afetam a estabilidade dinâmica das emulsões. Sendo assim, tamanhos menores de partículas contribuem para uma maior estabilidade, reduzindo a possibilidade de coalescência e facilitando a interação com o composto de interesse (Ferreira-Ribeiro *et al*., 2022; Ricaurte *et al.*, 2018; Ferreira e Nunes, 2019). Além disso, Ferreira e Nunes (2019), destacaram que diâmetros entre 100 e 1000 nm são mais apropriados para óleos nanoencapsulados. O tamanho de partícula em escala nanométrica aumenta a relação superfície-volume, levando a uma área superficial maior, o que permite melhor solubilidade, maior biodisponibilidade e liberação de compostos ativos na concentração e taxa desejadas (Prakash et al., 2018).

O PDI avalia a uniformidade dos diâmetros das partículas, sendo que valores próximos a 0 indicam que a amostra é monodispersa, enquanto valores próximos a 1 indicam que a amostra possui uma maior variedade de tamanho (Ferreira-Ribeiro *et al*., 2022). Ferreira e Nunes (2019) também sugerem que valores de PDI entre 0,1 e 0,25 representam uma menor variedade de tamanho, enquanto valores acima de 0,5 representam uma variedade mais ampla. A variação destes valores pode ser influenciada pela técnica utilizada para o nanoencapsulamento e pelo tipo e concentração do polímero utilizado, visto que altas concentrações tendem a formar nanopartículas com tamanhos heterogêneos (Sales *et al*., 2023).

O potencial zeta indica o potencial elétrico das partículas, sendo considerado como a diferença na carga eletrocinética da superfície da gota em relação ao seu meio de dispersão (Ferreira e Nunes, 2019; Ferreira-Ribeiro *et al*., 2022). É utilizado para indicar a estabilidade da suspensão em dispersões coloidais, em que valores maiores que 30 mV e menores que - 30 mV promovem alta estabilidade e impedem a agregação das partículas. Assim como o PDI, para o nanoencapsulamento de óleos, os valores de potencial zeta variam com a técnica e com material de parede utilizado, devido às suas características químicas (Ferreira e Nunes, 2019).

A eficiência do nanoencapsulamento (EE) está associada à quantidade de óleo presente dentro da nanopartícula, de modo que uma alta EE sinaliza que o óleo foi encapsulado adequadamente, aumentando a sua estabilidade contra a oxidação (Campo *et al*., 2017). Assim como as outras análises, a EE também pode ser influenciada pelo tipo de óleo, emulsificante e material de parede utilizado na nanopartícula (Campo *et al*., 2017; Hemmatkhah, Zeynali and Almasi, 2020).

3.3 Ensaios em célula com nanopartículas de óleos comestíveis

Ensaios em célula vem sendo amplamente explorados na literatura científica, especialmente devido ao crescente interesse em entender os efeitos biológicos e a segurança das nanopartículas. As principais análises que são realizadas a nível celular em nanopartículas de óleos comestíveis são: citotoxicidade e viabilidade celular (Mota-Ferreira *et al*., 2016; Lu *et al*., 2020), atividade antioxidante celular (Wu *et al.*, 2020; Zheng *et al.*, 2020; Lu *et al*., 2020; Chang *et al.*, 2020) e os possíveis efeitos e alterações na morfologia celular (Mota-Ferreira *et al*., 2016). Além disso, alguns estudos utilizam ensaios com células específicas, como as células de adenocarcinoma colorretal humano (Caco-2) (Reis *et al*., 2020), que imitam as células intestinais e avaliam a absorção e os efeitos em células relacionadas à ingestão de alimentos. Outros avaliam a influência do processo do nanoencapsulamento, onde é realizada a comparação entre o óleo livre e nanoencapsulado, e como o nanoencapsulamento pode influenciar na citotoxicidade e atividade antioxidante celular (Chang *et al*., 2020; Ali *et al*., 2020).

Em comparação aos ensaios químicos, como o ensaio da capacidade de absorção de oxigênio radical (ORAC), 2,2-difenil-1-picrilhidrazil (DPPH) e eliminação radical de 2,2'-azino-bis (3-etilbenzotiazolina-6-sulfônico) (ABTS), muito usados para avaliar a atividade antioxidante em óleos, a atividade antioxidante celular (AAC) considera fatores como absorção celular, metabolismo, localização e distribuição de compostos bioativos dentro das células e, portanto, pode prever melhor o comportamento antioxidante em sistemas biológicos (Chen *et al*., 2015; Lu *et al*., 2020). AAC envolve monitorar a capacidade dos compostos de prevenir a oxidação induzida pelo radical peroxil dentro da célula. O gerador de radicais livres dicloridrato de 2,2'-azobis (2-amidinopropano) (ABAP) é adicionado ao sistema, iniciando a formação de radicais peroxil, permitindo a quantificação da AAC de compostos, como suplementos dietéticos, alimentos e fitoquímicos (Wolfe e Liu, 2007; Liu *et al*., 2019).

Originalmente, o ensaio de AAC utilizavam células PC12 (glândula adrenal de ratos), porém hoje as células HepG2 (carcinoma hepatocelular humano), são relatadas com frequência na literatura (Wolfe & Liu, 2007; Wolfe *et al*., 2008; Song *et al*., 2010). Embora a linha celular HepG2 seja de origem humana e, portanto, seja uma melhoria em relação às células PC12, as células do fígado não são consideradas ideais para medir a eficácia dos antioxidantes dietéticos. Por esta razão, há um movimento no sentido da utilização de células diferenciais de adenocarcinoma colorretal humano (Caco-2) (Kellet, Greenspan e Pegg, 2018).

Desta forma, o uso de células Caco-2 tem sido explorado na literatura científica para ensaios de AAC (Kellet, Greenspan e Pegg, 2018) devido à sua semelhança com células epiteliais do intestino delgado, incluindo morfologia, estrutura microvilar e características de permeabilidade (Sergent *et al*., 2005; Wan, Dong, Yu, Sun e Li, 2015).

A literatura científica sobre AAC em nanopartículas de óleos comestíveis ainda é escassa. Foram encontrados apenas quatro estudos que avaliaram a AAC de óleos vegetais (Liu *et a*l., 2020; Xu *et al*., 2021; Liu *et al*., 2019; Zheng *et al*., 2020). No entanto, esses estudos não envolveram a produção de nanopartículas, e avaliaram a atividade antioxidante de constituintes específicos dos óleos analisados, tais como: tocoferois, esteróis e fenólicos totais em diferentes tipos de óleos vegetais; α-tocoferol e γ -orizanol em óleo de farelo de arroz e óleo de coco; fitoesteróis, esqualeno, γ-orizanol e polifenóis em óleo de farelo de arroz; e tocoferol em óleo de milho. Apenas um estudo avaliou a AAC em nanoemulsões de óleo de polpa de espinheiro marítimo (Chang *et al*., 2020), porém em células HepG2.

Um aspecto crítico no uso de nanomateriais é a sua segurança. Avaliações toxicológicas de nanocarreadores vêm se tornado cada vez mais importantes, especialmente no que diz respeito aos nanomateriais presentes no meio ambiente e àqueles destinados ao uso médico, farmacológico e em alimentos. Assim, o aumento na produção de nanopartículas, e a constante descoberta de novas aplicações para os nanomateriais, torna o conhecimento sobre a toxicidade dos sistemas transportadores uma grande demanda (Reis *et al*., 2020).

Embora o azeite de dendê seja frequentemente utilizado em alimentos, a segurança de suas partículas em escala nanométrica não está bem estabelecida na literatura. A redução de tamanho pode levar a propriedades únicas não encontradas em tamanhos maiores, e efeitos nocivos como disbiose, citotoxicidade e genotoxicidade foram relatados em nanopartículas de diferentes materiais (Ashraf *et al*., 2021). Assim, culturas celulares podem ser utilizadas para avaliar alterações celulares após exposição à substância investigada, revelando potenciais efeitos tóxicos, como peroxidação lipídica, ruptura de membrana e inflamação gastrointestinal (Schappo, Ferreira-Ribeiro e Nunes, 2021).

McClements e Xiao (2017) conduziram um estudo de revisão abordando os principais mecanismos potenciais de toxicidade associados a diferentes nanopartículas de grau alimentício. Os autores enfatizaram que a composição, dimensão, propriedades interfaciais e o estado de agregação são fatores cruciais que podem interferir na ação biológica das nanopartículas. Além disso, destacaram a importância de considerar a interação entre as nanopartículas e a matriz alimentar, pois esta interação pode influenciar em suas propriedades físico-químicas, e, consequentemente, modificar os fatores de absorção e toxicidade.

De acordo com Schappo, Ferreira-Ribeiro e Nunes (2021), que desenvolveram um estudo de revisão sobre a toxicidade de nanopartículas de óleos, com ênfase na ciência de alimentos, os estudos sobre toxicidade em óleos ainda são raros na literatura, porém os autores enfatizam que os resultados são promissores. Na maioria dos estudos, as nanopartículas de óleo são consideradas seguras, e várias amostras apresentaram algum efeito protetor nas células, especialmente efeitos anticancerígenos e antioxidantes.

Uma maneira de avaliar a toxicidade de materiais é por meio de ensaios utilizando culturas de células (humanas ou animais) (*in vitro*). Os ensaios *in vitro* são métodos rápidos, reprodutíveis e sensíveis. A avaliação envolve expor o potencial agente tóxico a uma cultura de células e observar alterações celulares por meio de diversos mecanismos (Rogero *et al*., 2003). A maioria dos estudos utiliza os ensaios brometo de 3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazólio (MTT), Sulforodamina B e Alamar Blue®. O ensaio MTT é o mais prevalente nos estudos, pois é simples, econômico e tradicional. Neste ensaio o anel tetrazólio MTT é clivado, levando à formação de cristais roxos de formazan [(4,5-dimetiltiazol-2-il)-3,5-difenilformazan], que são solubilizados e então quantificados por espectrofotometria, indicando se houve algum dano às mitocôndrias das células (Godoi *et al*., 2017; Mota-Ferreira, 2016).

4 CONCLUSÃO

O azeite de dendê híbrido Unaué HIE OxG se destaca pela sua maior resistência a doenças, alta produtividade em óleo e baixo custo de produção, quando comparado ao óleo de palma bruto. Além disso, vale destacar a sua composição nutricional, com maior teor de carotenoides, tocotrienóis, vitamina E e ácidos graxos insaturados (ácido oleico), o que torna este óleo um antioxidante promissor. A nanotecnologia surge como uma alternativa para preservação destes compostos, e o planejamento experimental auxilia na otimização dos processos, para a formação de nanopartículas mais estáveis e com melhores características físico-químicas. Os coprodutos de vegetais notadamente demonstram um potencial como materiais de parede, além de serem sustentáveis e agregar valor nutricional, e, portanto, poderiam ser utilizados no nanoencapsulamento de óleos. A avaliação da atividade antioxidante celular e citotoxicidade das nanopartículas de óleos comestíveis em células Caco-2 diferenciadas é relevante. Neste sentido, o referencial teórico desde estudo deixou claro que a análise dos efeitos biológicos destes antioxidantes, assim como a segurança das nanopartículas e sua capacidade de evitar danos às células epiteliais intestinais é importante, além de contribuir para o desenvolvimento de estratégias sustentáveis, eficazes e seguras para o azeite de dendê híbrido, visando a sua aplicação na indústria alimentícia.

REFERÊNCIAS

AHN, J. *et al.* Optimization of microencapsulation of seed oil by response surface methodology. **Food Chemistry**, v. 107, n. 1, p. 98-105, 2008.

ALI, H. *et al.* Effect of nanoencapsulation on volatile constituents, and antioxidant and anticancer activities of Algerian Origanum glandulosum Desf. essential oil Hatem. **Science reports**, v. 10, p. 2812, 2020.

ANTONIOLI, G. *et al.* Poly (lactic acid) nanocapsules containing lemongrass essential oil for postharvest decay control: In vitro and in vivo evaluation against phytopathogenic fungi. **Food Chemistry**, v. 326, p. 126997, 2020.

ANTONIASSI, R. *et al.* Óleo de palma de alto oleico produzido no Brasil no ano de 2016. **Embrapa Agroindústria de Alimentos.** Comunicado Técnico, n. 229, 2018.

ARABPOOR, B. *et al.* Multifunctional coating composed of Eryngium campestre L. essential oil encapsulated in nano-chitosan to prolong the shelf-life of fresh cherry fruits. **Food Hydrocolloids,** v. 111, p. 106394, 2021.

ADORNE, M.; BUTTENBENDER, S.; GUTERRES, S. S.; POHLMANN, A. R. Avaliação físico-química de nanocápsulas poliméricas multiparedes estabilizadas por polissorbato 80/lecitina revestidas com diferentes concentrações de quitosana para futura funcionalização com espécies moleculares. In: CONGRESSO BRASILEIRO DE POLÍMEROS, 12., 2013, Florianópolis. Anais...: Florianópolis, Associação Brasileira de Polímeros, 2013.

ASHRAF, A.S. *et al.* Innovations in nanoscience for the sustainable development of food and agriculture with implications on health and environment. **Science of The Total Environment**, v. 768, p. 144-990, 2021.

ASSUNÇÃO, L. S. *et al.* Combination of carotenoids from Spirulina and PLA/PLGA or PHB: New options to obtain bioactive nanoparticles. **Food Chemistry**, v. *346*, 128742, 2021.

BAJPAI, V. *et al*. Prospects of using nanotechnology for food preservation, safety, and security. **Journal of Food and Drug Analisys**, v. 26, p. 1201- 1214, 2018.

BAYRAKTAR O, ERDOGAN I, KOSE MD, KALMAZ G. Nanocarriers for plant-derived natural compounds. nanostructures for antimicrobial therapy: Nanostructures in therapeutic medicine series. **Elsevier Inc.**, p. 395-412, 2017.

BELTRÁN, J.D.; RICAURTE, L., ESTRADA, K. B.; QUINTANILLA-CARVAJAL, M. X. Effect of homogenization methods on the physical stability of nutrition grade nanoliposomes used for encapsulating high oleic palm oil. **LWT - Food Science and Technology**, v. 118, 108801, 2020.

BEZERRA, P. Q. M. *et al*. Innovative functional nanodispersion: Combination of carotenoid from Spirulina and yellow passion fruit albedo. **Food Chemistry**, v. 285, p. 397–405, 2019.

BRASIL. Ministério da Saúde. Secretaria de Vigilância Sanitária. Instrução Normativa nº 11, de 1° de março de 2023. Estabelece as funções tecnológicas, os limites máximos e as condições de uso para os aditivos alimentares e os coadjuvantes de tecnologia autorizados para uso em alimentos.

CAMPO, C. *et al.* Nanoencapsulation of chia seed oil with chia mucilage (Salvia hispânica L.) as wall material: Characterization and stability evaluation. **Food Chemistry**, v. 234, p. 1-9, 2017.

CHANG, M. *et al.* Sea buckthorn pulp oil nanoemulsions fabricated by ultra-high pressure homogenization process: A promising carrier for nutraceuticals. **Journal of Food Engineering**, v. 287, p. 110129, 2020.

CHEN, X. *et al.* The Stability, Sustained Release and Cellular Antioxidant Activity of Curcumin Nanoliposomes. **Molecules**, v. 20, p. 14293-14311, 2015.

CHOON, H. T. *et at.* Red Palm Oil: A Review on Processing, Health Benefits and Its Application in Food. **Journal of Oleo Science**, v. 70, p. 1201-1210, 2021.

CODEX ALIMENTARIUS. **Codex Standard for named vegetable oils** (Codex Stan - CXS 210-199) (p. 14), 2023.

CONTRI,R. V.; KULKAMP-GUERREIRO, I. C.; SILVA, D. J.; FRANK, L. A.; POHLMANN, A. R.; GUTERRES, S. S. Nanoencapsulation of Rose-Hip Oil Prevents Oil Oxidation and Allows Obtainment of Gel and Film Topical Formulations. **AAPS PharmSciTech - American Association of Pharmaceutical Scientists**, v.17, p. 863 - 870, 2015.

CUNHA, R. N. V. DA; LOPES, R. BRS MANICORÉ Híbrido Interespecífico entre o Caiaué e o Dendezeiro Africano Recomendado para Áreas de Incidência de Amarelecimento-Fatal. **Embrapa Agroindústria de Alimentos.** Comunicado Técnico, p. 85, 2010.

DE ALMEIDA, E.S. *et al.* Thermal and Physical Properties of Crude Palm Oil with Higher Oleic Content**. Applied Sciences**, v. 11, p. 7094, 2021.

DEVARAJAN, V.; RAVICHANDRAN, V. Nanoemulsions: As modified drug delivery tool. **Pharmacie Globale (IJCP)**, v. 4, 2011.

DOMIAN, E. *et al.* Influence of agglomeration on physical characteristics and oxidative stability of spray-dried oil powder with milk protein and trehalose wall material. **Journal of Food Engineering**, v. 125, p. 34-43, 2014.

DONATO, M.; RIBEIRO, C.D.F.; BLOCK, J.M.; NUNES, I.L. Efeito da adição de óleo de palma bruto nanoencapsulado na estabilidade oxidativa de molho para salada em teste de oxidação acelerada. **Research, Society and Development,** v. 9, e4229107841, 2020.

DORDEVIC, V. *et al.* Trends in Encapsulation Technologies for Delivery of Food Bioactive Compounds. **Food Engineering Reviews**, v. 7, p. 453-490, 2014.

DOS SANTOS, C. D.; MENEZES, R. C. R.; & DAL BOSCO, S. M. Releitura de pão de queijo: Versão vegetariana com farinha de oliveira. **Revista Verde de Agroecologia e Desenvolvimento Sustentável**, v. 16, n. 2, p. 159-163, 2021.

European Palm Oil Alliance. Facts on palm oil. Facts on palm oil - Sustainable Palm Oil Choice, 2022.

FANG, Z.; BHANDARI, B. Encapsulation of polyphenols – a review. **Trends Food Sci Technol,** 21:510–523, 2010.

FAO (Food and Agriculture Organization of the United Nations). Food Losses and Waste in Latin America and the Caribbean. **Bulletin**, v.2, p.1-39. 2015.

FAOSTAT. Crops and livestock products. **Food and Agriculture Organization of the United Nations**, 2023.

FERREIRA, C. D., & NUNES, I. L. Oil nanoencapsulation: development, application, and incorporation into the food market. **Nanoscale Research Letters**, *14*, 2019.

FERREIRA RIBEIRO, C. D. *et al.* Novel bioactive nanoparticles from crude palm oil and its fractions as foodstuff ingredients. **Food Chemistry**, v. *373*, 2022.

FESSI, H.; PUISIEUX, J. P..; DEVISSAGUET, N.; AMMOURY, N.; BENITA, S. Nanocapsule formation by interfacial polymer deposition following solvent displacement. **International Journal of Pharmaceutics**, v. 55, p. 1-4, 1989.

FREIBERGER, E. B. *et al*. Encapsulation of roasted coffee oil in biocompatible nanoparticles. **LWT - Food Science and Technology**, v. 64, p. 381- 389, 2015.

FROIIO, F. *et al.* Essential oils-loaded polymer particles: Preparation, characterization, and antimicrobial property. **Polymers**, v. 11, *p.* 6, 2019.

GALINDO-RODRIGUEZ, S.; ALLÉMAN, E.; FESSI, H.; DOELKER, E. Physicochemical parameters associated with nanoparticle formation in the salting- out, emulsification-diffusion, and nanoprecipitation methods. **Pharmaceutical Research**, v. 21, p. 1428-1439, 2004.

GHAFOOR, K. *et al*. Nutritional composition, extraction, and utilization of wheat germ oil: A review. **Eur J Lipid Sci Technol,** v. 119, p. 1–9, 2017.

GHAREHBEGLOU, P. et al. Pectin-whey protein complexes vs. small molecule surfactants for stabilization of double nano-emulsions as novel bioactive delivery systems. **J. Food Eng**, v. 245, p. 139-148, 2019.

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

GODOI, S. N. *et al.* Evaluation of Stability and in Vitro Security of Nanoemulsions Containing Eucalyptus Globulus Oil. **Biomed Res.** Int. 2017.

GONÇALVES, A. *et al.* Cowpea (*Vigna unguiculata* L. Walp), a renewed multipurpose crop for a more sustainable agri- food system: nutritional advantages and constraints. **Science of Food and Agriculture**, 2016.

GRANATA, G. *et al.* Essential oils encapsulated in polymer-based nanocapsules as potential candidates for application in food preservation. **Food Chemistry**, v. 269, p. 286–292, 2018.

HANDFORD, E. C. *et al.* Implications of nanotechnology for the agri-food industry: Opportunities, benefits and risks. **Trends In Food Science & Technology**, v. 40, n. 2, p. 226-241, 2014.

HE, X; DENG, H; HWANG, H. The current application of nanotechnology in food and agriculture. **Journal of Food and Drug Analisys**, v. 27, p. 1-21, 2019.

HEMMATKHAH, F.; ZEYNALI, F.; ALMASI, H. Encapsulated cumin seed essential oil-loaded active papers: characterization and evaluation of the effect on quality attributes of beef hamburger. **Food Bioproc Tech**, v. 13, p.533–547, 2020.

HERNÁNDEZ-CARRIÓN, M., MOYANO, M., & QUINTANILLA-CARVAJAL, M. X. Design of high-oleic palm oil nanoemulsions suitable for drying in refractance window™. **Journal of Food Processing and Preservation**, v. *45*, n. 1, p. 1–9, 2020.

HOSNY, *et al*. Utilization of nanotechnology and experimental design in development and optimization of Aloe vera gel loaded with Finasteride–Garlic Oil– Nanotransfersomes. **Journal of Drug Delivery Science and Technology**, v. 68, 103130, 2022.

ILYASOGLU, H.; El, S.N. Nanoencapsulation of EPA/DHA with sodium caseinateegum arabic complex and its usage in the enrichment of fruit juice. **LWT,** v. 56, p. 461–468, 2014.

