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MICROENCAPSULACÃO DA OLEORESINA DE *CAPSICUM* POR SPRAY DRYING: CARACTERIZAÇÃO, TOXICIDADE E AVALIAÇÃO DA SUA ATIVIDADE BIOLÓGICA UTILIZANDO MODELO *IN VIVO*

MICROENCAPSULATION OF *CAPSICUM* OLEORESIN BY SPRAY-DRYING: CHARACTERIZATION, TOXICITY ASSAY, AND ITS BIOLOGICAL ACTIVITY USING THE *IN VIVO* MODEL

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Tese de Doutorado apresentada à Faculdade de Engenharia de alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para obtenção do título de Doutora em Engenharia de Alimentos.

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

Orientadora: PROFA. DRA. MIRIAM DUPAS HUBINGER

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“Existe uma coisa que uma longa existência me ensinou: toda a nossa ciência, comparada à realidade, é primitiva e inocente; e, portanto, é o que temos de mais valioso”.

Albert Einstein

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RESUMO

A obesidade é um problema da sociedade moderna que vem contribuindo para a redução da expectativa de vida e o aumento dos gastos dos públicos com saúde. Diante disso, as pesquisas na área de alimentos estão sendo impulsionadas a desenvolver alimentos funcionais que sejam capazes de contribuir para um consumo consciente e moderado de alimentos, que além de nutrir, favoreçam à saciedade. A oleoresina de *Capsicum* (pimenta) é um produto que vem sendo estudado por estimular a secreção dos hormônios ligados à indução da saciedade após sua ingestão e promover efeitos antiobesogênicos. No entanto, as limitações encontradas para o uso deste aditivo na forma livre estão relacionadas à sua baixa solubilidade em meio aquoso e sua pungência acentuada. Como alternativa a esses problemas, este estudo teve como principal objetivo produzir e caracterizar micropartículas de oleoresina de *Capsicum* utilizando diferentes carboidratos como materiais encapsulantes e avaliar o efeito dessas microparticulas no consumo alimentar e metabolismo dos camundongos. A tese foi dividida em cinco etapas. Primeiramente, foram obtidos novos materiais de parede a base de malte de cevada. O malte foi modificado usando ácido esteárico (2% m/m) em combinação com diferentes densidades de energia da sonda de ultrassom e como resultado, quatro novos agentes encapsulantes com boas propriedades emulsificantes foram obtidos. O malte modificado (MALT) em combinação com a goma arábica (GA) e o amido de milho modificado com OSA (EMCAP) foram testados individualmente e em combinação (GA:EMCAP; GA:MALT; EMCAP: MALT) na formação da emulsão de oleoresina de *Capsicum.* A combinação do MALT com a GA e com o EMCAP resultou em emulsões mais estáveis, com baixos valores de tensão interfacial (<8,7 mN/m) e alta retenção de capsaicina (>80%). Na sequência, todas as emulsões de oleoresina de pimenta foram submetidas à secagem por *spray drying*. A atividade biológica das micropartículas de oleoresina de *Capsicum* mostrou que a formulação (GA:MALT) com concentração de capsaicina variando entre 0,0022 e 0,0044% suplementadas em dietas hiperlipídicas resultaram em menor ganho de peso total dos camundongos. Nenhum efeito tóxico das microparticulas, oleoresina e materiais de parede contra as células CaCO-2 e HepG2 foi observado. O estudo de digestibilidade *in vitro* mostrou que a formulação MALT: GA apresentou alta entrega de capsaicina na fase intestinal. A partir dos resultados apresentados, o último ensaio *in vivo* provou que os grupos tratados com as microparticulas ricas em oleoresina de pimenta apresentaram níveis de leptina similares ao grupo magro. Os níveis de lipídeos totais, colesterol e triglicerídeos no fígado e colesterol no plasma também foram menores para os grupos tratados com as micropartículas quanto comparados ao grupo controle gordo. Desta forma, pode-se dizer que as micropartículas da oleoresina de *Capsicum* podem ser utilizadas como ingrediente promissor na prevenção dos efeitos adversos causados pelo consumo de dietas hiperlipídicas e consequentemente poderiam ser utilizadas como ferramenta para o controle da obesidade.