JANG, S., XU, Z. Lipophilic and Hydrophilic Antioxidants and Their Antioxidant Activities in Purple Rice Bran. **Journal of Agricultural and Food Chemistry,** v. 57, n. 3, p. 858 – 862, 2009.

KELLETT, M. E.; GREENSPAN, P.; PEGG, R. B. Modification of the cellular antioxidant activity (CAA) assay to study phenolic antioxidants in a Caco-2 cell line. **Food Chemistry**, v. 244, p. 359-363, 2018.

KLETTENHAMMER, S. *et al.* Novel Technologies Based on Supercritical Fluids for the Encapsulation of Food Grade Bioactive Compounds. **Foods**, v. 9, 1395, 2020.

KONAN, Y. N., GURNY, R., & ALLÉMANN, E. Preparation and characterization of sterile and freeze-dried sub-200 nm nanoparticles. **International Journal of Pharmaceutics**, v. 233, p.239-252, 2002.

KUMAR, N.; MANDAL, A. Surfactant stabilized Oil-in-Water Nanoemulsion: Stability, Interfacial Tension and Rheology study for Enhanced Oil Recovery Application. **Energy Fuels**, v. 32, p. 6452-6466, 2018.

KUMAR, P. *et al.* Nanotechnology and its challenges in the food sector: a review. **Materials Today Chemistry** v. 17, p. 100332, 2020.

LAMMARI, N. *et al.* Encapsulation of Essential Oils via Nanoprecipitation Process: Overview, Progress, Challenges and Prospects. **Pharmaceutics**, v. 12, p. 431, 2020.

LI, Z. *et al.* Phytochemicals, antioxidant capacity and cytoprotective effects of jackfruit (*Artocarpus heterophyllus* Lam.) axis extracts on HepG2 cells. **Food Bioscience**, v. 41, p. 100933, 2021.

LIU, R. J. *et al.* Evaluation of the Antioxidant Properties of Micronutrients in Different Vegetable Oils. **European Journal of Lipid Science and Technology**, v. 122, p. 1900079, 2020.

LIU, R. J. *et al.* Effect of refining process on physicochemical parameters, chemical compositions and in vitro antioxidant activities of rice bran oil. **LWT-Food Science and Technology**, v. 109, p. 26–32, 2019.

LUCCI, P. *et al.* Palm oil and cardiovascular disease: a randomized trial of the effects of hybrid palm oil supplementation on human plasma lipid patterns. **Food & Function**, v. 7, p. 347–354, 2016.

LU, M. *et al.* Physical properties, and cellular antioxidant activity of vegetable oil emulsions with different chain lengths and saturation of triglycerides. **LWT - Food Science and Technology**, v. 121, p. 108948, 2020.

LUIZ, M. T., *et al.* Design of experiments (DoE) to develop and to optimize nanoparticles as drug delivery systems. **European Journal of Pharmaceutics and Biopharmaceutics**, v. 165, p. 127-148, 2021.

MACHADO, A. R. *et al.* A. Importance of lecithin for encapsulation processes. **African Journal of Food Science**, v. 8, n.4, p. 176-183, 2014.

MAHANTA, C. L.; KALITA, D. Processing, and utilization of jackfruit seeds. **Processing and Impact on Active Components in Food**, p. 395−400, 2015.

MAHDAVI, S. A.; JAFARI, S. M.; GHORBANI, M.; ASSADPOOR, E. Spray-drying microencapsulation of anthocyanins by natural biopolymers: A review. **Drying Technology**, v. 32, p. 509-518, 2014.

MARINHO, L. Q. M. CARACTERIZAÇÃO DE FARINHA DE TEGUMENTO DE FEIJÃO CAUPI (Vigna unguiculata). 2019. Trabalho de Conclusão de Curso (Bacharelado em Nutrição) – Escola de Nutrição, Universidade Federal da Bahia, Salvador, 2019.

MAURYA, P.; MOGRA, R. Assessment of consumption practices of jackfruit (*Artocarpus heterophyllus* lam.) seeds in villages of Jalalpur block district Ambedarnagar (U.P.) India. **Advances in Life Sciences**, p. 73-75, 2016.

MCCLEMENTS, J. D; XIAO, H. Is nano safe in foods? Establishing the factors impacting the gastrointestinal fate and toxicity of organic and inorganic food-grade nanoparticles. **Science of Food**, v. 6, p. 1-13, 2017.
MONTGOMERY, D. C. Design, and analysis of experiments. 7 ed. New York: John Wiley & Sons, 2009.

MORA-HUERTAS, C. E.; FESSI, H.; ELAISSARI. Influence of process and formulation parameters on the formation of submicron particles by solvent displacement and emulsification–diffusion methods Critical comparison. **Advances in Colloid and Interface Science**, v. 163, p. 90-122, 2010.

MOTA-FERREIRA, L. *et al.* Pomegranate Seed Oil Nanoemulsions with Selective Antiglioma Activity: Optimization and Evaluation of Cytotoxicity, Genotoxicity and Oxidative Effects on Mononuclear Cells. **Pharmaceutical Biology**, v. 54, n. 12, p. 2968–2977, 2016.

MOZZON, M.; FOLIGNI, R.; TYLEWICZ, U. Chemical Characteristics and Nutritional Properties of Hybrid Palm Oils. *In*: WAISUNDARA, V. **Palm Oils, Intechopen**, cap. 9, p. 149-170, 2018.

NAGAVARMA, B. V. N. *et al.* Different Techniques for Preparation Of Polymeric Nanoparticles- A Review. **Asian Journal of Pharmaceutical and Clinical Research**, v. 5, Suppl 3, p. 16-23, 2012.

OJEDA, M. *et al.* Hybrid palm oil (*Elaeis oleifera* × *Elaeis guineensis*) supplementation improves plasma antioxidant capacity in humans. **European Journal of Lipid Science and Technology,** v. 119, 2016.

OLIVEIRA, C. F.F. *et al.* Combined Effect of High-Pressure and Conventional Heating on Pectin Extraction from Passion Fruit Peel. **Food and Bioprocess Technology**, v.9, n.6, p.1021-1030, 2016.

OLIVEIRA, T. S. *et al.* A Patent Data Analysis in Nanotechnology Applied to Essential Oils. **Recent Patents on Nanotechnology**, v. *16*, n. 2, p. 92–106, 2022.

PATHAK, N. *et al.* Valorization of jackfruit waste into value added products and their potential applications. **Frontiers in Nutrition**. v. 9:1061098, 2022.

PINTO, S. S. *et al.* Produção e Composição de Cachos e Incidência do Anel Vermelho em Híbridos Interespecíficos de Caiaué com Dendezeiro no Sul da Bahia. **Agrotrópica**, v. 31, n. 1, p. 5 -16, 2019.

POJIĆ, M., MIŠAN, A. & TIWARI, B. Eco-innovative technologies for extraction of proteins for human consumption from renewable protein sources of plant origin. **Trends in Food Science & Technology**, v. 75, p. 93-104, 2018.

PRAKASH, A.; REVATHY, B.; NITHYANAND, P.; VELLINGIRI, V. Essential oil based nanoemulsions to improve the microbial quality of minimally processed fruits and vegetables: A review. **Food Res. Int**. 111: 509–523, 2018.

RAEISI, S., OJAGH, S. M., QUEK, S. Y., POURASHOURI, P., & SALAÜN, F. Nano-encapsulation of fish oil and garlic essential oil by a novel composition of wall material: Persian gum-chitosan, **LWT**, 108494, 2019.

RAO, J.; McCLEMENTS, D. J. Lemon oil solubilization in mixed surfactant solutions: Rationalizing microemulsion & nanoemulsin formation. **Food Hydrocolloids**, v. 26, p. 268-276, 2012.

REIS, L. *et al.* Evaluation of Cytotoxicity of Nanolipid Carriers with Structured Buriti Oil in the Caco-2 and HepG2 Cell Lines. **Bioprocess Biosystems. Engineering**, v. 43, n. 6, p. 1105–1118, 2020.

RICARDINO, I. E. F; SOUZA, M. N. C; NETO, I. F. S. Vantagens e possibilidades do reaproveitamento de resíduos agroindustriais. **Alimentos: Ciência, Tecnologia e Meio Ambiente**, v. 1, n. 8, 2020.

RICAURTE, L. *et al.* Physical, Thermal and Thermodynamical Study of High Oleic Palm Oil Nanoemulsions. **Food Chemistry**, V. 256, P. 62-70, 2018.

RICAURTE, L. *et al.* Production on High-Oleic Palm Oil Nanoemulsions by High-Shear Homogenization (Microfluidization). Innovative **Food Science and Emerging Technologies**. v.35, p. 75-85, 2016.

RICAURTE, L. *et al.* Edible gelatin-based nanofibres loaded with oil encapsulating high-oleic palm oil emulsions. **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, v. 595, 124673, 2020.

RICAURTE, L. *et al.* Compound distribution, structural analysis and nanomechanical properties of nanofibers loaded with high-oleic palm oil nanoemulsions for packaging application. **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, v. 636, 2022.

RITCHIE, H.; ROSER, M. Palm Oil. **Our World in Data**. Palm Oil - Our World in Data, 2020

RODRÍGUEZ, J. C. *et al.* Effects of the Fruit Ripening Stage on Antioxidant Capacity, Total Phenolics, and Polyphenolic Composition of Crude Palm Oil from Interspecific Hybrid *Elaeis oleifera* × *Elaeis guineensis*. **Journal of Agricultural and Food Chemistry**, v. 64, n. 4, p. 852–859, 2016.

ROGERO, S. O. *et al.* Teste in vitro de citotoxicidade: Estudo comparativo entre duas metodologias. **Materials Research**, v. 6, n. 3, p. 317 – 320, 2003.

ROMERO, H. M. *et al.* High-Oleic Palm Oil (HOPO) Production from Parthenocarpic. **Agronomy**, n. 11, v. 290, p. 1–18, 2021

Roundtable on Sustainable Palm Oil. RSPO smallholders. Get involved as a smallholder - Roundtable on Sustainable Palm Oil (RSPO), 2022. Disponível em: https://rspo.org/as-a-smallholder/. Acesso em: 11 de dezembro de 2022.

SALES, I. S. *et al.* Edible and essential oils nanoparticles in food: a review on the production, characterization, application, stability, and market scenario. **Critical Reviews in Food Science and Nutrition**, v. 6, p. 1-28, 2023.

SAMBANTHAMURTHI, R.; SUNDRAM, K.; TAN, Y. Chemistry, and biochemistry of palm oil. **Progress in Lipid Research**, v. 39, p. 507-558, 2000.

SANGUANSRI, P.; AUGUSTIN, M. A. Nanoscale materials development e a food industry perspective. **Trends in Food Science & Technology**, v. 17, p. 547-556, 2006.

SANTOS, S. S. *et al.* Formulation and in vitro evaluation of coconut oil-core cationic nanocapsules intended for vaginal delivery of clotrimazole. **Colloids and Surfaces B: Biointerfaces**. v. 116, p. 270-276, 2014.

SARAIVA, B. R., VITAL, A. C. P., ANJO, F. A., CESARO, E. & MATUMOTO-PINTRO, P. T. Valorização de resíduos agroindustriais: fontes de nutrientes e compostos bioativos para a alimentação humana. **Pubsaúde**, v. 1, p. 7, 2018.

SCHAFFAZICK, S. R.; GUTERRES, S. S.; FREITAS, L. L.; POHLMANN, A. R. Caracterização e estabilidade físico-química de sistemas poliméricos nanoparticulados para administração de fármacos. **Química Nova**. v. 26, n. 5, p. 726-737, 2003.

SCHAPPO, F. B. *et al.* The Toxicity of Oil Nanoparticles: A Review Focused on Food Science. **Food Reviews International**, v. 39, n. 1, p. 1-17, 2021.

SERGENT, T. *et al.* Differential modulation of ochratoxin A absorption across Caco-2 cells by dietary polyphenols, used at realistic intestinal concentrations. **Toxicology Letters**, 159,60–70, 2005.

SILVA, V. *et al.* Technological Prospection of Oil Nanoparticles: Primary Characteristics and Profiles. **Recent Patents on Nanotechnology**, v. 15, 2-14, 2021.

SINGH, T. *et al.* Application on nanotechnology in food science: perception and overview. **Frontiers in Microbiology**, v. 8, p. 1–7, 2017.

SONG, W. *et al.* Cellular antioxidant activity of common vegetables. **Journal of Agricultural and Food Chemistry**, 58, 6621–6629, 2010.

SRIDHAR, K.; INBARAJ, S.; CHEN, B-H. Recent Advances on Nanoparticle Based Strategies for Improving Carotenoid Stability and Biological Activity. **Antioxidants**, v. 10, p. 713, 2021.

SUZIHAQUE, M. U. H., *et al.* Jackfruit seed as an alternative replacement for starch flour. **Materials Today: Proceedings**, v. *63*, p. S451–S455, 2022.

TEMELLI, F. Perspectives on the use of supercritical particle formation technologies for food ingredients**. Journal Supercritical Fluids**, v. 134, p. 244–251, 2018.

UPADHYAY, N. *et al.* Assessment of nanoencapsulated *Cananga odorata* essential oil in chitosan nanopolymer as a green approach to boost the antifungal, antioxidant and in situ efficacy. **International Journal of Biological Macromolecules**, *171*, 480–490, 2021.

VOORA, V.; BERMÚDEZ, S.; FARRELL, J. J.; LARREA, C.; LUNA, E. Global Market Report. Palm oil prices and sustainability. **International Institute for Sustainable Development**, 2023.

XAVIER-JUNIOR *et al*. Experimental design approach applied to the development of chitosan coated poly(isobutylcyanoacrylate) nanocapsules encapsulating copaiba oil. **Colloids and Surfaces A: Physicochem. Eng. Aspects**, v. 536, p. 251–258, 2018.

XU, Y. *et al.* Effects of chain length and saturation of triglycerides on cellular antioxidant activity of vegetable oil emulsions. **LWT - Food Science and Technology**, v. 146, p. 111437, 2021.

WAGHMARE, R. *et al.* Jackfruit seed: an accompaniment to functional foods. **Brazilian Jounal Food Technology,** Campinas, v. 22, e2018207, 2019.

WALIA, N.; DASGUPTA, N.; RANJAN, S.; CHEN, L.; RAMALINGAM, C. Fish oil-based vitamin D nanoencapsulation by ultrasonication and bioaccessibility analysis in simulated gastro-intestinal tract. **Ultrason Sonochem**, v. 39: p. 623–635, 2017.

WAN, H. *et al.* A Caco-2 cell-based quantitative antioxidant activity assay for antioxidants. **Food Chemistry**, v. 175, p. 601–608, 2015.

WEISSMAN, S. A.; ANDERSON, N. G. Design of Experiments (DoE) and Process Optimization. A Review of Recent Publications. **Organic Process Research & Development**, v.19, p. 1605-1633, 2014.

WOLFE, K. L.; LIU, R. H. Cellular antioxidant activity (CAA) assay for assessing antioxidants, foods, and dietary supplements. **Journal of Agricultural and Food Chemistry**, v. 55, p. 8896–8907, 2007.

WOLFE, K. L.; LIU, R. H. Structure−activity relationships of flavonoids in the cellular antioxidant activity assay. **Journal of Agricultural and Food Chemistry**, v. 56, p. 8404–8411, 2008.

World Wildlife Fund (WWF). 8 things to know about palm oil, 2022. Disponível em: https://www.wwf.org.uk/updates/8things-know-about-palm-oil. Acesso em: 11 de dezembro de 2022.

WU, G. *et al.* The bioactive compounds and cellular antioxidant activity of Herbaceous peony (*Paeonia lactiflora Pall*) seed oil from China. **Journal of Food Science**, v. 85, p. 11, 2020.

YADA, R. Y. *et al.* Engineered nanoscale food ingredients: Evaluation of current knowledge on material characteristics relevant to uptake from the gastrointestinal tract. **Comprehensive Reviews in Food Science and Food Safety**, v. 13, p. 730-744, 2014.

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

ZHENG, L. *et al.* Antioxidant Activity Evaluation of Tocored through Chemical Assays, Evaluation in Stripped Corn Oil, and CAA Assay. **European Journal of Lipid Science and Technology**. v. 122, p. 1900354, 2020.

Capítulo II

Manuscrito: Optimization and characterization of hybrid crude palm oil Unaué HIE OxG nanoparticles with vegetable by-products as encapsulant

Optimization and characterization of hybrid crude palm oil Unaué HIE OxG nanoparticles with vegetable by-products as encapsulants

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Abstract: Hybrid crude palm oil (HCPO) HIE OxG is prominent for its fatty acid and antioxidant composition (carotenoids, tocopherols, and tocotrienols), lower production cost, and high pest resistance compared to crude palm oil. Biodegradable and sustainable encapsulants derived from vegetable byproducts were used to formulate HCPO nanoparticles. Nanoparticles with hybrid crude palm oil and jackfruit seed flour as the wall material (N-JSF) and with hybrid crude palm oil and jackfruit axis flour as the wall material (N-JAF) were optimized using a 22 experimental design. They exhibited nanoscale diameters (<250 nm) and were characterized based on their zeta potential, apparent viscosity, pH, color, and total carotenoid content. The nanoparticles demonstrated monodisperse distribution, good uniformity, and stability (polydispersity index <0.25; zeta potential N-JSF -19.50 ± 1.47mV and N-JAF -12.50 ± 0.17mV), high encapsulation efficiency (%) (N-JSF 86.44 ± 0.01 and N-JAF 90.43±1.34), and optimal carotenoid retention (>85%). These nanoparticles show potential for use as sustainable and clean-label HCPO alternatives in the food industry.

Keywords: Elaeis guineensis; Elaeis oleifera; Jackfruit seed; Jackfruit central axis; Experimental design; Nanotechnology.

1. Introduction

Crude palm oil (CPO) is extracted from the mesocarp of the fruits of palm species Elaeis guineensis and is dominant in oil production globally. CPO production reached 74.7 million tons from cultivation of 29 million hectares of oil palm in 2020 and increased by 1.9% (18.45 million tons) in 2022 [1]. According to the United States Department of Agriculture (USDA) [2], the leading producers are Indonesia (44.76 million tons), Malaysia (20.14 million tons), and Thailand (2.69 million tons). Brazil ranks 7th among the major oil-producing countries (576.76 tons) [2].

However, owing to the low resistance of CPO to diseases associated with cultivation practices, hybrid crude palm oil (HCPO) has been cultivated. HCPO is obtained from crossbreeding of the American and African oil palms (Elaeis oleifera and Elaeis guineensis, respectively). HCPO contains a higher percentage of unsaturated fatty acids than CPO, along with a longer shelf life and the plant has a better pests resistance. The primary producers of this hybrid form are Colombia (12% of the total cultivation area for this oil), Ecuador, and Costa Rica [3].

An exclusive Brazilian variety of interspecific hybrid (HIE OxG), named Unaué, derived from crossbred African oil palm (Elaeis guineensis) and American Caiaué (Elaeis oleifera), and is gaining prominence. This enhances the sustainability of regional oil palm cultivation, particularly for small-scale producers. Notably, Unaué surpasses hybrids from other

countries in oil productivity, cost efficiency, and pests and diseases resistance, particularly the lethal red ring diseases [4].

In the Pará region of Brazil, cultivated area dedicated to HIE OxG palm oil production is estimated at approximately 11,500 ha, with potential production of over 40,000 tons of oil annually [5, 6]. Additionally, approximately 30,000 ha of palm oil plantations aimed at producing hybrid palm oil is situated in the lower southern region of Bahia, Brazil [7].

HCPO has been explored in scientific literature for its antioxidant potential, making it a functional oil for disease prevention and health promotion [8,4,9]. Additionally, its prominence is due to its nutritional composition, with high amounts of vitamin E, carotenoids, and unsaturated fatty acids and lower levels of saturated fatty acids [7,10,11]. According to CODEX (2023) [12], this oil contains at least 48% oleic acid and is recognized as "palm oil of higher oleic acid content".

This oil has a high carotenoid level, ranging from 500 to 10,000 μg/g, primarily composed of β-carotene (52–60%) and α-carotene (33–36%) [13]. Tocopherols and tocotrienols content of the oil vary from 562 to 1417 μg/g, with γ-tocotrienol as the being the predominant component (406 to 887 μg/g) [12]. Despite the potential of the oil as a natural additive owing to its fatty acid and antioxidant (carotenoid, tocopherol, and tocotrienol) composition, the susceptibility of the bioactive compounds to degradation during processing and storage poses a functional loss risk [14].

Nanoencapsulation can increase the stability of bioactive compounds and control their release. In this process, small particles of core materials are enclosed within a nanometer-scale wall material (WM) (encapsulant) (smaller than 1 μm). The selection of the nanoencapsulation method depends on the specific oil being encapsulated and the material used as the encapsulant [15,16]. Additionally, studies have been conducted to develop nanoemulsions containing HCPO for use in packaging, and evaluated their physical, thermal, and thermodynamic properties of nanoemulsions evaluated [17,18,19,20,21].

Furthermore, considering the choice of WM, choice of solvent, encapsulant, and emulsifier is crucial in aiding the formation of stable nanoparticles. In this context, the development, characterization, and optimization of HCPO nanoparticles and the use of different encapsulants have been explored [21]. The type and concentration of the encapsulant and oil were the main independent variables studied, and particle size, polydispersity index, and zeta potential were the main response variables analyzed [20,22].

Additionally, there is an increasing interest in biopolymers and vegetable by-product flours with biodegradable features for encapsulating bioactive compounds and oils [23,24,25].

Approximately 40–50% gross weight from fruit and vegetable processing is deemed as waste. Despite being rich in vitamins, minerals, nutrients, and fibers, these by-products are undervalued and typically discarded [26].

Passion fruit albedo (Passiflora edulis Sims f. Flavicarpa) flour contains approximately 20% pectin, which is known for its effective binding, gelling, and stabilizing capacities, as well as having levels of soluble fiber and phenolic compounds [27]. Cowpea shell (Vigna unguiculata) flour has high protein and fiber and low lipid content [28], and jackfruit (Artocarpus heterophyllus) seed flour (JSF) provides plentiful starch (63%) and protein (8%), in addition to high content of soluble and insoluble fibers, minerals, vitamins, and bioactive compounds (flavonoids) [29,30]. Phenolic compounds and flavonoids have been identified in jackfruit axes flour (JAF), demonstrating their antioxidant potential [31].

Therefore, this study aimed to evaluate the most favorable conditions for the nanoencapsulation of HCPO HIE OxG with vegetable byproducts as encapsulants. This would facilitate the use of this oil as a sustainable alternative in the food industry.

2. Materials and Methods

2.1 Materials

The HIE OxG hybrid fruit was developed by Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) Western Amazon. The HCPO, known as Unaué, was formulated, and provided by the Comissão Executiva do Plano da Lavoura Cacaueira (CEPLAC) in the city of Una-BA. They were stored at -20°C in an amber bottle until the time of analysis. The cowpea shells (Vigna unguiculata) used in the preliminary tests were purchased from Sabor baiano®. Jackfruit (Artocarpus heterophyllus) and yellow passionfruit (Passiflora edulis Sims f. Flavicarpa) were obtained from local markets in Salvador, Bahia, Brazil. Tween 20, Tween 80 and grain alcohol were purchased from Shynth (São Paulo, Brazil).

2.2 Methods

2.2.1 Preparation of encapsulants

Passion fruit albedo flour, used in the preliminary tests for nanoparticle formulation, was prepared according to Oliveira et al. [32], with modifications. Passion fruits were peeled and washed in running water, and the albedo (mesocarp) was cut into pieces of approximately

1 cm. Subsequently, they were dried in a forced-air oven at 50°C for 8 hours and processed in a knife mill with a 35-mesh sieve. The sample was refrigerated at 4°C until the time of analysis.

To obtain the cowpea shell flour used in preliminary tests, the shells were thawed and placed in a dehydrator (PE 14 Junior Analogical, Pardal, Petrópolis, Brazil) at 40°C for a period of 72 hours. After drying, the shells were passed through a knife mill (Pulverisette 15, Fritsch, Markt Einersheim, Germany) with a 60-mesh sieve to obtain the flour.

To prepare JSF and JAF, the fruit was used at the green-ripening stage. The seed and central axis were separated individually from the fruit, followed by blanching (2 min in boiling water at 100°C, then cooled for 3 minutes in cold water) and freezing (-10°C) in a vacuum-sealed package until the day of flour preparation. After thawing under refrigeration (4°C), the seeds and central axis were dehydrated in a forced-air circulation dehydrator (Q317M-32, Quimis, Brazil) at 1.25 m/s² at 50°C for 36 hours. At the end of each drying process, the dehydrated samples were ground in a knife mill (Pulverisette 15, Fritsch, Markt Einersheim, Germany), with a mesh opening of 0.5 mm, and vacuum-packed. The samples were refrigerated at 4°C until the time of analysis.

2.2.2 Preliminary tests for the formulation of hybrid crude palm oil nanoparticles (N-HCPO)

Preliminary tests were conducted to assess the effects of different techniques, encapsulants, and solvent quantities on N-HCPO production. Various parameters were analyzed, including (a) type of WM (passion fruit albedo flour, cowpea shell flour, JSF, JAF), (b) solvent volume (50 mL, 75 mL, 100 mL), and (c) different techniques (nanoprecipitation and homogenization) (Figure 1).

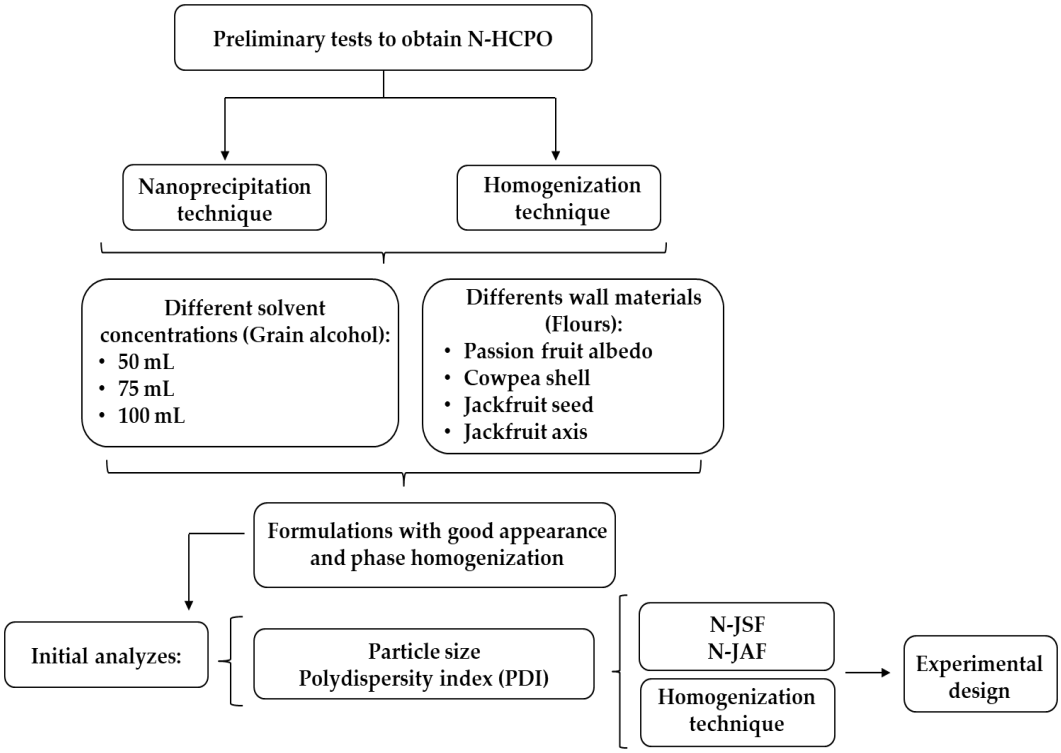

Figure 1. Flowchart of preliminary tests for obtaining N-HCPO.
N-HCPO = hybrid crude palm oil nanoparticles; N-JSF = nanoparticles with jackfruit seed flour as the encapsulant; N-JAF = nanoparticles with jackfruit axis flour as the encapsulant.

The homogenization and nanoprecipitation methods were as given below:

The N-HCPO were prepared using the homogenization method of Ferreira-Ribeiro et al. [33], with some modifications. Tween 20 (250 µL) and HCPO (250 mg) were dissolved in ethyl alcohol (different concentrations of the solvent were tested) under agitation (Ika, RH Basic 2, Brazil) for 15 min. This organic phase was then added dropwise to 100 mL of an aqueous solution containing 500 mg of WM (different flours containing vegetable by-products were tested, which were used individually and not combined) during homogenization on a helical agitator (IKA@, model RW 20 digital, Diagtech, Brazil) at a speed of 900 rpm for 30 minutes. Subsequently, the alcohol was evaporated in a rotary evaporator (35°C) (Büchi RII, DE, USA) until complete solvent evaporation.

Nanoprecipitation and solvent displacement methods were adapted from Granata et al. [16]. The organic phase (OP) was prepared by dissolving 310 mg of HCPO in ethyl alcohol, 35 mg of Tween 20, and 90 mg of WM (different flours containing vegetable byproducts were tested; these WMs were used individually and not combined), which was magnetically stirred for 15 minutes at 1250 rpm (Tecnal TE – 085, São Paulo, Brazil). The OP was then filtered (0.22 µm membrane) and added dropwise to the aqueous phase (AP) containing 50 ml of

distilled water and 75 mg of Tween 80, while being kept under magnetic stirring (500 rpm; 10 min) (Tecnal TE – 085, São Paulo, Brazil). Subsequently, the alcohol was evaporated using a rotary evaporator (35°C) (Tecnal TE - 211, São Paulo, Brazil).

Formulations that exhibited good appearance and phase homogenization were analyzed for particle size and PDI to identify the nanoparticles with the smallest average diameter and monodisperse distribution (Table 1, Supplementary Material). Thus, formulations with jackfruit seed flour (N-JSF) and jackfruit axis flour (N-JAF) as WMs prepared using the homogenization technique (Figure 1 and 2) were selected and used in the experimental design stage.

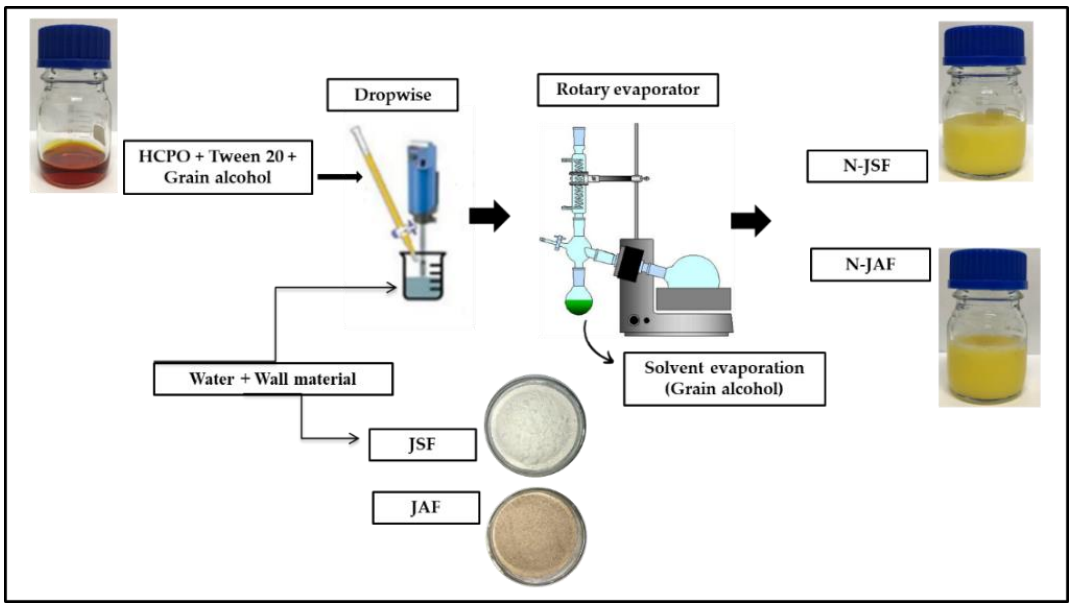

Figure 2. Formulation of N-JSF and N-JAF using the Homogenization Method.
OPBH = hybrid crude palm oil; JSF = jackfruit seed flour; JAF = jackfruit axis flour; N-JSF = nanoparticles with jackfruit seed flour as encapsulant; N-JAF = nanoparticles with jackfruit axis flour as encapsulant.

2.2.3 Optimization of the procedure to obtain N-OPBH by 2^2 factorial design

Two central composite rotatable designs (CCRD) were used; one with JSF as the WM, and the other with JAF, totaling 22 experiments (11 experiments for each design). The experiments were conducted in a randomized manner. Thus, a complete 2^2 factorial experimental design was developed, with three central points and four axial points at a distance $\alpha = \pm 1.412$. The levels (in coded values) were −1, 0, and +1, where 0 corresponded to the central point.

The design was based on the following independent variables: the amount of HCPO (200, 250, and 300 mg) and encapsulant (JSF or JAF) (300, 500, and 700 mg). The amounts of

solvent (ethyl alcohol), distilled water, and emulsifier (Tween 20) were kept constant during the process. Particle size and PDI were used as experimental responses.

The responses of the variables were analyzed using Statistica Software version 7, and the adopted significance level was set at 5%. The chosen levels were based on preliminary tests.

2.2.4 Particle Size, Polydispersity Index (PDI), and Zeta Potential (ζ)

The particle size, PDI, and zeta potential of N-HCPO were measured by dynamic light scattering and phase analysis light scattering (Zetasizer Nano ZS, Malvern Instruments, Malvern, UK) at 25°C. Particle size data were reported as the mean diameter and PDI. Zeta potential values were measured based on electrophoretic mobility [34].

2.2.5 Encapsulation Efficiency (EE)

EE was determined using the ultrafiltration/centrifugation technique described by Froiio et al. [35], with modifications. In an Amicon Ultra 0.5/30 kDa filter (Millipore, Carrigtwohill, Ireland), 500µL of nanoparticles were inserted and then centrifuged at 14,000xg for 30 min (Labnet Spectrafuge 24D, US). The sediment obtained after separating the supernatant was diluted in 500µL of acetone to determine the HCPO content, and this procedure was repeated twice. EE was directly determined using a calibration curve of HCPO in acetone (λmax=448 nm; Abs = 0.2735x concentration + 0.014, R^2=0.9908) (Equation 1):

$$EE = \left(\frac{M}{Mo}\right) \times 100 \quad (1)$$

where M (mg) is the amount of HCPO loaded into the nanoparticles (determined from the calibration curve), and Mo (mg) is the initial amount of HCPO added to the organic phase for the nanoparticle formulation.

2.2.6 Transmission electron microscopy (TEM)

The morphology of N-HCPO was determined by Transmission Electron Microscopy (TEM). A drop of the nanoemulsion was placed on a grid (Formvar carbon support film, 200 mesh) for 1 min. Subsequently, a drop of a 1% phosphotungstic acid solution was applied for 30 s. The grid was then examined under a transmission electron microscope (TEM; JEOL 1230, Tokyo, Japan) operating at 80 kV with an average magnification of 80,000 × and a scale of 100 nm [36].

2.2.7 Apparent viscosity, pH, color parameters and total carotenoids (TC)

The apparent viscosity of free oil and N-HCPO was measured using a concentric cylinder rheometer (Haake Rheotest model 2.1, Medingen, Germany), coupled with a wash bath for temperature control (at 25°C) and a shear rate of 25–1000 sec-1. The rheological data were fitted to the Ostwald–de Waele model (Equation 2) [37].

$$\mu = K\gamma\ (n - 1) \tag{2}$$

where μ is the apparent viscosity, K is the consistency index, γ is the shear rate, and n is the flow behavior index. The results were expressed as centipoises (cP).

A commercial pH meter (Sanxin, PHS-3D pH meter, Shanghai, China) was used to determine the nanoparticles pH at 25°C, without prior sample dilution and after instrument calibration [33].

The colors of free oil and N-HCPO were determined using a colorimeter CR-400 (Minolta, Osaka, Japan). The data are presented in CIELab Coordinates, which define color in a three-dimensional space with color values [L* (lightness), a* (red/green), and b* (yellow/blue)] [22].

The extraction of oil from the nanoparticles for the determination of total carotenoid content was carried out according to Ferreira-Ribeiro et al. [33], with modifications. For this purpose, the N-OPBH was centrifuged with iso-octane and isopropyl alcohol (2:2:1) (SOLAB, SL-706, Piracicaba, Brazil) at 3,500 rpm/25°C /10min/5 times. The obtained supernatants, after consolidation, were filtered through qualitative filter paper 150mm, added to anhydrous sodium sulfate, and subjected to rotary evaporation of solvents at 35°C (Büchi RII, New Castle, DE, USA), followed by drying under a stream of nitrogen. The mass of HCPO obtained after extraction was weighed and subsequently diluted in petroleum ether, and the carotenoid content was determined by UV-Vis spectrophotometry, quantifying β-carotene (λmax=450 nm; A1%1 cm =2592), according to Equation 3 [34]:

$$Total\ carotenoids\ content\ \left(\frac{\mu g}{g}\right) = A \times \frac{V(mL) \times 10^4}{A_{1cm}^{1\%}\, xP(g)} \tag{3}$$

where A is the absorbance, V is the total extract volume, P is the sample weight, and A1%1 cm = 2592 (absorption coefficient of β-carotene in petroleum ether).

2.2.8 Statistical analysis

The analyses were conducted in triplicates (± standard deviation). The means were assessed using analysis of variance (ANOVA) and compared through the Tukey's test ($p \leq 0.05$) and Student's t-test ($p \leq 0.05$) with the software SAS® OnDemand for Academics. The results from the experimental design were subjected to statistical tests using Statistica Software (Statsoft, Statistica 7.0, Tulsa, USA).

3. Results and Discussion

3.1 Optimization of the procedure to obtain N-JSF and N-JAF by 2^2 factorial design

Consensus in the literature regarding factors that predominantly influence the average diameter and uniformity of particle size is lacking. Techniques, such as nanoprecipitation and homogenization, allow the use of different encapsulants, surfactants, and varied concentration of the organic phase, some of which may interfere with nanoparticles characteristics [38,39].

In this context, preliminary tests were conducted to assess the influence of various parameters (different techniques, encapsulants, and solvent volumes) on obtaining hybrid crude palm oil nanoparticles (N-HCPO). Formulations with good appearance and phase homogenization were analyzed for particle size and polydispersity index (PDI). According to the results (Table S1, Supplementary Material), N-JSF and N-JAF prepared using the homogenization technique showed smaller particle sizes ($P < 0.05$) than those prepared using the nanoprecipitation technique. According to Fereira-Ribeiro et al. [33], particle size affects the physicochemical properties of the material, compound kinetic release, and biodistribution. Therefore, smaller particles tend to better interact with compounds of interest in future applications.

According to Ricaurte et al. [18], the smaller the PDI, the lower the tendency for particle aggregation, confirming the quality of the nanoencapsulation method. PDI < 0.25 indicates that the nanoemulsions had a narrow and monodisperse distribution with good uniformity in nanoparticle diameter [18, 33]. Only the formulation with passion fruit albedo as the WM, prepared using the homogenization technique, showed a high PDI. Nanoparticles prepared by homogenization with cowpea shell flour, JSF, or JAF showed PDI ≤ 0.25, with no statistical difference between the samples ($p > 0.05$) (Table S1, Supplementary Material).

With regard the formulations, using the nanoprecipitation technique, only nanoparticles with JSF and central axis flour as encapsulants showed PDI ≤ 0.25. However, these were not used in the experimental design because of their larger particle size compared to those of the homogenization technique ($p < 0.05$). Thus, N-JSF and N-JAF, prepared using the

homogenization technique, were selected for the experimental design stage, considering that in addition to presenting smaller diameters among all analyzed formulations, the nanoemulsions had a monodisperse distribution and good uniformity (Table 1, Supplementary Material).

Table 1 shows particle size and PDI results for N-HCPO with different concentrations of HCPO and WM evaluated using a 2^2 factorial design.

Table 1. 2^2 factorial design for N-HCPO with jackfruit seed flour or jackfruit axis flour as wall materials, and results obtained from response parameters particle size (nm) and polydispersity index (PDI) responses.

Formulations	Independent variables		Response parameters		Response parameters	
	X1 (HCPO)	X2 (WM)	Size (nm)*	PDI*	Size (nm)**	PDI**
1	-1	-1	209.33 ± 2.85	0.10 ± 0.01	250.76 ± 3.27	0.092 ±0.00
2	-1	1	204.76 ± 2.98	0.13 ± 0.00	224.80 ± 1.04	0.087 ± 0.00
3	1	-1	234.20 ± 2.50	0.13 ± 0.00	283.70 ± 4.92	0.126 ± 0.02
4	1	1	241.00 ± 1.49	0.15 ± 0.03	308.20 ± 5.14	0.144 ± 0.01
5	-1.41	0	196.90 ± 4.43	0.14 ± 0.01	248.43 ± 1.55	0.108 ± 0.01
6	1.41	0	206.50 ± 2.10	0.13 ± 0.02	317.03 ± 2.61	0.128 ± 0.00
7	0	-1.41	194.46 ± 0.72	0.07 ± 0.01	212.03 ± 1.25	0.098 ± 0.00
8	0	1.41	218.13 ± 2.01	0.12 ± 0.01	230.03 ± 2.37	0.113 ± 0.02
9 (CP)	0	0	264.56 ± 1.12	0.12 ± 0.02	266.50 ± 1.25	0.116 ± 0.01
10 (CP)	0	0	264.40 ± 3.05	0.12 ± 0.02	266.00 ± 5.57	0.117 ± 0.00
11 (CP)	0	0	264.73 ± 4.52	0.12 ± 0.01	265.70 ± 2.33	0.118 ± 0.00

*WM=JSF; **WM=JAF
CP = central point
HCPO = Hybrid crude palm oil; WM = Wall material
HCPO: −1=200 mg; +1=300 mg; 0 = 250 mg; -1.41=179.28 mg; 1,41=320.71. WM: −1=300 mg; +1=700 mg; 0= 500 mg; -1,41=217.15 mg; 1,41=782.84 mg. The results were expressed as the average of triplicate measurements.

According to the Pareto diagrams (Figure S1a and S1b, Supplementary Material) for both experimental designs, the lower the amount of oil and encapsulant added to the formulation, the smaller the particle size. This trend was also evident when analyzing the response surface graphs for variable amounts of oil and encapsulant on N-JSF (Figure 3a) and N-JAF (Figure 3c) particle sizes. One of the most critical factors affecting the dynamic stability of emulsions is particle size, which influences coalescence. Smaller particles tend to exhibit higher stability, thereby reducing the possibility of coalescence [33].