Palavras-chave*: carboidratos, capsaicina, pimenta, secagem por atomização, obesidade, leptina, metabolismo.*

ABSTRACT

In modern society, increasing obesity is a problem for reducing public life expectancy. Thus, food research is driven to develop conscious foods contributing to moderate food intake and nutrition. Studies regarding *Capsicum* oleoresin are carried out to evaluation on its effect on anti-obesity diseases. However, the consumption and usage of neat chili oleoresin in food are limited due to its low solubility and high pungency. *Capsicum* oleoresin microencapsulation is an alternative to overcoming these limitations. Microparticles of *Capsicum* oleoresin were produced using different carbohydrates as wall materials. Microparticles were evaluated regarding food consumption and metabolism in mice. This work was divided into five steps. First, the malt was modified using stearic acid (2% w/w) at different ultrasonic energy densities. As a result, four new encapsulating agents with good emulsifier properties were obtained. In sequence, modified malt (MALT with gum arabic (GA) and modified malt with EMCAP) were tested isolated and in combination (GA: MALT; EMCAP: MALT; GA: EMCAP) to produce *Capsicum* oleoresin emulsions. The combination of MALT with GA and with EMCAP resulted in more stable emulsions with low interfacial tension values (<8.7 mN/m) and high capsaicin retention (>80%). All *Capsicum* oleoresin emulsions oleoresin were submitted to spray drying. The biological activity of *Capsicum* oleoresin microparticles showed that the formulation GA: MALT with capsaicin concentration ranging from 0.0022 to 0.0044%, supplemented in high-fat diets, effectively reduced weight gain in mice. No toxic effects of microparticles, oleoresin, and wall materials against CaCO-2 and HepG2 cells were observed. The digestibility assay demonstrated that the MALT: GA formulation delivered a higher capsaicin content in the intestinal phase. Based on the results, the last *in vivo* trial proved that the groups fed with the *Capsicum* oleoresin-rich microparticles had leptin levels like the lean group. The liver's lipids levels, cholesterol and triglycerides, and plasma cholesterol were also lower for the groups treated with the microparticles compared to the fat control group. *Capsicum* oleoresin microparticles can be considered a promising ingredient in preventing the adverse effects caused by the consumption of high-fat diets and, consequently, could be used as a tool for obesity control.

Keywords: *carbohydrates, capsaicin, chili, spray-drying, obesity, leptin, metabolism.*

SUMÁRIO

CAPÍTULO 1

Introdução geral, objetivos e estrutura da tese

1.1 INTRODUÇÃO GERAL

Segundo a Organização Mundial da Saúde (OMS) o percentual de pessoas com excesso de peso em 2021 no Brasil representava 62% da população, sendo que 22% encontravam-se em estado de obesidade, aumento que foi acentuado pela pandemia COVID-19 (OMS, 2021). Doenças crônicas não transmissíveis, como doenças cardiovasculares, pulmonares, hipertensão arterial e *diabetes mellitus* têm sua incidência aumentada em decorrência do aumento de sobrepeso e obesidade na população, gerando danos socioeconômicos aos sistemas públicos de saúde e de seguridade social (ABESO, 2016).

Apesar de uma gama de ingredientes alimentícios ser fonte de compostos naturais, muitos deles se enquadram em alimentos que são desconhecidos pela população. Ricos em compostos bioativos, alguns alimentos, quando consumidos, podem auxiliar na redução da absorção de lipídeos e carboidratos, melhorando a sensibilidade à insulina e prevenindo a obesidade. No entanto, muitas vezes esses alimentos são subutilizados e seus benefícios são pouco conhecidos.

A oleoresina de *Capsicum*, um derivado da pimenta conhecida popularmente como oleoresina de pimenta, é rica em antioxidantes, carotenóides, compostos fenólicos e capsaicinóides, e possui potencial para ser utilizada no tratamento da obesidade, controle de apetite e diabetes (AGUIAR ET AL., 2022; MOREB ET AL., 2020). No entanto, sua baixa solubilidade, alta pungência e instabilidade dos seus compostos bioativos na presença de luz, oxigênio e calor, são fatores limitantes para sua aplicação direta em alimentos (BERKE & SHIEH, 2010).

Uma das alternativas que viabiliza o uso de oleoresinas na formulação de ingredientes é a sua encapsulação. A encapsulação consiste em "empacotar" ou imobilizar materiais líquidos, sólidos ou gasosos utilizando um ou mais polímeros (SOBEL, VERSIC, GAONKAR, 2014). Através da encapsulação, os materiais são reduzidos a tamanho nano ou micro e dependendo da técnica de encapsulação utilizada podem ser formadas cápsulas, esferas ou partículas (GHARSALLAOUI ET AL., 2007).

Nesse sentido, as oleoresinas podem ser encapsuladas através da técnica de emulsificação. As emulsões de oleoresinas são formadas por uma fase oleosa constituída pela oleoresina, que é dispersa na fase contínua composta por polímeros e água (AGUIAR ET AL., 2016). Essas emulsões, quando estáveis, podem ser aplicadas em produtos líquidos, como corantes naturais, agentes antioxidantes e antimicrobianos (CEREZAL-MESQUITA ET AL., 2021; IPAR, SINGHAL, DEVARAJAN, 2022). Já foi relatada na literatura a produção de emulsões com *blends* de duas

diferentes oleoresinas com o propósito de aumentar as propriedades antioxidantes para aplicação em alimentos (FERRAZ ET AL., 2021).

Estudos recentes vêm viabilizando a aplicação de oleoresina de especiariais através da emulsificação por microfluidização (AGUIAR ET AL., 2021), ultrassom (AGUIAR ET AL., 2016), rotor-stator (FERRAZ ET AL., 2021) e alta-pressão (ZHOU ET AL., 2020). A aplicação de emulsões formadas com oleoresina de pimenta em dietas animais evidenciou um efeito anti-obesogênico causado pelo aumento da bioacessibilidade dos capsaicinoides (KIM ET AL., 2014; JOSEPH ET AL. 2020).

Ademais, em combinação com a técnica de emulsão, a produção de oleoresina em pó por *spray drying* também vem ganhando cenário. A técnica de *spray drying* é simples quando comparada a liofilização, permite a obtenção de partículas com diferentes propriedades físico-quimicas através da modificação dos parâmetros de processo, sendo considerado um método escalonável (PATEL ET AL., 2022). Em outras palavras, pode-se dizer que a produção de partículas pelas técnicas de emulsificação seguida por *spray drying* tem como propósito aumentar a bioacessibilidade dos compostos melhorar a estabilidade da cor do ingrediente em pó frente às condições de armazenamento, ampliar sua aplicação em alimentos, melhorar as propriedades sensoriais do produto e favorecer a entrega dos compostos bioativos na fase intestinal (JAFARI, ASSADPOOR, HE e BHANDARI, 2008; VULIĆ ET AL., 2019; SANTOS ET AL., 2021). Apesar das vantagens relacionadas à encapsulação por *spray drying,* poucos estudos realizaram a produção da oleoresina de pimenta pelo referido método, sendo a produção desse ingrediente por esta técnica um interessante objeto de estudo.

Associado à encapsulação temos a escolha dos polímeros aplicados como agentes encapsulantes/emulsificantes/carreadores. Quando se trata da encapsulação por emulsão de um material lipofílico seguido pela secagem por *spray drying* a seleção do tipo de material é muito importante, uma vez que o material encapsulante precisa boa capacidade emulsificante, alta estabilidade, ter afinidade com o composto bioativo a ser encapsulado e apresentar baixa viscosidade (JAFARI, ASSADPOOR, HE e BHANDARI, 2008).

Dentre os materiais disponíveis para uso como material encapsulante pelo processo de *spray drying* podemos citar as proteínas, a gelatina, a maltodextrina, as gomas e os amidos modificados (McCLEMENTS, 2012). Além destes, novos materiais vêm sendo desenvolvidos com o propósito de melhorar a estabilidade da emulsão e aumentar a eficiência de encapsulação

do composto de interesse. Como exemplo, podemos mencionar o malte de cevada, um cereal rico em amido, β-glucana, proteína, compostos antioxidantes como ácido benzoico, os taninos e os flavonoides (LEITAO et al., 2012; MONTANUCI et al, 2013; FOROGASI et al., 2015; MONTANUCI et al., 2016) que após sua modificação físico-química pode tornar um potencial material encapsulante. A vantagem do uso do cereal maltado como agente encapsulante está associada ao seu alto valor nutricional quando comparado aos grãos não maltados, e aos benefícios que podem promover a saúde, como a redução do índice glicêmico, efeito anti-inflamatório e redução do estresse oxidativo (NELSON et al., 2016; LÓPEZ-MARTÍNEZ et al., 2017; MAETENS et al., 2017).

Nesse sentido, cabe ressaltar que a encapsulação da oleoresina de pimenta utilizando diferentes polissacarídeos como materiais encapsulantes ainda é pouco explorada. Os carboidratos apresentam muitas vantagens como materiais encapsulantes, tais como baixo custo, fácil obtenção, são não-alergênicos, apresentam boa solubilidade e alguns deles apresentam boa capacidade emulsificante (MARCILLO-PARRA, 2021, SAMBORSKA ET AL., 2021). Diante dos polissacarídeos disponíveis para encapsulação por emulsificação seguida por *spray drying*, os materiais convencionais como goma arábica, amido de milho esterificado com octanil succinico (OSA) em combinação com novos agentes encapsulantes como o malte de cevada modificado podem ser explorados a fim de trazer relevantes informações sobre seus efeitos nas propriedades da oleoresina de pimenta microencapsulada, pois essa combinação pode influenciar diretamente nas propriedades das micropartículas em relação à sua eficiência de encapsulação, solubilidade, estabilidade, propriedades de barreira, formação de filme, higroscopicidade, temperatura de transição vítrea, porosidade e morfologia (VASISHT, 2014). Aliado a isso, essas combinações podem ser interessantes na formulação de um novo ingrediente alimentar rico em capsaicina para aplicação em alimentos de base lipídica com propriedades funcionais relacionadas à redução de apetite, controle de peso e melhora no metabolismo dos lipídios.