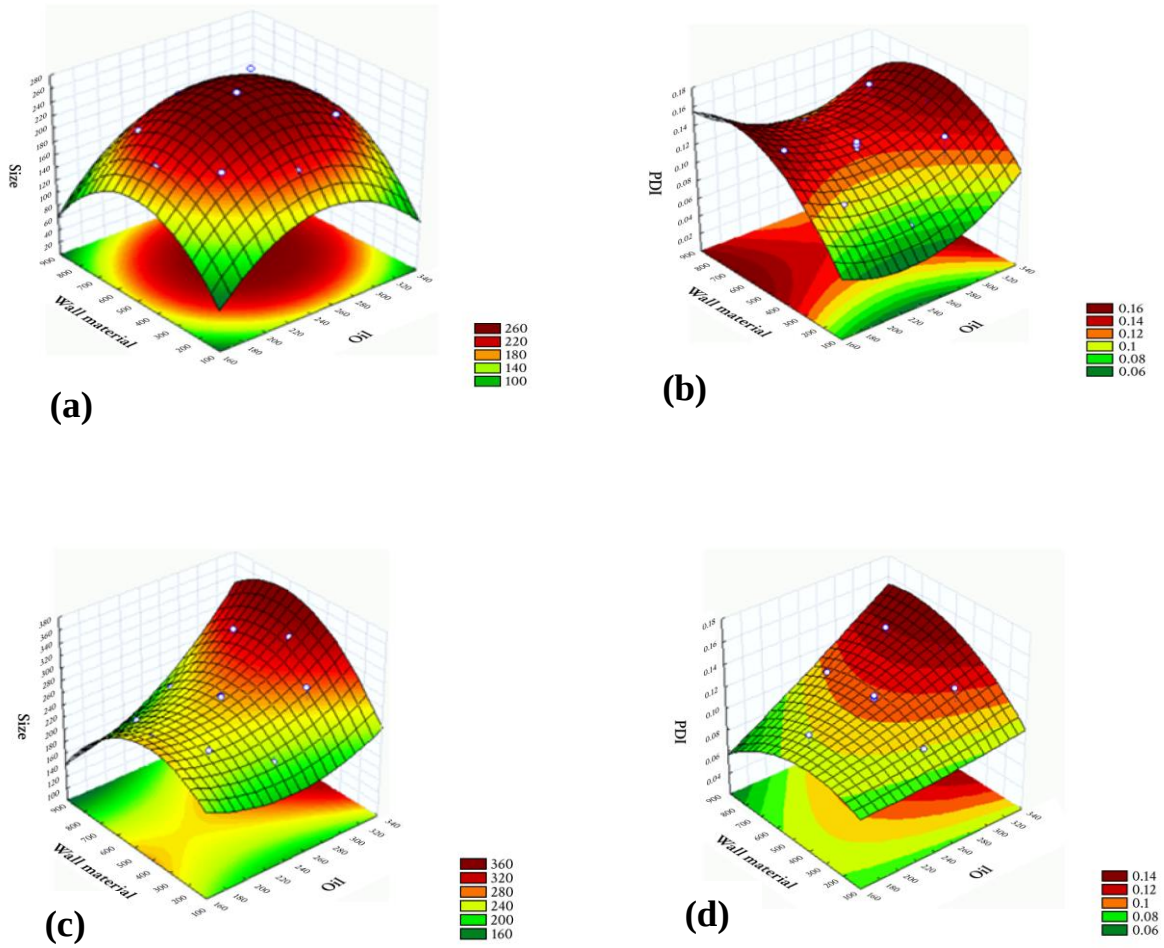

Figure 3. Response surface for the variables amount of oil and wall material on particle size (a) and PDI (b) for N-JSF, and on particle size (c) and PDI (d) for N-JAF.

In addition, the interaction between WM and oil in the nanoparticle formulation was significant and positive, as indicated by the Pareto plots (Figure S1a and S1b, Supplementary Material). An increase in the concentration of these variables resulted in larger nanoparticle sizes. Despite this positive interaction, its impact on particle size was smaller than the individual effects of the variables (oil and WM). According to Table 1, formulation 7, which had the lowest amount of WM, had the smallest particle size in both experimental designs. This characteristic could be advantageous for the future applicability of nanoparticles, as this formulation has a higher ratio of HCPO:WM (mass ratio of 0.8:1; JSF or JAF: HCPO), providing a better potential for greater retention of bioactive compounds.

It is important to note that the amount of oil added to the formulation had a significant and positive effect on particle size (Figure S1a and S1b, Supplementary Material), similar to WM. This trend was also observed when analyzing the response surface for the variable

amounts of oil and WM on particle size for N-JSF (Figure 3a) and N-JAF (Figure 3c). As the concentration of oil added to the formulation increased, particle size tended to increase. Formulations 3, 4 (N-JSF and N-JAF), and 6 (N-JAF), which contained higher oil concentrations, also had larger particle diameters (Table 1).

Ricaurte et al. [20] observed similar results when studying the physical characteristics and thermal and thermodynamic stability of high-oleic palm oil nanoemulsions. The oil concentration was the variable that most affected the response to the analyzed parameters, and higher concentrations of oil led to larger particle sizes. These authors reported that higher oil concentrations resulted in a greater number of dispersed droplets in the aqueous phase, forming macromolecular layers with the encapsulant covered by hydrophilic residues. This process causes the formation of disulfide bonds and thiol/disulphide exchange reactions, leading to the collision of oil droplets and consequently increasing the particle size.

Ricaurte, Perea-Flores, Martinez, & Quintanilla-Carvajal [22], nanoencapsulated high oleic palm oil (HOPO) by using high-shear homogenization (microfluidization), with whey protein as the WM and Tween 20 as the emulsifier. In this study, the oil concentration directly influenced the particle size, and formulations with higher oil concentrations led to larger particle.

Regarding the PDI, according to the Pareto charts (Figure S2a and S2b, Supplementary Material), it was noted that the lower the amount of oil and WM added, the lower the PDI; a similar trend was also observed for particle size. In Figure 3, this trend is also evident when analyzing the response surface for the amount of oil and the encapsulant in the PDI for N-JSF (Figure 3b) and N-JAF (Figure 3d). PDI is a measure that reflects the range of particle size distributions [22]. Values close to 0 indicate that the sample is monodisperse with good uniformity in the nanoparticle diameter, whereas values closer to 1 indicate that the sample has a wide range of nanoparticle sizes [40].

According to Hernández-Carrión, Moyano, & Quintanilla-Carvajal [21], the distribution of particles in an emulsion is directly affected by the coalescence and aggregation phenomena of polymers and other constituents present in the WM, influencing not only the particle size but also the homogeneity with which particles of different sizes being distributed in the aqueous phase. Floury et al. [41] reported that the α and β bonds of secondary structures and the break in tertiary and quaternary structures of protein during the nanoencapsulation process can lead to the aggregation of their structures and consequently higher PDI values. The flours used as WM in this study contained protein and other constituents, such as starch

(polysaccharide) in their composition, which may explain the greater tendency for aggregation and, consequently, higher PDI in formulations with higher WM.

Therefore, considering that a lower PDI value indicates more uniform nanoparticle diameters and less tendency to aggregate, a formulation with lower concentrations of oil and WM may lead to a reduction in PDI and particle size. In Table 1, formulation 7 (in both experimental designs) with the lowest WM achieved the lowest PDI values.

Similar results were reported by Ricaurte, Perea-Flores, Martinez, & Quintanilla-Carvajal [22] and Ricaurte et al. [20]. These authors studied encapsulated HCPO and evaluated the characteristics of nanoemulsions along with the effects of variables through factorial design. The concentrations of WM and oil were the variables that most affected the PDI, increasing the PDI value as the amount of these variables increased.

Therefore, considering the best results obtained in the experimental design, formulation 7 was selected and characterized for its zeta potential, encapsulation efficiency, morphology, apparent viscosity, pH, color parameters, and total carotenoid content, as discussed below.

3.2 Characterization of N-JSF and N-JAF

3.2.1 Zeta potential (ζ)

According to Ferreira and Nunes [42], zeta potential indicates the electrical potential of the particles and has a significant effect on the stability of the colloidal system. This parameter is strongly influenced by the composition of the nanoparticles and the medium in which they are distributed [43]. Zeta potential values above +30 mV and below -30 mV indicate stable suspensions without particle aggregation [44].

N-JSF presented a zeta potential of -19.50 ± 1.47 mV, and N-JAF -12.50 ± 0.17 mV, indicating good stability of the nanoemulsions and suggesting that repulsive forces were predominant between the droplets in this system [18]. Variations in zeta potential are generally attributed to the chemical characteristics of the WMs [42]. In this study, the negative charges may be related to the presence of carboxyl groups in the composition of the flours used as encapsulants [31,45]. Additionally, the emulsifier used in the preparation of the nanoemulsions, Tween 20, generates a negative charge due to the adsorption of hydroxide ions at the oil-water interfaces and the formation of hydrogen bonds between it and the hydroxide ions [46].

Ricaurte et al. [18] reported a zeta potential of -24.8 ± 0.5 mV in nanofibers of HCPO and gelatin. Passion fruit albedo (fruit byproduct) and commercial pectin were used as encapsulants by Bezerra et al. [24] in the production of nanodispersions of carotenoid extract

from Spirulina. They reported higher zeta potential values compared to the present study (-41.36 ± 1.43 to -43.64 ± 1.83 mV with passion fruit albedo flour; -24.57 ± 0.66 mV to -27.39 ± 0.86 mV with commercial pectin). The highly negative zeta potential of the nanodispersions with passion fruit albedo flour may be due to the greater number of carboxyl groups that were not replaced by methyl groups, generating more negative charges. Additionally, the encapsulated compound was different from that used in the present study (carotenoid extract).

3.2.2 Encapsulation efficiency (EE)

The encapsulation efficiency (EE; the amount of oil in nanoparticles) was 90.43 ± 1.34 and 86.44 ± 0.01% for N-JAF and N-JSF, respectively. EE is related to the stability and protection against oxidation [23]. Additionally, EE can vary according to the formulation components, such as oil, emulsifiers, and WM [23,43]. Values exceeding 80%, as demonstrated in both studied nanoparticles, revealed that the technology employed for the nanoencapsulation process has a high capacity to protect the oil inside the nanoparticle, suggesting a greater potential for oxidative stability. This result is promising to consider the high phytochemical content of HCPO [18].

Sathasivam et al. [47] found an EE of 83–96% in palm oil nanoparticles with carboxymethyl cellulose obtained from sago biomass as the encapsulant. Ferreira-Ribeiro et al. [33] reported EE values of 86.65 ± 1.18–88.13 ± 1.11% in nanoparticles of crude palm oil and fractions, palm olein and palm stearin, with casein or gum Arabic as encapsulants. Ilyasoglu and El (2014) [48] found EE values of 60–80% in fish oil nanoparticles produced by homogenization, and Esfahani et al. [49] reported EE values of 69–98% for different formulations of omega-3 fatty acid nanoparticles with gelatin or gum Arabic using the homogenization technique.

3.2.3 Morphology

The importance of analyzing the morphology of nanoparticles is related to the comprehensive structural characterization of the material, which allows for the direct observation of dispersed particles. Regardless of the WM used, the morphologies determined by transmission electron microscopy (TEM) of N-JSF and N-JAF was similar, exhibiting a spherical, regular shape without cracks or aggregates (Figure 4).

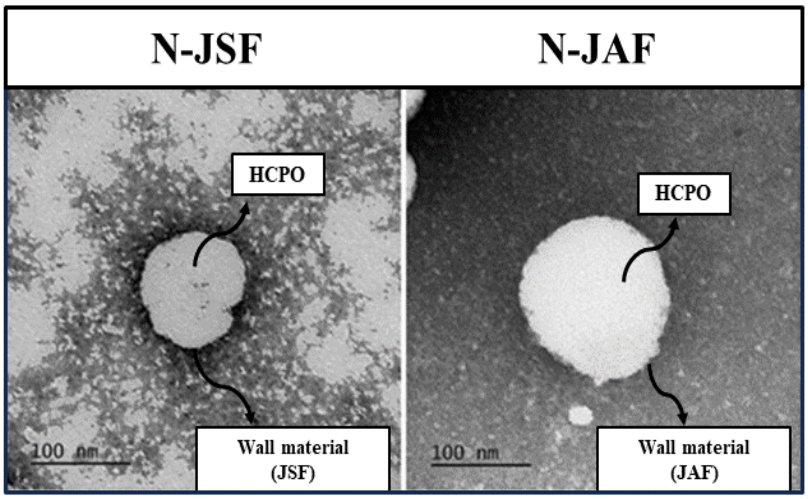

Figure 4. Transmission electron microscopy (TEM) of the nanoparticle containing hybrid palm oil and jackfruit seed flour as wall material (N-JSF) and nanoparticle containing hybrid palm oil and jackfruit axis flour as wall material (N-JAF) (scale = 100nm).

Similar results were observed for nanoparticles of chia seed oil [23], crude palm oil and its fractions [33], hybrid palm oil [50], and shrimp oil [51].

3.2.4 Apparent viscosity, pH, color parameters and total carotenoids

The apparent viscosity, pH, color parameters, and total carotenoids (TC) of the nanoparticles are listed in Table 2.

Table 2. Apparent viscosity, pH, color parameters and total carotenoids (TC) of the nanoparticles.

Samples	Apparent viscosity (cP)	pH	Color parameters			TC (µg/g)
			L*	a*	b*	
Free oil	23.04 ± 0.18 [a]	3.52 ± 0.08 [c]	27.98 ± 0.09 [b]	10.98 ± 0.00 [a]	11.69 ± 0.01 [a]	921.94 ± 28.39 [a]
N-JSF	22.89 ± 2.38 [a]	5.75 ± 0.05 [a]	39.01 ± 3.91 [a]	0.64 ± 0.09 [b]	8.20 ± 2.53 [b]	809.76 ± 41.53 [b]
N-JAF	20.89 ± 0.82 [a]	5.49 ± 0.05[b]	41.98 ± 0.03 [a]	0.55 ± 0.14 [b]	10.40 ± 0.39 [ab]	799.94 ± 45.60 [b]

N-JSF = Nanoparticles of HCPO with jackfruit seed flour as encapsulant. N-JAF = Nanoparticles of HCPO with jackfruit axis flour as encapsulant.
The data are expressed as mean ± standard deviation (n = 3).
Different letters in the same column indicate significant differences ($p < 0.05$).

The mean apparent viscosity of the nanoparticles at 25°C was 21.89 ± 1.20 cP, and no significant difference ($p > 0.05$) was observed among the samples (Table 2). All the analyzed samples exhibited non-Newtonian behavior, indicating that as the shear rate increased, apparent

viscosity decreased. Ferreira-Ribeiro et al. [33] prepared nanoparticles of crude palm oil and fractions, palm olein, and palm stearin using a homogenization technique with casein and gum Arabic as encapsulants. They found apparent viscosity values ranging from 14.58 ± 1.22–27.10 ± 1.41 cP, like that is reported in this study, and the samples also exhibited non-Newtonian behavior.

The rheological characteristics of emulsions can be influenced by the composition and structure of the nanoemulsion (WM type, concentration, and interaction between the dispersed particles and oil concentration) [52]. Jackfruit seeds have a high content of starch and pectin, and the central axis of the jackfruit contains these constituents [53]. Thus, starch and pectin may have influenced the viscosity of the nanoemulsions. Bezerra et al. [24] using both passion fruit albedo-derived pectin and commercial pectin as encapsulants, highlighted that differences in viscosity could be related to various factors, such as the particles hydrodynamic volume and molar mass, and interactions between the WM, solvent, and encapsulated material. Because of its hydrocolloidal characteristics, pectin tends to contribute to the production of more viscous emulsions if it contains esterified carbonyl groups.

Ricaurte et al. [20] encapsulated HOPO by microfluidization with the aim of evaluating the physical, thermal, and thermodynamic stabilities of nanoemulsions. One of the parameters assessed was the apparent viscosity, which ranged between 1.15 and 80.42 cP. The highest apparent viscosity was obtained for the nanoemulsion with high whey protein content as an encapsulant, palm oil, and gelatin. The results showed that the concentrations of these constituents significantly affected the viscosity of the emulsion and hence, its applicability in the food industry.

Ricaurte, Santagapita, Díaz, & Quintanilla-Carvajal [18] observed the same trend, when HOPO was encapsulated using the electrospinning technique. In this study, the diameters, and morphologies of the nanoparticles, as well as their physicochemical properties, were investigated. Viscosity values ranged between 64.7 ± 0.1 and 502.1 ± 0.1 cP, and the viscosity increased as the amount of WM increased. Ricaurte, Perea-Flores, Martinez, & Quintanilla-Carvajal [22] also nanoencapsulated HOPO using high-shear homogenization (microfluidization) using whey protein as WM and Tween 20 as an emulsifier; they found apparent viscosity values of 1.9–553.3 cP at 19 °C. Under refrigeration at 4 °C, they reported values of 0.88–112.2 cP during a 4-day storage period. They noted that samples with higher concentrations of whey and oil exhibited higher apparent viscosities.

Viscosity is an important parameter that can influence the processing and quality control in the food industry. Depending on the goal of incorporating nanoemulsion into the food matrix,

this parameter can either favor or disfavor the viscosity of the final product. More viscous food matrices, such as yogurt (viscosity of 35–55 cP), would benefit from the addition of more viscous nanoemulsions. Conversely, more fluid matrices such as milk (viscosity of approximately 2 cP) would benefit from the addition of less viscous nanoemulsions [33]. As statistical differences between the samples are lacking, it can be suggested that both nanoparticles could be used in the food industry to replace free oil without interfering with the viscosity of the product. For example, yogurt has a viscosity similar to that found in this study (21.89 ± 1.20 cP).

The pH of free oil was 3.52 ± 0.08, indicating acidity, and that of N-JAF was lower (5.49 ± 0.05) than that of N-JSF (5.75 ± 0.05) ($p < 0.05$) (Table 2). However, both formulations had an acidic pH, and nanoencapsulation led to an increase in pH compared to that of the free oil. The difference in pH between free oil and nanoparticles can be explained by the WMs used. The mean pH of JAF is 5.54 ± 0.29 and 5.83 ± 0.06 for JSF [53], values similar to those observed for the nanoparticles.

It is important to note that pH is a crucial indicator of nanodispersion quality and can guide the application of the resultant nanomaterials, especially in food applications. Significantly low pH values indicate strong acidity that can lead to a decrease in the stability of pH-sensitive compounds, such as carotenoids. Additionally, acidity values can also interfere with the taste, and possible changes in pH can indicate the presence of bacteria or chemical reactions, compromising the final quality [54,36]. Thus, owing to their more acidic pH, N-JSF and N-JAF could be used in the preparation of naturally more acidic foods such as yogurt, which usually has a pH of 3.6–4.5, or even in salad dressings with pH values of 3.2–4.0 [55,56].

According to Campo et al. [23], pH of a medium influence's zeta potential. pH values below 2 tend to favor a slightly positive zeta potential, leading to a reduction in the electrostatic repulsion between particles by reducing groups with similar charges. However, a pH above 2 gradually increases the magnitude of the negative charge, as observed in this study.

As stated by Ferreira-Ribeiro et al. [33], pH values ranged from 3.82 ± 0.04 to 5.36 ± 0.01 for nanoparticles of crude palm oil and its fractions, palm olein, and palm stearin, similar to those in this study. The more acidic pH reported by the authors (3.82 ± 0.04) can be explained by the difference in the WM used (casein), which has a more amphiphilic characteristic, thereby influencing the reduction in pH.

Considering color parameters, as HCPO is rich in carotenoids, it showed a greater tendency towards red (a* 10.98 ± 0.00) and yellow (b* 11.69 ± 0.01), confirmed by the color analysis (CIELab) (Table 2). In comparison with African palm oil (Elaeis guineensis) analyzed

by de Almeida et al. [57], a value of 20.57 for b*, HCPO showed a less yellowish color (b* 11.69 ± 0.01). This may be because the hybrid oil had a lower fraction of stearin and a more yellowish palm oil.

The nanoparticles showed a greater tendency towards yellow (higher b* values) than red, and there was no statistical difference between the a* and b* parameters ($p > 0.05$). However, when compared to the free oil, there was a decrease in a* for both nanoparticles (0.64 ± 0.09 N-JSF and 0.55 ± 0.14 N-JAF) and in b* only for N-JSF (8.20 ± 2.53) ($p < 0.05$). Ferreira-Ribeiro et al. [33] who encapsulated crude palm oil and fractions, palm olein, and palm stearin. They reported a* and b* values ranging from 0.52 ± 0.02 to 1.51 ± 0.05 and 5.61 ± 0.07 to 8.36 ± 0.13, respectively, for both fractions analyzed, similar to those in the present study (a* = 0.64 ± 0.09 N-JSF and 0.55 ± 0.14 N-JAF and b* = 8.20 ± 2.53 N-JSF and 10.40 ± 0.39 N-JAF).

The nanoemulsions presented an average L* value of 40.52 ± 2.97 which was not changed even with the change in the WM in the preparation of nanoparticles. This parameter was significantly higher in the nanoemulsions than in the free oil ($p < 0.05$), probably because of the WMs used (flours present a whitish color) in addition to the water added to the formulation, which can generate a lighter color emulsion.

Color is an important quality attribute in the food industry and serves as the basis for the acceptance of a wide variety of products, positively or negatively influencing the perception of other sensory attributes. Despite great interest in natural colorants owing to their functionalities, their instability has made the industry invest in synthetic colorants [58]. Therefore, concerning the future applicability of N-JSF and N-JAF in the food industry, these nanoparticles can serve as more stable and promising alternatives for adding colorants to processed foods, due to the protection of encapsulated pigments, especially carotenoids as natural pigments.

The TC content of HCPO before encapsulation was 921.94 ± 28.39 µg/g (Table 2), and this changed to 809.76 ± 41.53 and 799.94 ± 45.60 µg/g for N-JSF and N-JAF, respectively, after encapsulation, in accordance with literature findings (500–10,000 µg/g of oil) [13]. Thus, the percentage retention of carotenoids after encapsulation was 87.83 and 86.77%, respectively, demonstrating the excellent preservation of carotenoids in encapsulated oil compared with that of the free oil. This, once encapsulated in nanoparticles, the likelihood of their degradation and consequent loss of functionality is reduced, making them more suitable for application in the food and possibly pharmaceutical industries [59,60]. Therefore, carotenoids and other phytochemicals have been studied for protection against non-communicable chronic diseases

(NCDs), where oxidative stress is the main contributor [61], and in the development of clean-label food products.

The percent carotenoids retention in nanoparticles with crude palm oil and its fractions, palm olein, and palm stearin, was up to 68% as reported by Ferreira-Ribeiro et al. [33]. This low value may be related to the encapsulation process (use of acetone), extraction method, sensitivity of carotenoids to oxidation, and isomerization during the analysis. However, precautions to achieve their optimal retention were performed in this study.

4. Conclusions

Nanoparticles of hybrid crude palm oil HIE OxG produced by homogenization using a non-toxic solvent (ethyl alcohol) and vegetable byproducts as encapsulants demonstrated an appropriate average diameter (<250 nm), uniformity, good stability, high encapsulation efficiency, and excellent preservation of carotenoids in the encapsulated oil compared to that of the free oil. These results demonstrate the potential for the application of nanoparticles with hybrid crude palm oil and jackfruit seed flour as wall material and nanoparticles with hybrid crude palm oil and jackfruit axis flour as wall material (N-JSF and N-JAF, respectively), as sustainable alternatives for the use of hybrid crude palm oil in the food industry.

5. Patents

The patent document "Nanoparticles of Hybrid Crude Palm Oil Unaué HIE OxG (Elaeis guineensis x Elaeis oleifera) obtained by homogenization method," stemming from the preliminary tests of this study, has been filed with the National Institute of Industrial Property (INPI) (Process no. BR 10 2022 019533 1) [62].

Supplementary Materials: The following Supporting Information can be downloaded from: www.mdpi.com/xxx/s1, Table S1: Results of preliminary tests for the development of N-HCPO with different wall materials; Figure S1: Pareto diagram for the amount of oil and wall material on particle size for N-JSF (a) and N-JAF (b); Figure S2: Pareto diagram for the amount of oil and wall material on PDI for N-JSF (a) and N-JAF (b).

Author contributions: L. S. A. Formal analysis, investigation, methodology, data curation, writing – original draft, writing – review and editing, and visualization. C. O. S. supervision, project administration, writing, review and editing, and visualization. F. S. Writing – review and editing, visualization. T. S. O. Methodology. D. J. A. Methodology. L. F. P. S.

Methodology. I. L. N wrote, reviewed, and edited. B. A. S. M. Writing, reviewing, and editing. C. D. F. R. Funding acquisition, Conceptualization, Project administration, Supervision, Writing – review and editing, and visualization.

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Conflicts of Interest: The authors declare no conflict of interest associated with any aspect of this study.

References

1. Food and Agriculture Organization of the United Nations (FAO). FAOSTAT - Crops. Roma, **2022**. Available on: https://www.fao.org/faostat/en/#data/QCL. Accessed on 12 November 2023.
2. United States Departament of Agriculture (USDA). Brazil Palm Oil: Potential and Pitfalls About. In Commodity Intelligence Report. **2022**, 19, Issue June 2019, 1–9. https://ipad.fas.usda.gov/highlights/2012/08/Mexico_corn/
3. Romero, H. M.; Daza, E.; Ayala-Díaz, I.; Ruiz-Romero, R. High-Oleic Palm Oil (HOPO) Production from Parthenocarpic. Agronomy. **2021**, 290, 1–18.
4. Rodríguez, J. C.; Gómez, D.; Pacetti, D.; Núnnez, O.; Gagliardi, R.; Frega, N. G.; Ojeda, M. L.; Loizzo, M. R.; Tundis, R.; Lucci, P. Effects of the Fruit Ripening Stage on Antioxidant Capacity, Total Phenolics, and Polyphenolic Composition of Crude Palm Oil from Interspecific Hybrid *Elaeis oleifera* × *Elaeis guineensis*. J. Agric. Food Chem. **2016**, 64, 852–859.
5. Cunha, R. N. V. da; Lopes, R. BRS Manicoré: Híbrido Interespecífico entre o Caiaué e o Dendezeiro Africano Recomendado para Áreas de Incidência de Amarelecimento-Fatal. Embrapa Agroindústria de Alimentos. Techinical communication, **2010**.
6. Antoniassi, R.; Machado, A. F. de F.; Wilhelm, A. E.; Guedes, A. M. M.; Bizzo, H. R.; Oliveira, M. E. C.; Yokoyama, R.; Lopes, R. Óleo de palma de alto oleico produzido no Brasil no ano de 2016 (Embrapa Agroindústria de Alimentos, Rio de Janeiro, Brazil). Techinical communication, **2018**.

7. Pinto, S. S.; Lopes, R.; Cunha, R. N. V. da; Filho, L. P. dos S.; Moura, J. I. L. Produção e Composição de Cachos e Incidência do Anel Vermelho em Híbridos Interespecíficos de Caiaué com Dendezeiro no Sul da Bahia. Agrotrópica. **2019**, 31, 5 -16.
8. de Almeida, E. S.; Damaceno, D. S.; Carvalho, L.; Victor, P. A.; dos Passos, R. M.; Pontes, P. V. A.; Cunha-Filho, M.; Sampaio, K. a.; Monteiro, S. Thermal and physical Properties of crude palm oil with higher oleic contente. Appl. Sci. **2021**, 11, 7094.
9. Lucci, P.; Borrero, M.; Ruiz, A.; Pacetti, D.; Frega, N. G.; Diez, O.; Ojeda, M.; Gagliardi, R.; Parra, L.; Angel, M. Palm oil and cardiovascular disease: a randomized trial of the effects of hybrid palm oil supplementation on human plasma lipid patterns. Food function, **2016**, 7, 347-354.
10. Choon, H. T.; Lee, C. J.; Tan, S. N.; Poon, D. T. S.; Chong, C. Y. E.; Pui, L. P. Red Palm Oil: A Review on Processing, Health Benefits and Its Application in Food. J. Oleo Sci. **2021**, 70, 1201-1210. doi: 10.5650/jos.ess21108.
11. Sambanthamurthi, R.; Sundram, K.; Tan, Y. Chemistry, and biochemistry of palm oil. Prog. Lipid Res. **2000**, 39, 507-558.
12. Codex Alimentarius. Codex Standard for named vegetable oils (CODEX STAN 210 - CXS 210-199), **2023**.
13. Mozzon, M.; Foligni, R.; Tylewicz, U. Chemical Characteristics and Nutritional Properties of Hybrid Palm Oils. In: Waisundara, V. Palm Oils, Intechopen, **2018**, 9, 149-170.
14. Ghaderi-Ghahfarokhi, M., Barzegar, M., Sahari, M. A., & Azizi, M. H. Nanoencapsulation Approach to Improve Antimicrobial and Antioxidant Activity of Thyme Essential Oil in Beef Burgers During Refrigerated Storage. Food Bioproc Tech. **2016**, 9(7), 1187–1201. https://doi.org/10.1007/s11947-016-1708-z
15. Javanmardi, Z., Koushesh Saba, M., Nourbakhsh, H., & Amini, J. Efficiency of nanoemulsion of essential oils to control Botrytis cinerea on strawberry surface and prolong fruit shelf life. Int. J. Food Microbiol. **2023**, 384, 109979. https://doi.org/10.1016/j.ijfoodmicro.2022.109979
16. Granata, G., Stracquadanio, S., Leonardi, M., Napoli, E., Consoli, G. M. L., Cafiso, V., Stefani, S.; Geraci, C., Essential oils encapsulated in polymer-based nanocapsules as potential candidates for application in food preservation. Food Chem. **2018**, 269, 286–292.
17. Ricaurte, L., Perea-Flores, M. de J., Méndez-Méndez, J. V., Santagapita, P. R., & Quintanilla-Carvajal, M. X. Compound distribution, structural analysis and nanomechanical properties of nanofibers loaded with high-oleic palm oil nanoemulsions for packaging application. Colloids Surf. A Physicochem. Eng. Asp. **2022**, 636, 128148. https://doi.org/10.1016/j.colsurfa.2021.128148
18. Ricaurte, L., Santagapita, P. R., Díaz, L. E., & Quintanilla-Carvajal, M. X. Edible gelatin-based nanofibres loaded with oil encapsulating high-oleic palm oil emulsions. Colloids Surf. A Physicochem. Eng. Asp. **2020**, 595, 124673. https://doi.org/10.1016/j.colsurfa.2020.124673
19. Beltrán, J.D.; Ricaurte, L., Estrada, K. B.; Quintanilla-Carvajal, M. X. Effect of homogenization methods on the physical stability of nutrition grade nanoliposomes used for encapsulating high oleic palm oil. LWT - Food Sci. Technol. **2020**, 118, 108801.
20. Ricaurte, L.; Hernández-Carrión, M.; Moyano-Molano, M.; Clavijo-Romero, A.; Quintanilla-Carvajal, M. X. Physical, thermal and thermodynamical study of high oleic palm oil nanoemulsions. Food Chem. **2018**, 256, 62–70.
21. Hernández-Carrión, M., Moyano, M., & Quintanilla-Carvajal, M. X. Design of high-oleic palm oil nanoemulsions suitable for drying in refractance windowTM. J. Food Process. Preserv. **2020**, 45(1), 1–9. https://doi.org/10.1111/jfpp.15076
22. Ricaurte, L., Perea-Flores, M. D. J., Martinez, A., & Quintanilla-Carvajal, M. X. Production of high-oleic palm oil nanoemulsions by high-shear homogenization (microfluidization). Innov Food Sci Emerg Technol. **2016**, 35, 75–85. https://doi.org/10.1016/j.ifset.2016.04.004

23. Campo, C., Santos, P. P., Costa, T. M. H., Paese, K., Guterres, S. S., Rios, A. de O., & Flôres, S. H. Nanoencapsulation of chia seed oil with chia mucilage (Salvia hispanica L.) as wall material: Characterization and stability evaluation. Food Chem. **2017**, 234, 1–9. https://doi.org/10.1016/j.foodchem.2017.04.153
24. Bezerra, P. Q. M., Matos, M. F. R. de, Ramos, I. G., Magalhães-Guedes, K. T., Druzian, J. I., Costa, J. A. V., & Nunes, I. L. Innovative functional nanodispersion: Combination of carotenoid from Spirulina and yellow passion fruit albedo. Food Chem. **2019**, 285, 397–405. https://doi.org/10.1016/j.foodchem.2019.01.181
25. Upadhyay, N., Singh, V. K., Dwivedy, A. K., Chaudhari, A. K., & Dubey, N. K. Assessment of nanoencapsulated Cananga odorata essential oil in chitosan nanopolymer as a green approach to boost the antifungal, antioxidant and in situ efficacy. Int. J. Biol. Macromol. **2021**, 171, 480–490. https://doi.org/10.1016/j.ijbiomac.2021.01.024
26. dos Santos, C. D., Menezes, R. C. R., & Dal Bosco, S. M. Releitura de pão de queijo: Versão vegetariana com farinha de oliveira. Rev. Verde Agroecologia Desenvolv. Sustent. **2021**, 16(2), 159-163.
27. Gharehbeglou, P. et al. Pectin-whey protein complexes vs. small molecule surfactants for stabilization of double nano-emulsions as novel bioactive delivery systems. J. Food Eng. **2019**, 245, 139-148.
28. Gonçalves, A.; Goufo, P.; Barros, A.; Domínguez-Perles, R.; Trindade, H.; Rosa, E. A. S.; Ferreira, L.; Rodrigues, M. Cowpea (Vigna unguiculata L. Walp), a renewed multipurpose crop for a more sustainable agri-food system: nutritional advantages and constraints. J. Sci. Food Agric. **2016**, 96, p. 2941 - 2951.
29. Maurya, P.; Mogra, R. Assessment of consumption practices of jackfruit (Artocarpus heterophyllus lam.) seeds in villages of Jalalpur block district Ambedarnagar (U.P.) India. Advances in Life Sciences. **2016**, 73-75.
30. Suzihaque, M. U. H., Zaki, N. A. M., Alwi, H., Ibrahim, U. K., Abd Karim, S. F., & Anuar, N. K. Jackfruit seed as an alternative replacement for starch flour. Materials Today: Proceedings. **2022**, 63, S451–S455. https://doi.org/10.1016/j.matpr.2022.04.117
31. Li, Z., Lan, Y., Miao, J., Chen, X., Chen, B., Liu, G., Wu, X., Zhu, X., & Cao, Y. Phytochemicals, antioxidant capacity and cytoprotective effects of jackfruit (Artocarpus heterophyllus Lam.) axis extracts on HepG2 cells. Food Biosci. **2021**, 41, 100933. https://doi.org/10.1016/j.fbio.2021.100933
32. Oliveira, L. F., Nascimento, M. R. F., Borges, S. V., Ribeiro, P. C., Do, N., & Ruback, V. R. Alternative use of yellow passion fruit peel (Passiflora edulis f. Flavicarpa) for sweet production in syrup. Food Sci. Technol. **2002**, 22, 259–262.
33. Ferreira Ribeiro, C. D., Schappo, F. B., Sales, I. da S., Assunção, L. S., Otero, D. M., Magalhães-Guedes, K. T., Machado, B. A. S., Block, J. M., Druzian, J. I., & Nunes, I. L. Novel bioactive nanoparticles from crude palm oil and its fractions as foodstuff ingredients. Food Chem. **2022**, 373. https://doi.org/10.1016/j.foodchem.2021.131252
34. Jiménez, M., Domínguez, J. A., Pascual-Pineda, L. A., Azuara, E., & Beristain, C. I. Elaboration and characterization of O/W cinnamon (Cinnamomum zeylanicum) and black pepper (Piper nigrum) emulsions. Food Hydrocoll. **2018**, 77, 902–910. https://doi.org/10.1016/j.foodhyd.2017.11.037
35. Froiio, F., Ginot, L., Paolino, D., Lebaz, N., Bentaher, A., Fessi, H., & Elaissari, A. Essential oils-loaded polymer particles: Preparation, characterization and antimicrobial property. Polymers. **2019**, 11(6). https://doi.org/10.3390/polym11061017
36. Assunção, L. S., Bezerra, P. Q. M., Poletto, S. H. V., Rios, A. de O., Ramos, I. G., Ferreira-Ribeiro, C. D., Machado, B. A. S., Druzian, J. I., Costa, J. A. V., & Nunes, I. L. Combination of carotenoids from Spirulina and PLA/PLGA or PHB: New options to obtain bioactive nanoparticles. Food Chem. **2021**, 346, 128742. https://doi.org/10.1016/j.foodchem.2020.128742
37. de Jesus Assis, D., Brandão, L. V., de Sousa Costa, L. A., Figueiredo, T. V. B., Sousa, L. S., Padilha, F. F., & Druzian, J. I. A Study of the Effects of Aeration and Agitation on the

Properties and Production of Xanthan Gum from Crude Glycerin Derived from Biodiesel Using the Response Surface Methodology. Appl. Biochem. Biotechnol. **2014**, 172(5), 2769–2785.

38. Antonioli, G. et al. Poly(lactic acid) nanocapsules containing lemongrass essential oil for postharvest decay control: In vitro and in vivo evaluation against phytopathogenic fungi. Food Chem. **2020**, 326, 126997.
39. Quintanar-Guerrero, D., Allémann, E., Fessi, H., & Doelker, E. Preparation techniques and mechanisms of formation of biodegradable nanoparticles from preformed polymers. Drug Dev. Ind. Pharm. **1998**, 24(12), 1113–1128. https://doi.org/10.3109/ 03639049809108571.
40. Lancheros, R. J., Beleño, J.Á., Godoy-Silva, R. D., & Guerrero, C. A. Producción de nanopartículas de PLGA por el método de emulsión y evaporación para encapsular N-Acetilcisteína (NAC). J. Fac. Sci. **2014**, 92, 161–168.
41. Floury, J., Desrumaux, A., & Lardières, J. Effect of high-pressure homogenization on droplet size distributions and rheological properties of model oil-in-water emulsions. Innov. Food Sci. Emerg. Technol. **2000**, 1(2), 127–134. https://doi.org/10.1016/S1466 -8564(00)00012 -6
42. Ferreira, C. D., & Nunes, I. L. Oil nanoencapsulation: development, application, and incorporation into the food market. Nanoscale Res. Lett. **2019**, 9, 14. https://doi.org/10.1186/s11671-018-2829-2
43. Hemmatkhah, F.; Zeynali, F.; Almasi, H. Encapsulated cumin seed essential oil-loaded active papers: characterization and evaluation of the effect on quality attributes of beef hamburger. Food Bioproc Tech. **2020**, 13, 533–547.
44. Mohanraj, V.J.; Chen, Y. Nanoparticle – A review. Trop. J. Pharm. **2006**, 5, 561–573.
45. Zagtap, U. B.; Bapat, V. A. Green synthesis of silver nanoparticles using Artocarpus heterophyllus Lam. Seed extract and its antibacterial activity. Ind Crops Prod. **2013**, 46, 132-137.
46. Xin, X., Zhang, H., Xu, G., Tan, Y., Zhang, J., & Lv, X. Influence of CTAB and SDS on the properties of oil-in-water nano-emulsion with paraffin and span 20/tween 20. Colloids Surf. A Physicochem. Eng. Asp. **2013**, 418, 60–67. http:// dx.doi.org/10.1016/j.colsurfa.2012.10.065
47. Sathasivam, T., Muniyandy, S., Chuah, L. H., & 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
48. Ilyasoglu, H.; El, S.N. 2014. Nanoencapsulation of EPA/DHA with sodium caseinate e gum arabic complex and its usage in the enrichment of fruit juice. LWT - Food Sci. Technol. **2014**, 56, 461–468.
49. Esfahani, R., Jafari, S. M., Jafarpour, A., & Dehnad, D. Loading of fish oil into nanocarriers prepared through gelatin-gum Arabic complexation. Food Hydrocoll. **2019**, 90, 291–298. https://doi.org/10.1016/j.foodhyd.2018.12.044
50. Samyn, P., Van Nieuwkerke, D., Schoukens, G., Stanssens, D., Vonck, L., & Van Den Abbeele, H. Hybrid palm-oil/styrene-maleimide nanoparticles synthesized in aqueous dispersion under different conditions. J. Microencapsul. **2015**, 32(4), 336–348. https://doi.org/10.3109/02652048.2015.1028493
51. Gulzar, S., & Benjakul, S. (2020). Characteristics and storage stability of nanoliposomes loaded with shrimp oil as affected by ultrasonication and microfluidization. Food Chem. **2020**, 310, 125916.
52. Polychniatou, V., & Tzia, C. Study of formulation and stability of co-surfactant free water-in-olive oil nano- and submicron emulsions with food grade non-ionic surfactants. JAOCS J. Am. Oil Chem. Soc. **2014**, 91, 79–88. https://doi.org/10.1007/s11746-013-2356-3
53. Sousa, A. P. M. de, Campos, A. R. N., Santana, R. A. C. de, Dantas, D. L., Macedo, A. D. B. de, Silva, J. R. B. da, Malaquias, A. B., Albuquerque, T. da N., Silva, G. B. da, & Gomes, J. P. Parâmetros de qualidade física e química do eixo central, mesocarpo e semente de jaca

submetidos a diferentes processos de secagem. Res., Soc. Dev. **2022**, 11(4), 1-12. https://doi.org/10.33448/rsd-v11i4.27328

54. Lobato, K. B. S. et al. Evaluation of stability of bixin in nanocapsules in model systems of photosensitization and heating. LWT - Food Sci. Technol. **2015**, 60, 8-14, 2015.
55. Rodas, A. B. M. et al. Caracterização físico-química histológica e viabilidade de bactérias lácticas em iogurtes com frutas. Ciênc. Tecnol. Aliment. **2001**, 21, 304-309.
56. Donato, M. et al. Efeito da adição de óleo de palma bruto nanoencapsulado na estabilidade oxidativa de molho para salada em teste de oxidação acelerada. Res., Soc. Dev. **2020**, 9, 1-27.
57. de Almeida, D. T., Viana, T. V., Costa, M. M., Silva, C. de S., & Feitosa, S. Effects of different storage conditions on the oxidative stability of crude and refined palm oil, olein and stearin (*Elaeis guineensis*). Food Sci. Technol. **2019**, 39, 211–217. https://doi.org/10.1590/fst.43317
58. Ousji, O; Sleno, L. Identification of In Vitro Metabolites of Synthetic Phenolic Antioxidants BHT, BHA, and TBHQ by LC-HRMS/MS. Int. J. Mol. Sci. **2020**, 21, 95- 25.
59. Mortensen, A. Carotenoids and other pigments as natural colorants. Pure Appl. Chem. **2006**, 78, 1477-1491.
60. Souza, W. A.; Vilas-Boas, O. M. G. C. A deficiência de vitamina A no Brasil: um panorama. Rev Panam Salud Publica. **2002**, 12, 3.
61. Chandrasekara, A., Daugelaite, J., Shahidi, F. DNA scission and LDL cholesterol oxidation inhibition and antioxidant activities of Bael (Aegle marmelos) flower extracts. J. Tradit. Complement. Med., **2018**, 8, 428–435.
62. Assunção, L. S et al. Nanopartículas de óleo de palma bruto híbrido Unaué HIE OxG (*Elaeis guineensis x Elaeis oleifera*) obtidas por método de homogeneização. Federal University of Bahia, Brazil. BR Patent nº 10 2022 019533 1, 08 september 2022.

Capítulo III

Manuscrito: Nanoencapsulation of hybrid crude palm oil Unaué HIE OxG (Elaeis guineensis x Elaeis oleifera) with jackfruit by-products as encapsulants: a study of cellular antioxidant activity and cytotoxicity in Caco-2 cells

Nanoencapsulation of hybrid crude palm oil Unaué HIE OxG (*Elaeis guineensis* x *Elaeis oleifera*) with jackfruit by-products as encapsulants: a study of cellular antioxidant activity and cytotoxicity in Caco-2 cells

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Abstract: Hybrid crude palm oil (HCPO) HIE OxG nanoparticles, using jackfruit by-products as encapsulants were produced and characterized for cellular antioxidant activity (CAA) and cytotoxicity in differentiated human colorectal adenocarcinoma (Caco-2) cells. The nanoparticles exhibited nanoscale diameters (<250 nm), monodisperse distribution, good uniformity, and stability (Polydispersity index <0.25; zeta potential JSF-NP -12.46 ± 0.15mV and JAF-NP -13.73 ± 1.28mV). Cellular Antioxidant Activity (CAA) assay may better predict antioxidant behavior in biological systems, and CAA of JSF-NP and JAF-NP surpassed those of free HCPO at all concentrations, while none of the nanoparticles exhibited cytotoxic effects on differentiated Caco-2 cells. Notably, scientific literature lacks studies about CAA and cytotoxicity of HCPO nanoparticles with vegetable by-products as encapsulants. As such, the present study demonstrated the bioactivity and biological safety of these nanoparticles in intestinal cells, highlighting JSF-NP and JAF-NP as delivery systems for future HCPO applications.

Keywords: Palm oil with a higher content of oleic acid; *Artocarpus heterophyllus;* Jackfruit seed; Central axis of jackfruit; Cellular antioxidant activity; Toxicity.

1. Introduction

Hybrid crude palm oil (HCPO) is extracted from the fruit, resulting of the crossbreeding between palms from the African species (*Elaeis guineensis*) and the American species (*Elaeis oleifera*). Since 1997 in eastern Ecuador, there have been reports on HCPO due to better resistance to diseases associated with cultivation practices, compared to crude palm oil (CPO) from the fruit of African palm oil (Tezara et al., 2021). Colombia, Ecuador, and Costa Rica are the main producers of HCPO, with 12% of the total cultivation area located in Colombia (Romero et al., 2021).

A new variety cultivated only in Brazil, named Unaué, has gained prominence and is contributing to the sustainability of oil palm cultivation, especially for small producers. It combines high oil productivity from the oil palm, low production costs, and greater resistance to pests and diseases compared to the hybrids produced in other countries, especially the so-called red ring, a common and highly lethal disease. This variety is obtained through crossbreeding of the African oil palm (*Elaeis guineensis*) and the American Caiaué and is known as the HIE OxG interspecific hybrid. In Brazil's Pará region, the cultivated area exclusively dedicated to HIE OxG palm oil production is estimated to be around 11,500 hectares, with the potential to yield over 40,000 tons of oil annually (Pinto et al., 2019).

The HCPO stands out due to its nutritional composition as opposed to CPO, with higher amounts of carotenoids (500 to 10,000 µg/g), with β-carotene (52–60%) and α-carotene (33–36%) being the main components (Mozzon et al., 2018). It also has a higher content of vitamin E unsaturated fatty acids and a lower content of saturated fatty acids (Pinto et al., 2019). Regarding the content of tocopherols and tocotrienols, values range from 562 to 1417 µg/g, with γ-tocotrienol as the main component (406 to 887 µg/g). With at least 48% oleic acid, this oil is recognized as "palm oil with a higher content of oleic acid" (Codex, 2022).

Considering the composition of HCPO in terms of fatty acids and phytochemicals, it is believed that this oil may exhibit a promising alternative to replace synthetic additives as a colorant and with antioxidant functions in foodstuffs. However, HCPO bioactive compounds are unstable and susceptible to oxidative deterioration, especially when exposed to oxygen, light, moisture, and temperature, resulting in a loss of nutritional quality, development of off-flavors, and decreased shelf-life (Ferreira-Ribeiro et al., 2022).

In this context, nanoencapsulation, a process in which nanosized particles (less than 1 µm) are formed, can protect the encapsulated oil, and facilitate its incorporation into food. It can also improve its bioavailability and protect bioactive compounds from deterioration, thus maintaining its natural characteristics (Singh et al., 2017). Additionally, there is a growing interest in biopolymers and flours derived from vegetable by-products, with biodegradable, sustainable characteristics. These flours may be used by the food industry, as encapsulating agents for oils and bioactive compounds (Campo et al., 2017; Ferreira-Ribeiro et al., 2022).

The fruit and vegetables segment stands out as a major contributor to waste in the agri-food industry, producing residues such as skins, seeds, stems, barks, and leaves (Danielski and Shahidi, 2023). The consumption and processing of jackfruit (*Artocarpus heterophyllus*) results in a high number of by-products such as peels, central axis, and seeds. About 25–35% of jackfruit constitutes the edible portion, leaving the remaining 65-75% as the residual fraction. (Pathak et al., 2022). The jackfruit seed flour (JSF) including a source of starch (63%) and protein (8%), with interesting levels of soluble and insoluble fibers, minerals, vitamins, and bioactive compounds (flavonoids) (Suzihaque et al., 2022). Phenolic compounds, including flavonoids have been determined in the jackfruit axis flour (JAF), demonstrating antioxidant potential (Li et al., 2021).

The antioxidant potential of HCPO has been explored in the scientific literature, especially as a functional oil for disease prevention and health benefits, such as cardiovascular disease risk reduction (Lucci et al., 2016). The antioxidant activity of oils can be assessed by chemical methods such as oxygen radical absorbance capacity (ORAC), 2,2-diphenyl-1-

picrylhydrazyl (DPPH), and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical scavenging (Liu et al., 2019). These assays mostly evaluate the antiradical capacity of molecules with antioxidant property, including hydrogen atom transfer (HAT) and singlet electron transfer (SET) capacities. In several instances, such mechanisms are the basis for the health-promoting benefits shown by naturally occurring bioactive compounds (Ayoub, Camargo, and Shahidi, 2016).

Although the above-mentioned methods have been utilized to evaluate *in vitro* antioxidant activity, they still exhibit some inherent shortcomings, such as neglecting important biological markers, including the uptake, metabolism, and bioavailability, determinant factors for *in vivo* effect. Thus, the biological activity of natural antioxidants at the cellular level should also be considered. In comparison with chemical assays, cellular antioxidant activity (CAA) considers cellular absorption, metabolism, location, and distribution of bioactive compounds within cells, and therefore, it may better predict antioxidant behavior in biological systems (Chen et al., 2015; Lu et al., 2020). CAA involves monitoring the ability of compounds to prevent peroxyl radical-induced oxidation within the cell. The free-radical generator 2,2'-azobis (2-amidinopropane) dihydrochloride (ABAP) is incubated with the cells, initiating peroxyl radicals' formation. The ability of dietary antioxidants to hamper the propagation of free radicals can measured and quantified (Wolfe and Liu, 2007; Liu et al., 2019). CAA assessment using differentiated human colorectal adenocarcinoma (Caco-2) cells has been explored in scientific literature (Kellet, Greenspan and Pegg, 2018) due to their similarity with small intestine epithelial cells, including morphology, microvillar structure, and permeability characteristics (Sergent et al., 2005; Wan, Dong, Yu, Sun, and Li, 2015).

Although CPO is frequently used in foods, the CAA and safety regarding its nanosized particles is not well-established in the literature. Size reduction can lead to unique properties not found in larger sizes, and harmful effects such as dysbiosis, cytotoxicity, and genotoxicity have been reported in nanoparticles of different materials. Thus, cell cultures can be used to assess cellular changes after exposure to the investigated substance, revealing potential toxic effects, such as lipid peroxidation, membrane rupture and gastrointestinal inflammation (Ashraf et al., 2021).

There is no literature on CAA and cytotoxicity in differential Caco-2 cells of HCPO nanoparticles with vegetables by-products as encapsulants. It is noteworthy that exploring CAA and the lack of cytotoxicity in oil nanoparticles in cells that closely resemble small intestine epithelial may be interesting for comprehending the biological antioxidant activity and safety consideration, demonstrating the potential harmlessness to epithelial cells. Therefore, in the

present work HCPO nanoparticles with jackfruit seed flour (JSF-NP) or jackfruit axis flour (JAF-NP) as encapsulants were developed, characterized, and the cellular antioxidant activity and cytotoxicity of them were evaluated in differential human colorectal adenocarcinoma (Caco-2) cells.

2. Material and methods

2.1 Material

The HIE OxG hybrid fruit was developed by *Empresa Brasileira de Pesquisa Agropecuária* (EMBRAPA) Western Amazon. The HCPO, known as Unaué, was formulated, and provided by the *Comissão Executiva do Plano da Lavoura Cacaueira* (CEPLAC) in the city of Una-BA, Brazil, stored at -20°C in an amber bottle until the time of analysis. Jackfruit (*Artocarpus heterophyllus*) were obtained from local markets in Salvador, Bahia. 2′,7′-dichlorofluorescin diacetate or 2′,7′-dichlorodihydro-fluorescein diacetate (DCFH-DA, ≥97% purity), 2,2′-azobis (2-amidi-nopropane) dihydrochloride (ABAP), dimethyl sulfoxide (DMSO), were acquired from Sigma-Aldrich Ltd. (Oakville, ON, Canada). Dulbecco's Modified Eagle Medium (DMEM), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), fetal bovine serum (FBS), L-glutamine, penicillin-streptomycin solution (10,000 units), and trypsin-EDTA (0.25%) were procured from Thermo Fisher Scientific (Nepean, ON, Canada) and Sigma-Aldrich (Oakville, ON, Canada). Other solvents and chemicals of analytical grade were obtained from Sigma-Aldrich Ltd. (Oakville, ON, Canada).

2.2 Methods

2.2.1 Preparation of encapsulants

For the preparation of JSF and JAF, the fruit was used in the green ripening stage. The seed and central axis were separated individually from the fruit, followed by blanching (2 min in boiling water at 100°C, then cooled for 3 min in cold water) and freezing (-10°C) in a vacuum-sealed package until the day of flour preparation. After thawing under refrigeration (4°C), the seeds and the central axis were dehydrated in a forced-air circulation dehydrator (Q317M-32, Quimis, Brazil) at 1.25 m/s² at 50°C for 36 h. At the end of each drying process, the dehydrated samples were ground in a knife mill (Pulverisette 15, Fritsch, Markt Einersheim,

Germany), with a mesh opening of 0.5 mm, and vacuum-packed. The samples were kept refrigerated (4°C) until the time of analysis.

2.2.2 Preparation of nanoparticles

The nanoparticles were prepared using the homogenization method described by Ferreira-Ribeiro et al. (2022), with some modifications. Tween 20 (250 µL) and HCPO (250 mg) were dissolved in ethyl alcohol (100 mL) under agitation (IKA@, RH Basic 2, US) for 15 min. This organic phase (tween 20, ethyl alcohol and HCPO) was then added dropwise to 100 mL of an aqueous solution containing 217.15mg of wall material (JSF or JAF - these wall materials were used individually and not in combination) during homogenization on a helical agitator (IKA@, model RW 20 digital, Diagtech, US) at a speed of 900 rpm for 30 min. Subsequently, the ethyl alcohol was evaporated in a rotary evaporator (35°C) (R-300, Büchi, Flawil, Switzerland) until complete solvent evaporation, verified by measuring the final volume of the nanoemulsion (100 mL). The resulting aqueous dispersion nanoparticles (≅ 2.5 mg/mL) were subjected to characterization by particle size, polydispersity index (PDI) and zeta potential (ζ).

2.2.3 Determination of particle size, polydispersity index (PDI) and zeta Potential (ζ)

The particle size, PDI, and zeta potential of nanoparticles were measured by dynamic light scattering and phase analysis light scattering (Zetasizer Nano ZS, Malvern Instruments, Malvern, United Kingdom) at 25ºC. Particle size data were reported as the mean diameter and PDI. Zeta potential values were measured based on electrophoretic mobility (Ferreira Ribeiro et al., 2022).

2.2.4 Transmission electron microscopy (TEM)

The morphology of nanoparticles was determined by transmission electron microscopy (TEM). A drop of the nanoemulsion was placed on a grid (Formvar carbon support films, 200 mesh) for 1 min. Subsequently, a drop of 1% phosphotungstic acid solution was applied for 30 sec. The grid was then examined under a transmission electron microscope (TEM; JEOL 1230, Tokyo, Japan) operated at 80 kV with an average magnification of 80,000 times and a scale of 100 nm.

2.2.5 Cell culture

Human colon adenocarcinoma (Caco-2) cells were kindly provided by Dr. Mark Berry (Biochemistry Department of Memorial University of Newfoundland, Canada) and used at passage numbers 36-40. Caco-2 cells were cultured using the protocol described by Xie, Kosińska, Xu, and Andlauer (2013). In summary, cells were grown as a monolayer and cultured in Advanced Dulbecco's modified Eagle medium (DMEM), heat-inactivated fetal bovine serum (FBS), 1% L-glutamine, 1% penicillin (10,000 U/mL), and 1% streptomycin (10,000 U/mL) under 5% CO2 at 37 °C. Caco-2 cells were used in experiments at passage numbers 36-40.

2.2.6 Preparation of samples to Cellular antioxidant activity (CAA) assay and Cell cytotoxicity assay

On the day of analysis, samples were diluted to final concentration ranging from 2.5 to 250 μg/mL in a serum-free culture medium. A solution with HCPO was prepared in ethyl acetate at a concentration of 2.5 mg/mL. A 12.5 mM stock solution of the fluorescent probe 2′,7′-dichlorofluorescin diacetate (DCFH-DA) in methanol was employed. Prior to each experiment, working solutions of 25 μM DCFH-DA in a serum-free culture medium were freshly prepared. A 60 mM 2,2′-azobis (2-amidi-nopropane) dihydrochloride (ABAP) stock solution in phosphate-buffered saline (PBS) was diluted to 600 μM before its use in experiments. All DCFH-DA and ABAP solutions were stored at -20 °C, while samples were refrigerated at 4 °C prior to their use.

2.2.6.1 Cellular antioxidant activity (CAA) assay

Cellular antioxidant measurements were conducted following the protocol of Wolfe and Liu (2007), with modifications suggested by Kellet et al. (2018). Upon reaching confluency in Corning 75-cm^2 culture flasks, Caco-2 cells were twice washed with sterile PBS (pH 7.4) and then detached from the surface using 0.05% trypsin-EDTA. Subsequently, cells were seeded (6.0×10^4) in 100-μL cell culture media per well in Corning Costar® 96-well, black, flat bottom tissue culture-treated dishes (VWR International, Suwanee, GA, USA) and incubated until reaching full confluency (24 h). Confluence was verified using a microscope, and wells along

the perimeter were intentionally left empty to minimize variation due to plate location. Subsequently, the growth medium was aspirated, and the cells were washed with PBS to eliminate any non-adherent and dead cells.

Following this, 50 μL of a 25 μM DCFH-DA working solution was dispensed into each well, followed by 50 μL of the antioxidant treatments (JSF-NP, JAF-NP, or HCPO) (in triplicate wells). As a control, 50 μL of DCFH-DA and 50 μL of serum-free culture media (without any antioxidant) were added to each of the three wells as replicates. Next, cells were incubated for 1 h at 37 °C. Following incubation, the cells were washed three times with PBS to ensure that any observed effects were solely attributed to compounds absorbed by the cells. Subsequently, 100 μL of ABAP (600 μM) were introduced. The cells were then promptly taken to a microplate reader (BioTek Synergy Mx microplate reader, BioTek In- struments, Inc. Winooski, VT, USA), where real-time fluorescence was initially recorded and then at 5 min intervals for 1 h (a total of 13 readings). The fluorescence excitation and emission wavelengths were taken at 485 and 538 nm, respectively. Results were expressed as cellular antioxidant activity (CAA) units.

2.2.6.2 Cell cytotoxicity assay

The colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was employed, as described by Gentile et al. (2012). DCFH-DA and the samples were applied to the cells using the same procedure as outlined for the CAA assay, with an incubation period of 1 h at 37 °C. Subsequently, the cells were thoroughly washed with PBS to eliminate all sample residues and traces of media. Control wells contained MTT and media, excluding samples. A volume of 100 μL of serum-free culture media was added to the cells along with 10 μL of 12 mM MTT. After 4 h of incubation, 85 μL of media were removed, and 50 μL of DMSO were introduced to each well to dissolve the generated cellular formazan chromagen. The plates were once again incubated at 37 °C for 10 min, and the absorbance of the purple-colored formazan was measured at 540 nm in a microplate reader (BioTek Synergy Mx microplate reader, BioTek In- struments, Inc. Winooski, VT, USA). For quantitative assessment, the absorbance of cells incubated with the samples was compared to that of the control (without any antioxidant). The treatments resulting in significantly ($p < 0.05$) lower absorbance readings was considered cytotoxic. All assays were conducted in triplicate. Moreover, cell relative viability (%) was calculated as the ratio of treated cells absorbance by the control absorbance.

2.2.6.3 Quantification of the CAA assay

The effectiveness of JSF-NP, JAF-NP, and HCPO was quantified by assessing the percent reduction in fluorescence. In short, curves were constructed using the 13 fluorescence response readings of each treatment during the 1-hour assay. The area under each curve (AUC) was determined through integration using Excel®. The percent reduction (or the CAA unit) was calculated as follow Eq. 1:

$$CAA\ unit = \%\ reduction\left(1 - \frac{AUC\ sample}{AUC\ control}\right) \times 100 \quad (1)$$

CAA units were calculated for free oil (HCPO) and nanoparticles of HCPO from triplicate determinations, and the results are presented as mean ± standard deviation.

2.2.7 Statistical analysis

The analyses were performed in triplicate (±standard deviation). The results concerning particle size, PDI, zeta potential and cytotoxic were assessed using analysis of variance (ANOVA) and compared through the Tukey's test ($p \leq 0.05$) and Student's t-test ($p \leq 0.05$) with the software SAS® OnDemand for Academics.

3. Results and discussion

3.1. Characterization of nanoparticles

3.1.1. Particle size, PDI and zeta potential

Dynamic light scattering (DLS) analysis showed that the mean diameters of JAF-NP was smaller than JSF-NP (225.63 ± 1.89 nm and 242.03 ± 2.38, respectively) ($p < 0.05$), confirming the nanoparticle classification for both samples (<1µm). Particle size affects the physicochemical properties of the material, kinetic release of the compound and biodistribution. Furthermore, suitable diameters for nanoencapsulated oils should range between 100 and 1000 nm (Ferreira and Nunes, 2019). In this context, the mean particle diameter obtained in the present study (< 250 nm) demonstrate that the homogenization technique and the use of

jackfruit by-products as encapsulants were effective for obtaining nanostructures with dimensions below 1000 nm. Particle diameters of CPO and its fractions, palm olein and palm stearin, nanoencapsulated with casein or gum Arabic as encapsulants, were reported to range from 155.66 ± 3.41 to 187.37 ± 2.19 nm (Ferreira-Ribeiro et al., 2022). Additionally, Ricaurte et al. (2020) noted a diameter of 198.3 ± 1.1 nm for hybrid CPO.

According to the results shown in Figure 1, both nanoparticles formed a stable and dispersed nanodispersions and showed unimodal curves which indicates that all nanoparticles had a uniform particle diameter distribution. This result was confirmed by the analysis of PDI (0.10 ± 0.02 JSF-NP and 0.11 ± 0.00 JAF-NP). There was no significant difference ($p > 0.05$) observed in the PDI among the nanoparticles. The PDI is a measure that reflects the range of the particle size distribution, and according to Ricaurte et al. (2020), the smaller the PDI, the lower the tendency for particle aggregation, confirming the quality of the nanoencapsulation process. A PDI < 0.25 indicates that the nanoemulsions have a narrow and monodisperse distribution, with good uniformity in nanoparticle diameter.

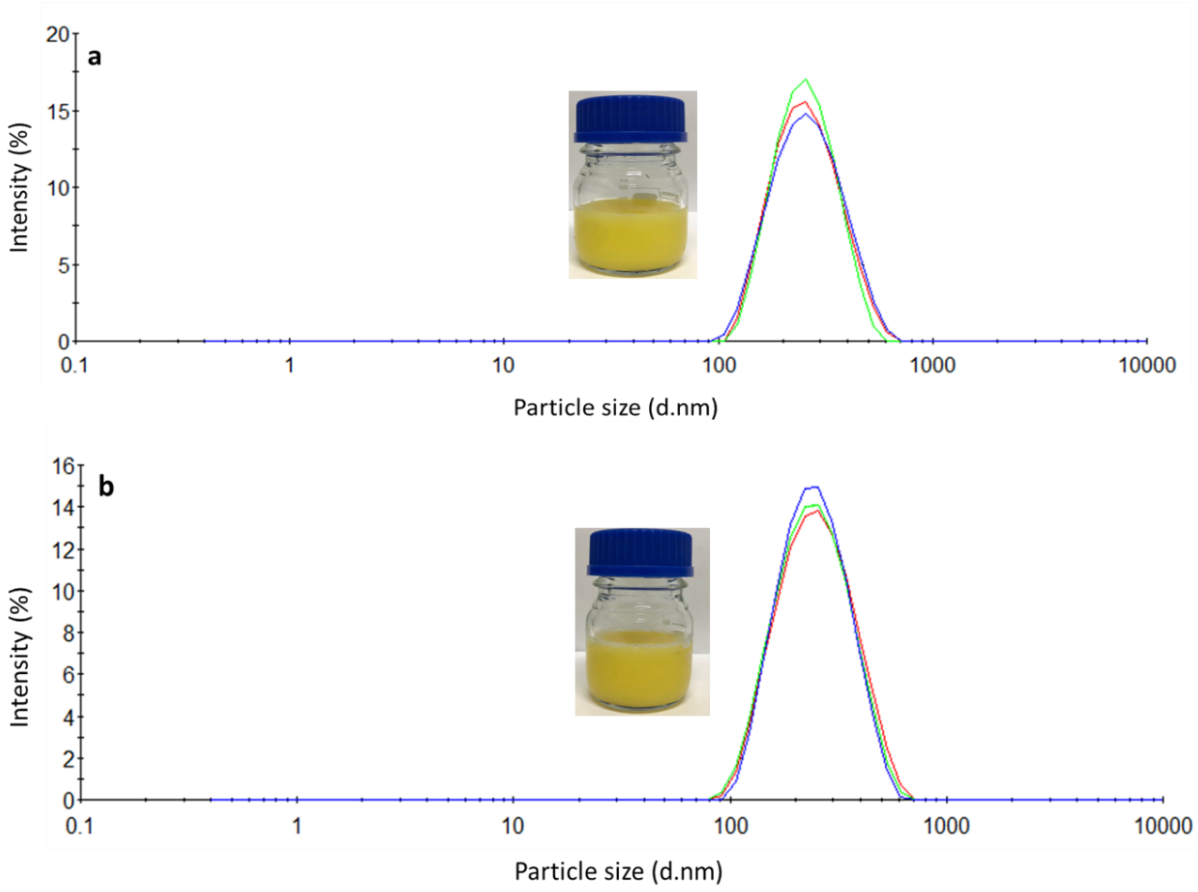

Figure 1. Particle size distribution of jackfruit seed flour nanoparticles (JSF-NP) (a); jackfruit axis flour nanoparticles (JAF-NP) (b) (in triplicate) and its nanoemulsions.
d.nm = diameter values in nanometers.

Similar results were documented by Cheong and Nyam (2016) in their investigations on nanoencapsulated kenaf seed oil. The use of sodium caseinate and pectin, along with sodium caseinate as the wall material, yielded comparable PDI values of 0.152 ± 0.01 and 0.147 ± 0.00, respectively.

As highlighted by Ferreira and Nunes (2019), the zeta potential serves as an indicator of the electrical potential of particles, exerting a significant influence on the stability of colloidal systems. This parameter is notably affected by the composition of nanoparticles and the surrounding medium. Zeta potential values above +30 mV and below -30 mV signify stable suspensions with minimal particle aggregation.

Zeta potential for JSF-NP, and JAF-NP were measured at -12.46 ± 0.15 mV, and -13.73 ± 1.28 mV, respectively, and no statistical difference ($p > 0.05$) was observed between the samples. These values indicate a good nanoemulsion stability, suggesting that repulsive forces between droplets predominate in this system. Moreover, the negative charges are likely related to the existence of carboxyl groups in the composition of the flours employed as encapsulants (Li et al, 2021). Additionally, the emulsifier used in the nanoemulsion preparation (Tween 20), introduces negative charge through the adsorption of hydroxide ions at the oil-water interfaces and the formation of hydrogen bonds with these ions (Xin et al, 2013). Ricaurte et al. (2020) reported a zeta potential of -24.8 ± 0.5 mV in hybrid crude palm oil nanofibers with gelatin and Ferreira-Ribeiro et al. (2022) obtained zeta potentials between -18.23 ± 1.11 mV and -36.33 ± 0.47 mV in the stability assessment of nanoparticles from crude palm oil and its fractions, palm olein and palm stearin.

3.1.2. TEM analyses

Figure 2 shows the morphological characteristics of nanoparticles formulated with jackfruit seed flour and hybrid crude palm oil (a), as well as jackfruit axis flour and hybrid crude palm oil (b). The nanoparticles were similar, regardless of the wall material used, exhibiting a spherical and regular shape without cracks or aggregates.

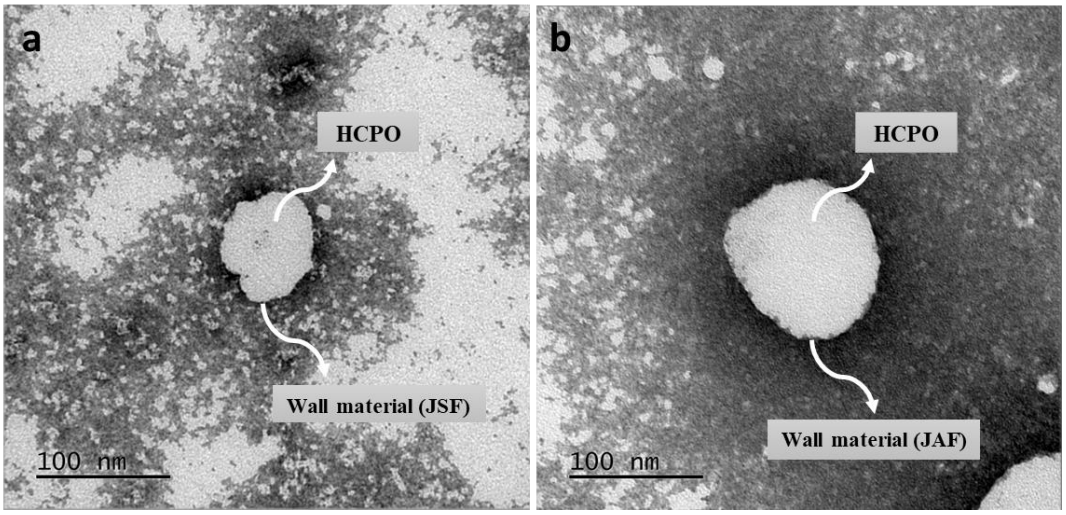

Figure 2. TEM images of nanoparticles containing (a) hybrid palm oil and jackfruit seed flour as wall material (JSF-NP) and (b) nanoparticles containing hybrid palm oil and jackfruit axis flour as wall material (JAF-NP) (scale = 100nm).

Similar results were reported by Ferreira-Ribeiro et al. (2022) and Campo et al. (2017), using the same technique and conditions of morphological characterization as those in the present study for crude palm oil and fractions, hybrid crude palm oil and chia seed oil, respectively.

3.2. Cellular antioxidant activity (CAA) assay

The cellular antioxidant activity (CAA) assay has higher physiological relevance than chemical methods since it factors in cellular uptake and metabolism, being a good predictor of the bioavailability of phytochemical compounds. Therefore, this assay is suitable for evaluating antioxidant behavior in biological systems (Chen et al., 2015). In this context, the ability of HCPO, before and after nanoencapsulation, to prevent peroxyl radical-induced oxidation in Caco-2 cells was assessed. Reduced fluorescence compared to the control indicates that the sample has substantial antioxidant activity in the cell, being able to prevent the conversion of DCFH (fluorescent probe) into its oxidized form. The higher the CAA unit (the relative reduction in fluorescence), the more effective the antioxidant in the cellular system (Kellet, Greenspan and Pegg, 2018).

In Figure 3, it can be observed that CAA levels for JSF-NP (33.82 ± 1.70; 56.57± 1.50; 48.21 ± 1.65; 34.97 ± 1.75; 59.62 ± 1.70; 36.79 ± 1.60) were higher than those of the HCPO free (-10.37 ± 2.70; 23.21 ± 2.60; 34.54 ± 2.75; 16.71 ± 2.76; 41.11 ± 2.60; 9.89 ± 2.65) at all concentrations studied, respectively. The highest CAA was seen at a concentration of 200

µg/mL (59.62 ± 1.70) for JSF-NP. A similar behavior was reported by Chang et al. (2020), who produced nanoemulsions of sea buckthorn pulp oil (SBPO) from different places of origin through the high-pressure homogenization process and evaluated CAA in HepG2 cells. The results indicated that all nanoemulsions showed good cellular antioxidant activity (310.54 ± 21.48 µmol of QE/g oil - SBPO-Q; 307.71 ± 21.86 µmol of QE/g oil - SBPO-G; 255.24 ± 10.55 µmol of QE/g oil - SBPO–H; 238.03 ± 10.09 µmol of QE/g oil - SBPO–H), and the difference in CAA values may be related to the chemical composition of oil from different locations. The authors reported that antioxidant activity is related to the combined effect of triacylglycerols and phytochemicals in oils, especially phenolic compounds, and that further studies are needed to examine this relationship.

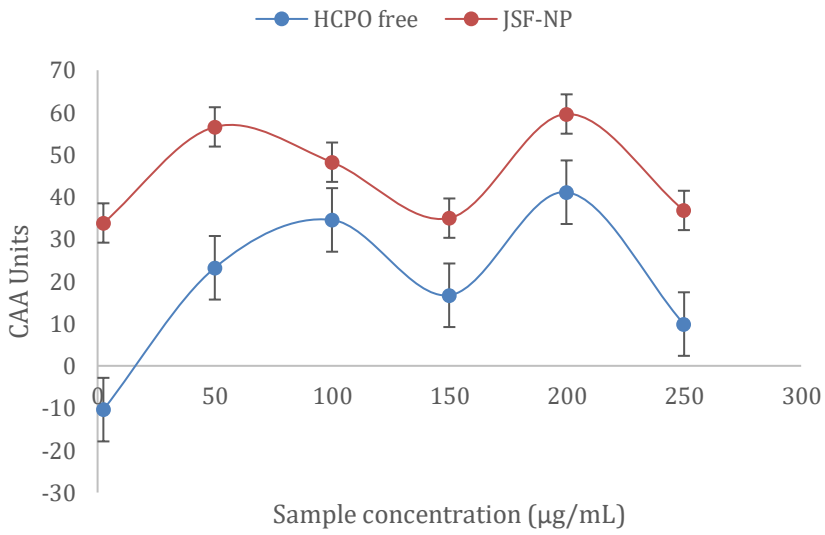

Figure 3. Cellular antioxidant activity (CAA) of HCPO and JSF-NP in Caco-2 cell. CAA units, a value reflecting cellular antioxidant capacity, were determined at the fluorescence excitation/emission wavelength pair of 485 nm and 538 nm. The data represent mean ± standard deviation based on triplicate determinations.

According to Yan et al. (2019), fatty acids with different chain lengths or degrees of saturation could alter the morphology and fluidity of the cell membrane, which may explain the difference in the absorption and bioavailability of antioxidant components in cells. Additionally, oils with a similar composition of fatty acids may exhibit different CAA due to their phytochemical composition.

Chung, Lee and Lee (2020), who developed resveratrol nanoparticles with chitosan and γ-poly (glutamic acid) (γ-PGA) as wall materials and assessed CAA in HepG2 cells, observed that the reduction in particle size led to an increase in the contact surface area between the

encapsulated material and the medium, also enhancing solubility and cellular absorption. This resulted in better CAA results compared to free oil (≈ 40 CAA units for free oil and ≈ 60; ≈ 45 CAA units for nanoparticles), similar to what was observed in the present study. Yu, Li, Shi and Huang (2011), who encapsulated curcuminoids in modified ε-polylysine, found similar results. The CAA value of the nanoparticle was higher when compared to the free curcuminoid (≈ 60 CAA units for the nanoparticle and ≈ 40 CAA units for free curcuminoid), resulting in greater solubility, more specific interaction with the cell, and less degradation of the encapsulated material.

The CAA values for JAF-NP were also higher than those of the free oil under most concentrations. The 200 µg/mL concentration was the only instance where the CAA of the nanoparticle was lower than that of the free oil (HCPO) (35.20 ± 1.50 JAF-NP and 41.11 ± 2.70 HCPO free) (Figure 4). These findings suggest that the nanoparticles can be used as a delivery system for HCPO, enhancing its bioavailability as compared to the free oil. Similar results were reported by Chang et al. (2020) when assessing CAA in nanoemulsions of sea buckthorn pulp oil and underscored the notable antioxidant activity compared to free oil, suggesting that nanoencapsulation holds potential as a carrier for nutraceuticals, effectively preserving the active compounds. It is noteworthy to mention that in the current study, besides the preservation of bioactive compounds facilitated by the encapsulation process, the flours used as encapsulants (JSF and JAF) also include antioxidants (phenolic compounds, including flavonoids). Consequently, there is a possibility of an additional impact on CAA (Suzihaque et al., 2022; Li et al., 2021).

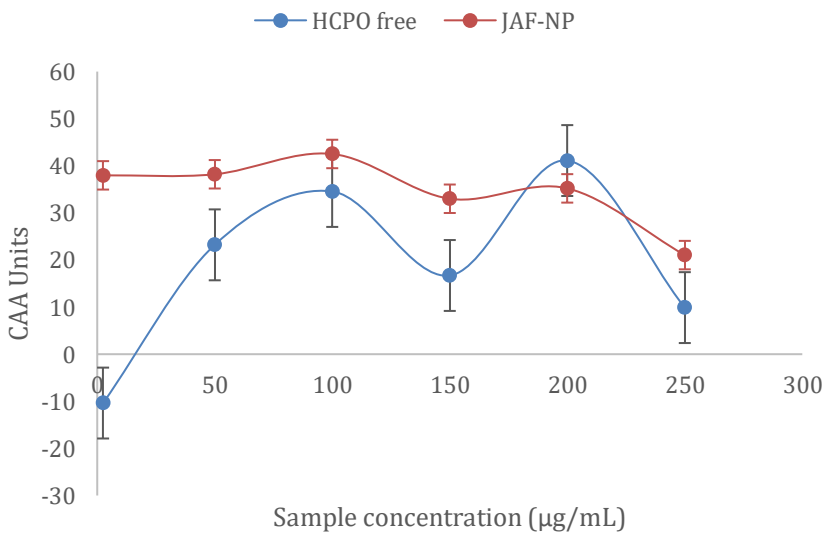

Figure 4. Cellular antioxidant activity (CAA) of HCPO and JAF-NP in Caco-2 cell. CAA units, a value reflecting cellular antioxidant capacity, were determined at the fluorescence

excitation/emission wavelength pair of 485 nm and 538 nm. The data represent mean ± standard e deviation based on triplicate determinations.

Fan et al. (2018) when encapsulating curcumin with bovine serum albumin (BSA)-dextran and assessing CAA in Caco-2 cells. The encapsulated compound showed higher CAA (65.35 units) than its free form (48.61 units), mainly attributed to the small size of the nanoparticles, leading to greater absorption of encapsulated curcumin and thus higher cellular antioxidant activity. According to the authors, the larger surface areas of the absorbed nanoparticles may lead to higher interaction with peroxyl radicals, neutralizing them and increasing CAA. Similar results were also reported by Yi et al. (2014), who produced β-carotene nanoparticles by homogenization-evaporation with different ratios of sodium caseinate, whey protein isolate, or soy protein isolate as wall materials and assessed CAA in Caco-2 cells.

Comparison with literature data is challenging, as only four studies evaluating the CAA of vegetable oils have been found. However, these studies did not involve the production of nanoparticles, and they assessed antioxidant activity of specific constituents of oils: tocopherols, sterols, and phenolic compounds in 15 different types of vegetable oils (Liu et al. 2020), α-tocopherol and γ-oryzanol in rice bran oil and coconut oil (Xu et al. 2021), phytosterols, squalene, γ-oryzanol, and polyphenols in rice bran oil (Liu et al. 2019), and tocopherol in dehulled corn oil (Zheng et al. 2020). Only one study assessed the CAA in nanoemulsions of sea buckthorn pulp oil (Chang et al., 2020), but in HepG2 cells. As a result, the evaluation of biological activity becomes essential to understand various aspects such as uptake, absorption, metabolism, and the location of antioxidant compounds within cells. Consequently, CAA has emerged as a crucial tool in investigating the potential bioactivity of antioxidants in natural sources, including vegetable oils. Thus, these findings emphasize the importance of studying the cellular antioxidant activity of bioactive-rich oil nanoparticles on different cell lines.

3.3. Cytotoxicity assay

Figure 5 shows the MTT results for JAF-NP, JSF-NP, and HCPO free using Caco-2 cells after 4 h of incubation. No statistical difference ($p > 0.05$) was observed between the different tested concentrations for the nanoparticles. In JSF-NP, concentrations of 150 and 200 µg/mL promoted proliferative effects on cells, increasing cell viability to 101.37 ± 10.90 and

113.18 ± 21.88%, respectively. The concentration of 100 µg/mL resulted in a slight decrease in average cell viability of approximately 10.44% compared to the control (without any antioxidant); however, as there was no statistical difference ($p > 0.05$) regarding the control, this concentration was not considered cytotoxic (Figure 3A). In JAF-NP, the concentration of 250 µg/mL increased cell viability to 104.12 ± 7.91%, indicating a proliferative effect on Caco-2 cells. At the concentration of 2.5 µg/mL, there was a reduction of 9.07% in cell viability, even though cytotoxicity was not recorded ($p > 0.05$) (Figure 5).

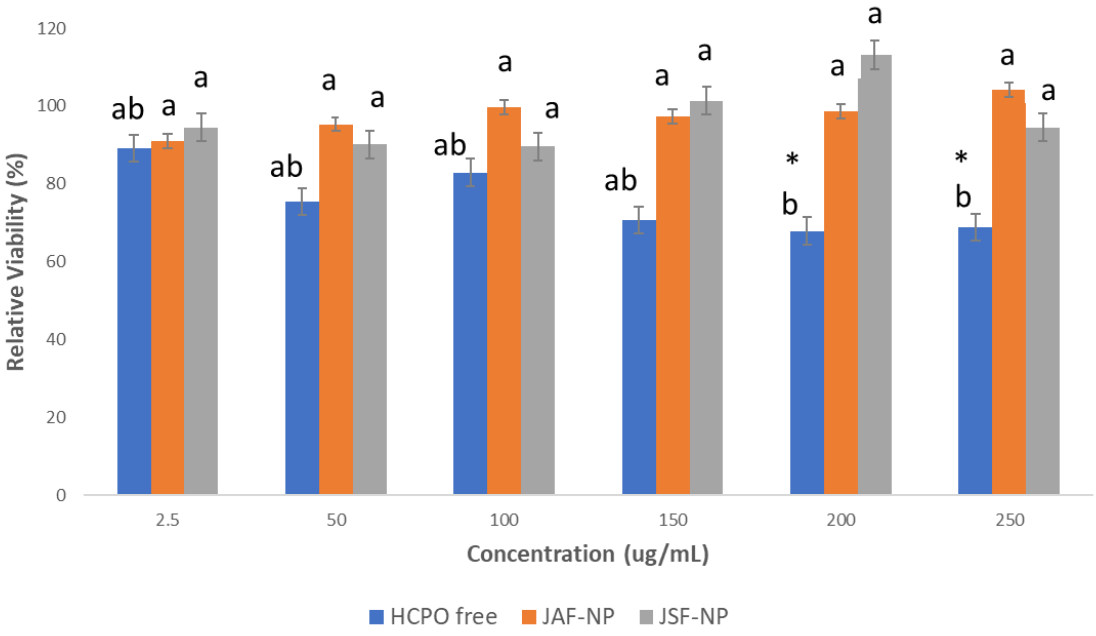

Figure 5. Results of cytotoxicity assay in JAF-NP, JSF-NP and HCPO free on differential Caco-2 cells according to MTT assay. Data reported as mean values for each sample ± standard deviation (n = 3). Different lowercase letters indicate significant differences ($p < 0.05$) in the concentrations tested. *Significant difference between different concentrations of the samples and negative control (cytotoxicity) ($p < 0.05$).

Cell viability increase at selected concentrations (150 and 200 µg/mL for JSF-NP and 250 µg/mL for JAF-NP) may be attributed to the stimulation of cells, probably due to the promotion of cell health as well as higher metabolic activity caused by HCPO nanoparticles. According to Ramiro-Puig and Castell (2009), some compounds with antioxidant properties, such as flavonoids, might exhibit immunostimulatory effects by modulation of gene expression. It is worth noting that antioxidant compounds are present in flours used as wall material (phenolic compounds, including flavonoids) and in HCPO (carotenoids, phenolic compounds) (Suzihaque et al., 2022; Li et al., 2021).

Regarding the free oil, there was a statistical difference ($p < 0.05$) at concentrations 200 and 250 µg/mL regarding the negative control. Therefore, HCPO was considered cytotoxic to Caco-2 cells at these studied concentrations, and there was no cell proliferation (> 100%) at any of the tested concentrations. According to Nano, Nobili, Girard-Pipau and Rampal (2003), a possible cytotoxic action may occur through contact between fatty acids and cell membranes, modifying the lipid profile of the cell membrane, altering lipid-mediated signaling pathways, lipid peroxidation, and fluidity, causing a lower cellular viability.

Furthermore, it was observed that nanoencapsulation, a process in which particles of nanometric size (smaller than 1 µm) are formed and protected by a wall material (encapsulant), may have provided protection for the encapsulated oil, which may explain the non-cytotoxicity of the nanoparticles compared to the free oil (Pathak, Vaidya, and Pandey, 2019).

Marchiori et al. (2017) reported similar results when assessing the cytotoxicity through the MTT assay of pomegranate oil nanocapsules. The study demonstrated 100% cell viability for the nanoencapsulated form, whereas the free oil exhibited a 30% reduction in cell viability at concentrations of 145 and 724 µg/mL.

Pomegranate seed oil was also encapsulated by Mota-Ferreira et al. (2016), using the spontaneous emulsification technique, and evaluated toxicity in mononuclear cells (MTT). Most concentrations tested showed increased cell viability compared to the control. Only at 0.5 mg/mL reduced cell viability was noted, and even in the nanoemulsions form, it showed less effect than the free oil (28.33 ± 0.73% for nanoemulsions and 89.57 ± 0.25% in free form), as in the present study. Reis et al. (2020), who developed nanoemulsions and nanostructured lipid carriers with buriti oil, assessed cytotoxicity by the Sulforhodamine B assay in Caco-2 and HepG2 cells (human hepatocellular carcinoma). In their study, the samples did not cause decrease of cell viability. Moreover, the authors described that the bioactive compounds in buriti oil likely acted as antioxidants, as cell glutathione consumption was not detected.

According to Schappo, Ferreira-Ribeiro, Farina and Nunes (2021), studies on toxicity of oil nanoparticles are still rare in the literature, but the authors emphasize that the results are promising. In most studies, oil nanoparticles are considered safe, as observed in the present study, and several samples exhibited some protective effects on cells, especially anticancer and antioxidant effects. However, comparison with literature data is challenging, primarily due to the scarce number of studies evaluating the cytotoxicity of oil nanoparticles using the MTT assay in differentiated Caco-2 cells under the conditions used in the present research. Some studies use different assays (Sulforhodamine B and Alamar Blue®) and cell cultures, such as THLE2, Hep-G2, among others. Thus, lack of data related to the toxicity of vegetable oil

nanoparticles underscores the need for additional studies. Furthermore, differentiated Caco-2 cells resemble small intestinal epithelial cells regarding to morphology, marker enzyme, microvillar structure, tight junction, permeability and functionally (Kellett, Greenspand and Pegg, 2018). Therefore, the absence of cytotoxicity from JSF-NP and JAF-NP may indicate biological safety to intestinal epithelial cells.

Conclusion

Nanoparticles produced with hybrid crude palm oil (HIE OxG) and jackfruit by-products as encapsulants exhibited favorable characteristics, including nanoscale diameters (<250 nm), uniformity and good stability. Cellular antioxidant activity (CAA) levels for JSF-NP and JAF-NP were higher than those of the free HCPO at all concentrations studied in differentiated human colorectal adenocarcinoma (Caco-2) cells. These findings suggest the potential of nanoparticles to be used as a delivery system for HCPO, enhancing its bioavailability as compared to the free oil. The nanoparticles did not show cytotoxicity toward Caco-2 cells and concentrations of 150, 200 and 250 µg/mL for JAF-NP and JAF-NP, respectively, promoted proliferative effects on cells, increasing relative viability. On the other hand, free HCPO at concentrations of 200 and 250 µg/mL were cytotoxic. Therefore, it was observed that nanoencapsulation may have provided protection to the oil and the non-cytotoxic nature of JSF-NP and JAF-NP indicates biological safety and mitigation of oxidative stress in intestinal epithelial cells.

Credit authorship contribution statement

Larissa Santos Assunção: Formal analysis, Investigation, Methodology, Data Curation, Roles/Writing - original draft, Writing - review & editing, Visualization. **Camila Duarte Ferreira Ribeiro:** Funding acquisition, Supervision, Writing - review & editing, Visualization. **Carolina Oliveira de Souza:** Writing - review & editing. **Renan Danielski:** Methodology, Formal analysis, Writing - review & editing, Visualization. **Sarika Kumari:** Methodology, Formal analysis, Writing - review & editing, Visualization. **Itaciara Larroza**

Nunes: Writing - review & editing. **Fereidoon Shahidi:** Conceptualization, Supervision, Writing - review & editing, Visualization.

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Conflict of interest

All authors declare that they have no conflicts of interest.

Ethical approval

Not applicable.

Informed consent

Not applicable.

References

Ashraf, A.S. et al. (2021) Innovations in nanoscience for the sustainable development of food and agriculture with implications on health and environment. *Science of The Total Environment*, v. 768, p. 144-990.

Ayoub, M., Camargo, A. C., & Shahidi, F. (2016). Antioxidants and bioactivities of free, esterified, and insoluble-bound phenolics from berry seed meals. *Food Chemistry*, v. 197, p. 221–232.

Chang, M. et al. (2020). Sea buckthorn pulp oil nanoemulsions fabricated by ultra-high pressure homogenization process: A promising carrier for nutraceutical. *Journal of Food Engineering*, v. 287, p. 110129.

Chen, X.; Zou, L.; Niu, J.; Liu, W.; Peng, S.; Liu, C. (2015) The Stability, Sustained Release and Cellular Antioxidant Activity of Curcumin Nanoliposomes. *Molecules*, 20, 14293-14311; doi:10.3390/molecules200814293

Cheong, A. M.; Nyam, K. L. (2016) Improvement of physical stability of kenaf seed oil-in-water nanoemulsions by addition of β-cyclodextrin to primary emulsion containing sodium caseinate and Tween 20. Journal of Food Engineering, v. 183, p-24-31.

Chung, J. H.; Lee, J.; Lee, H. G. (2020) Resveratrol-loaded chitosan–γ-poly (glutamic acid) nanoparticles: Optimization, solubility, UV stability, and cellular antioxidant activity. *Colloids and Surfaces B: Biointerfaces*, v. 186, p. 110702.

Codex Alimentarius. Standard for Named Vegetable Oils. CXS 210-1999, Amended in 2022.

Danielski, R.; Shahidi, F. (2023) Guava processing waste: Biological activity profile of a natural and sustainable source of phenolic antioxidants. *Food Bioscience*, v. 56, 103284.

Fan, Y.; Yi, J.; Zhang, Y.; Yokoyama, W. (2018) Fabrication of curcumin-loaded bovine serum albumin (BSA)-dextran nanoparticles and the cellular antioxidant activity. *Food Chemistry*, v. 239, p. 1210–1218.

Ferreira, C. D., & Nunes, I. L. (2019). Oil nanoencapsulation: development, application, and incorporation into the food market. *Nanoscale Research Letters*, 14. https://doi.org/10.1186/s11671-018-2829-2

Ferreira Ribeiro, C. D., Schappo, F. B., Sales, I. da S., Assunção, L. S., Otero, D. M., Magalhães-Guedes, K. T., Machado, B. A. S., Block, J. M., Druzian, J. I., & Nunes, I. L. (2022). Novel bioactive nanoparticles from crude palm oil and its fractions as foodstuff ingredients. *Food Chemistry*, DOI https://doi.org/10.1016/j.foodchem.2021.131252

Gentile, C., Allegra, M., Angileri, F., Pintaudi, A. M., Livrea, M. A., & Tesoriere, L. (2012). Polymeric proanthocyanidins from Sicilian pistachio (Pistacia vera L.) nut extract inhibit lipopolysaccharide-induced inflammatory response in RAW 264.7 cells. *European Journal of Nutrition*, 51, 353–363.

Kellett, M. E.; Greenspan, P.; Pegg, R. B. (2018) Modification of the cellular antioxidant activity (CAA) assay to study phenolic antioxidants in a Caco-2 cell line. *Food chemistry*, 244, p. 359-363.

Li, Z., Lan, Y., Miao, J., Chen, X., Chen, B., Liu, G., Wu, X., Zhu, X., & Cao, Y. (2021). Phytochemicals, antioxidant capacity and cytoprotective effects of jackfruit (Artocarpus heterophyllus Lam.) axis extracts on HepG2 cells. *Food Bioscience*, v. 41, p. 100933. https://doi.org/10.1016/j.fbio.2021.100933

Liu, R. J., Liu, R. R., Shi, L. K., Zhang, Z. Y., Zhang, T., Lu, M. Y., et al. (2019). Effect of refining process on physicochemical parameters, chemical compositions and in vitro

antioxidant activities of rice bran oil. *LWT-Food Science and Technology*, v. 109, p. 26–32. https://doi.org/10.1016/j.lwt.2019.03.096.

Liu, R.; Lu, M.; Zhang, T.; Zhang, Z.; Jin, Q.; Chang, M.; Wang, X. (2020) Evaluation ofthe Antioxidant Properties of Micronutrients in Different Vegetable Oils. *Eur. J. Lipid Sci. Technol*, v. 122, p. 1900079.

Lu, M.; Zhang, T.; Jiang, Z.; Guo, Y.; Qiu, F.; Liu, R.; Zhang, L.; Chang, M.; Liu, R.; Jin, Q.; Wang, X. (2020) Physical properties, and cellular antioxidant activity of vegetable oil emulsions with different chain lengths and saturation of triglycerides. *LWT - Food Science and Technology*, v. 121, p. 108948.

Lucci, P.; Borrero, M.; Ruiz, A.; Pacetti, D.; Frega, N. G.; Diez, O.; Ojeda, M.; Gagliardi, R.; Parra, L.; Angel, M. (2016) Palm oil and cardiovascular disease: a randomized trial of the effects of hybrid palm oil supplementation on human plasma lipid patterns. *Food function*, 7, 347-354.

Marchiori, M. C. L.; Rigon, C.; Copetti, P. M.; Sagrillo, M. R.; Cruz, L. (2017). Nanoencapsulation Improves Scavenging Capacity and Decreases Cytotoxicity of Silibinin and Pomegranate Oil Association. *AAPS PharmSciTech*, v. 18, n. 8. DOI: 10.1208/s12249-017-0810-5.

Mota-Ferreira, L.; Gehrcke, M.; Cervi, V. F.; Bitencourt, P. E. R.; Da Silveira, E. F.; Azambuja, J. H.; Ramos, A. P..; Nascimento, K.; Beatriz Moretto, M.; Braganhol, E. (2016) Pomegranate Seed Oil Nanoemulsions with Selective Antiglioma Activity: Optimization and Evaluation of Cytotoxicity, Genotoxicity and Oxidative Effects on Mononuclear Cells. *Pharm. Biol*, v. 54(12), p. 2968–2977.

Mozzon, M.; Foligni, R.; Tylewicz, U. (2018) Chemical Characteristics and Nutritional Properties of Hybrid Palm Oils. *In*: WAISUNDARA, V. *Palm Oils, Intechopen*, cap. 9, p. 149-170.

Nano, J.-L., Nobili, C., Girard-Pipau, F., & Rampal, P. (2003). Effects of fatty acids on the growth of Caco-2 cells. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 69(4), 207–215. https://doi.org/10.1016/s0952-3278(03)00083-8.

Pathak, C.; Vaidya, F.U.; Pandey, S.M. (2019). Mechanism for development of nanobased drug delivery system. *Appl Target Nano Drugs Deliv Syst* 35–67.

Pathak, N.; Sing, S.; Singh, P.; Sing, P. K.; Singh, R.; Bala, B. V.; T. B. V.; Gaur, R.; Tripathi, M. (2022) Valorization of jackfruit waste into value added products and their potential applications. *Frontiers in Nutrition*. 9:1061098. doi: 10.3389/fnut.2022.1061098

Pinto, S. S.; Lopes, R.; Cunha, R. N. V. da; Filho, L. P. dos S.; Moura, J. I. L. (2019) Produção e Composição de Cachos e Incidência do Anel Vermelho em Híbridos Interespecíficos de Caiaué com Dendezeiro no Sul da Bahia. *Agrotrópica*, v. 31, n. 1, p. 5 -16.

Ramiro-Puig E, Castell M. (2009). Cocoa: antioxidant and immunomodulator. *Br J Nutr*. 101:931–940.

Reis, L.; de, C.; Leão, K. M. M.; Ribeiro, A. P. B.; de Jesus, M. B.; Macedo, G. A.; Macedo, J. A. (2020) Evaluation of Cytotoxicity of Nanolipid Carriers with Structured Buriti Oil in the Caco-2 and HepG2 Cell Lines. *Bioprocess Biosyst. Eng*, 43(6), 1105–1118. DOI: 10.1007/s00449-020-02308-6.

Ricaurte, L., Santagapita, P. R., Díaz, L. E., & Quintanilla-Carvajal, M. X. (2020). Edible gelatin-based nanofibres loaded with oil encapsulating high-oleic palm oil emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, v. *595*, 124673. https://doi.org/10.1016/j.colsurfa.2020.124673

Romero, H. M.; Daza, E.; Ayala-Díaz, I.; Ruiz-Romero, R. (2021) High-Oleic Palm Oil (HOPO) Production from Parthenocarpic. Agronomy, n. 11, v. 290, p. 1–18.

Schappo, F. B.; Ferreira-Ribeiro, C. D.; Farina, M.; Nunes, I. L. (2021) The Toxicity of Oil Nanoparticles: A Review Focused on Food Science. *Food Reviews International*, v. 39(1), p. 1-17. DOI: https://doi.org/10.1080/87559129.2021.2008954

Sergent, T., Garsou, S., Schaut, A., De Saeger, S., Pussemier, L., Van Peteghem, C., Schneider, Y.-J. (2005). Differential modulation of ochratoxin A absorption across Caco-2 cells by dietary polyphenols, used at realistic intestinal concentrations. *Toxicology Letters*, 159,60–70.

Singh, T.; Shukla, S.; Kumar, P.; Wahla, V.; Bajpai, V.K.; Rather I.A. (2017). Application on nanotechnology in food science: perception and overview. *Front. Microbiol.* 8: 1–7.

Suzihaque, M. U. H., Zaki, N. A. M., Alwi, H., Ibrahim, U. K., Abd Karim, S. F., & Anuar, N. K. (2022). Jackfruit seed as an alternative replacement for starch flour. *Materials Today: Proceedings*, *63*, S451–S455. https://doi.org/10.1016/j.matpr.2022.04.117

Tezara, W.; Domínguez, T. S. T.; Loyaga, D. W.; Ortiz, R. N.; Chila, V. H. R.; Ortega, M. J. B. (2021) Photosynthetic activity of oil palm (*Elaeis guineensis*) and interspecific hybrid genotypes (*Elaeis oleifera* × *Elaeis guineensis*), and response of hybrids to water deficit. *Scientia Horticulturae, 287*.

Wan, H., Dong, L., Yu, X., Sun, H., & Li, Y. (2015). A Caco-2 cell-based quantitative antioxidant activity assay for antioxidants. *Food Chemistry*, 175, 601–608.

Wolfe, K. L., & Liu, R. H. (2007). Cellular antioxidant activity (CAA) assay for assessing antioxidants, foods, and dietary supplements. *Journal of Agricultural and Food Chemistry*, 55, 8896–8907.

Xie, Y., Kosińska, A., Xu, H., & Andlauer, W. (2013). Milk enhances intestinal absorption of green tea catechins in in vitro digestion/Caco-2 cells mode. *Food Research International*, 53, 793–800.

Xin, X., Zhang, H., Xu, G., Tan, Y., Zhang, J., & Lv, X. (2013). Influence of CTAB and SDS on the properties of oil-in-water nano-emulsion with paraffin and span 20/tween 20. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 418,60–67. http:// dx.doi.org/10.1016/j.colsurfa.2012.10.065

Xu, Y.; Zhang, T.; Lu, M.; Liu, R.; Guo, Y.; Liu, R.; Chang, M.; Wang, X. (2021) Effects of chain length and saturation of triglycerides on cellular antioxidant activity of vegetable oil emulsions. *LWT - Food Science and Technology*, v. 146, p. 111437.

Yan, Q.X., Tang, S.X., Zhou, C.S., Han, X.F., Tang, Z.L. (2019). Effects of free fatty acids with different chain lengths and degrees of saturability on the milk fat synthesis in primary cultured bovine mammary epithelial cells. J. Agric. *Food Chemistry*. 67 (31), 8485–8492. https://doi.org/10.1021/acs.jafc.9b02905.

Yi, J., Lam, T. I., Yokoyama, W., Cheng, L. W., & Zhong, F. (2014). Beta-carotene encapsulated in food protein nanoparticles reduces peroxyl radical oxidation in Caco-2 cells. *Food Hydrocolloids*, v. 43, p. 31–40.

Yu, H.; Li, J.; Shi, K.; Huang, Q. (2011) Structure of modified ε-polylysine micelles and their application in improving cellular antioxidant activity of curcuminoids. *Food Funct*. V. 2(7), p. 373–380. doi:10.1039/c1fo10053j.

Zheng, L.; Jin, J.; Karrar, E.; Xie, L.; Huang, J.; Chang, M.; Wang, X.; Zhang, H.; Jin, Q. (2020) Antioxidant Activity Evaluation of Tocored through Chemical Assays, Evaluation in Stripped Corn Oil, and CAA Assay. *Eur. J. Lipid Sci. Technol*. v. 122, p. 1900354.

Anexo 1.

Comprovante de submissão de artigo original na revista *Foods* (Capítulo II)

Optimization and characterization of hybrid crude palm oil Unaué HIE OxG nanoparticles with vegetable by-products as encapsulants

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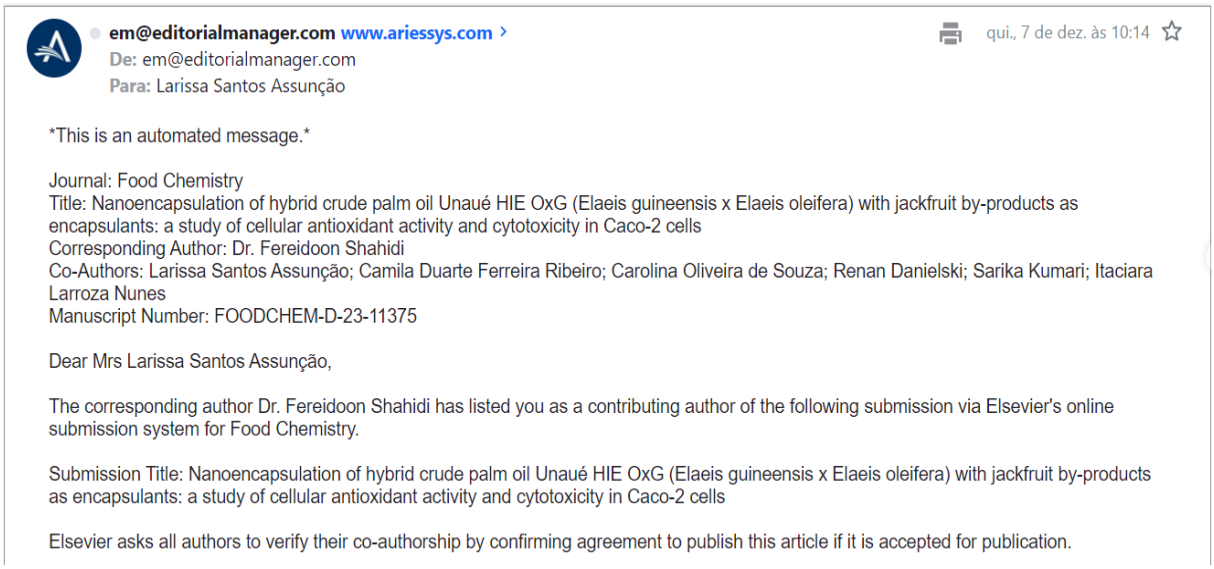
Received: 16 Dec 2023

Anexo 2.

Comprovante de submissão de artigo original na revista *Food Chemistry* (Capítulo III)

Nanoencapsulation of hybrid crude palm oil Unaué HIE OxG (Elaeis guineensis x Elaeis oleifera) with jackfruit by-products as encapsulants: a study of cellular antioxidant activity and cytotoxicity in Caco-2 cells



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Co-Authors: Larissa Santos Assunção; Camila Duarte Ferreira Ribeiro; Carolina Oliveira de Souza; Renan Danielski; Sarika Kumari; Itaciara Larroza Nunes
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Anexo 3.

Comprovante de depósito de documento de patente no Instituto Nacional da Propriedade Industrial (INPI) (Processo nº BR 10 2022 019533 1)

Nanopartículas de óleo de palma bruto híbrido Unaué HIE OxG (Elaeis guineensis x Elaeis oleifera), obtidas pelo método de homogeneização

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Dados do Pedido

Natureza Patente: 10 - Patente de Invenção (PI)

Título da Invenção ou Modelo de Utilidade (54): NANOPARTÍCULAS DE ÓLEO DE PALMA BRUTO HÍBRIDO UNAUÉ HIE OxG (ELAEIS GUINEENSIS X ELAEIS OLEIFERA), OBTIDAS PELO MÉTODO DE HOMOGENEIZAÇÃO

Resumo: A presente Patente de Invenção (PI) diz respeito ao desenvolvimento de nanopartículas (NP) pela técnica de homogeneização, do óleo de palma bruto híbrido (OPBH) ou azeite de dendê híbrido Unaué HIE OxG, utilizando como material de parede a farinha da semente de jaca (Artocarpus heterophyllus Lam.). A sua aplicação se dará nas indústrias alimentícia e farmacêutica, contribuindo para o aumento da utilização dessas matérias-primas. Na indústria de alimentos, elas poderão ser utilizadas seja para fins de fortificação de alimentos (alto teor de pro-vitamínico A) e/ou ação bioativa (potencial efeitocorante e antioxidante) e /ou conservante (potencial efeito antimicrobiano). As NP poderão ser inseridas, sem interferir nos atributos sensoriais dos alimentos, além disso, também poderão ser utilizadas em substituição ao uso de aditivos sintéticos, devido as propriedades antioxidantes e corantes do óleo. Essas NP poderão ser comercializadas pelo próprio produtor do OPBH, ou dos materiais de parede que as compõem, podendo ser inseridas como matéria-prima de outros alimentos, como produtos cárneos refrigerados ou congelados,margarinas, produtos de panificação, molhos e laticínios. Na indústria farmacêutica, as NP poderão ser incorporadas em cosméticos como loções hidratantes e sabonete, com vistas ao combate aos radicais livres, pelo efeito antioxidante, bem como por apresentar uma coloração possivelmente mais atrativa, sem o uso de corantes sintéticos. É importante ressaltar que a principal característica que difere essas NP das outras é de que não há nanopartículas elaboradas com óleo de palma bruto híbrido utilizando como material de parede a farinha da semente de jaca e álcool de cereais como solvente utilizado na elaboração destas NP. Dessa forma, essa pode ser uma invenção promissora e com impacto potencial do ponto de vista econômico, tecnológico, social, ambiental e científico.

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Documentos anexados

Tipo Anexo	Nome
Relatório Descritivo	01_Relatório descritivo_Patent_HCPO_NP_27.04.2022.pdf
Reivindicação	02_Reivindicações_Patent_HCPO_NP_27.04.2022.pdf
Resumo	03_Resumo_Patent_HCPO_NP_27.04.2022.pdf
Procuração	Procuração_SENAI_PROPRIEDADE_INDUSTRIAL_OLEO_DE_PALMA_UFBA.pdf
Procuração	Procuracao_UFSC__UFBA_SENAI-CIMATEC_Universidade_da_Georgia_assinado.pdf
Procuração	Procuração_UNIVERSIDADE-DA-GEÓRGIA_UFBA_SENAI-CIMATEC_UFSC_GG 97472_d.pdf
Procuração	Procuração_UFBA.pdf
Comprovante de pagamento de GRU 200	29409161952806010.pdf

Acesso ao Patrimônio Genético

☑ Declaração Negativa de Acesso - Declaro que o objeto do presente pedido de patente de invenção não foi obtido em decorrência de acesso à amostra de componente do Patrimônio Genético Brasileiro, o acesso foi realizado antes de 30 de junho de 2000, ou não se aplica.

Declaração de veracidade

☑ Declaro, sob as penas da lei, que todas as informações acima prestadas são completas e verdadeiras.

Anexo 4.

1º lugar no Prêmio Drª. Angeolina Rossi, na categoria de pós-graduação, área de Tecnologia de Alimentos

Nanopartículas otimizadas com azeite de dendê híbrido Unaué HIE OxG e coprodutos de jaca como encapsulantes

CERTIFICADO

Certificamos que o trabalho **"NANOPARTÍCULAS OTIMIZADAS COM AZEITE DE DENDÊ HÍBRIDO UNAUÉ HIE OxG E COPRODUTOS DE JACA COMO ENCAPSULANTES",** de autoria de **Larissa Santos Assunção, Tainara Santos Oliveira, Michelle Silva Rezende Santos, Luis Fernandes Pereira Santos, Carolina Oliveira de Souza e Camila Duarte Ferreira Ribeiro**, foi apresentado através de apresentação oral na categoria de **PÓS-GRADUAÇÃO**, na área de **TECNOLOGIA DE ALIMENTOS**, garantindo o **1º lugar** no **Prêmio Drª Angeolina Rossi**, evento realizado pelo **Conselho Regional de Nutricionistas da 5ª Região**, no dia 02/12/2023, de forma online, somando um total de 4h40 de atividades.

PRÊMIO Drª AngeolinaRossi 2023

Iza Charla da Silva Macedo
Presidente do CRN-5
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