1.2 OBJETIVOS

1.2.1 Objetivo geral

Desenvolver e caracterizar micropartículas da oleoresina de pimenta (*Capsicum*) obtidas por *spray dryer* e avaliar o efeito dessas micropartículas adicionadas em dieta *high-fat* sobre o consumo alimentar e metabolismo de camundongos.

1.2.2 Objetivos específicos

- Modificar o malte de cevada por ultrassom em diferentes densidades energéticas utilizando o ácido esteárico, a fim de obter um novo material com propriedades emulsificantes;

- Produzir e caracterizar emulsões de oleoresina de *Capsicum* em água utilizando goma Arábica (GA), amido de milho modificado-OSA (EMCAP) e malte modificado na forma isolada e combinada;

- Obter e caracterizar micropartículas de oleoresina de *Capsicum* por *spray drying* quanto às suas propriedades fisico-químicas, térmicas e morfológicas;

- Avaliar o efeito dos diferentes carboidratos nas propriedades das micropartículas de oleoresina de pimenta sobre sua toxicidade, usando modelos *in vivo* e *in vitro*.

- Estudar a estabilidade das partículas de oleoresina de *Capsicum* em diferentes temperaturas (25 e 45 ºC) quanto à cor instrumental e teor de capsaicina durante o armazenamento;

- Avaliar a digestibilidade *in vitro* das micropartículas de oleoresina de *Capsicum* através da quantificação de capsaicina.

- Avaliar o efeito da dieta contendo oleoresina de pimenta microencapsulada com os diferentes materiais de parede sobre o comportamento alimentar e metabólico de camundongos.

1.3 ESTRUTURA DA TESE

Esta tese de Doutorado foi dividida em 10 capítulos, conforme descrito a seguir:

No primeiro capítulo desta tese ***(Capítulo 1)*** é apresentada a introdução geral do trabalho, seguida pelos objetivos gerais e específicos que contribuíram para a realização dos artigos dessa tese.

O ***Capítulo 2*** apresenta a revisão bibliográfica desta tese. No referido capítulo, os principais e mais recentes trabalhos no tema foram citados e comentados. O objetivo deste capítulo foi de realizar o embasamento teórico para o desenvolvimento dos experimentos e comparação dos resultados.

O primeiro artigo publicado da tese está apresentado no ***Capítulo 3.*** O trabalho compreendeu a etapa de modificação do malte, utilizando ácido esteárico com diferentes densidades energéticas seguida da avaliação desses materiais como agentes emulsificantes. A respectiva etapa compõe o primeiro artigo da tese publicado na revista *Food and Bioprocess Technology (https://doi.org/10.1007/s11947-020-02569-9).*

O ***Capítulo 4*** deu continuidade ao trabalho publicado anteriormente. Nesta etapa, o malte após a modificação apresentou boas propriedades estabilizantes para emulsão óleo de canola em água, e por isso foi utilizado na produção de emulsões de oleoresina de *Capsicum* em água na forma isolada e em combinação com os materiais comerciais, como a goma arábica e o amido de milho modificado com OSA (ácido octanilsuccinico). Os resultados obtidos nessa fase foram escritos na forma de artigo, publicado na revista *Food and Bioprocess Technology (https://doi.org/10.1007/s11947-021-02728-6).*

O ***Capítulo 5*** dessa tese teve como objetivo apresentar um artigo contendo a produção e caracterização das micropartículas de oleoresina de *Capsicum* obtidas por *spray drying*. Este trabalho compreende o estudo de toxicidade dessas micropartículas em modelo experimental *in vivo*, e avalição do efeito dessas partículas no consumo alimentar de camundongos. A respectiva etapa compõe o terceiro artigo da tese que foi publicado na revista *Food Chemistry:X (https://doi.org/10.1016/j.fochx.2021.100179).*

Os resultados apresentados no ***Capítulo 6*** foram obtidos durante a realização do estágio de pesquisa na Technology University Dublin (Dublin, Irlanda) e University College Dublin, financiado pela FAPESP por meio da bolsa BEPE 2019/10432-1 durante os meses de dezembro de 2019 a março de 2021. A bolsa permitiu o desenvolvimento do projeto proposto por meio de

diversas novas linhas de investigação, incluindo cultura de células, transporte *in vitro* de micropartículas e ensaio de digestibilidade pelo método INFOGEST (MINEKUS ET AL., 2014). Os resultados apresentados nesta etapa mostram o efeito das micropartículas de oleoresina de *Capsicum e* da oleoresina de *Capsicum* não microencapsulada sobre as células Caco-2 e HepG2. O ensaio de toxicidade foi realizado com diferentes doses de micropartículas de oleoresina de *Capsicum* e comparadas com a oleoresina de *Capsicum* não encapsulada e com materiais de parede isolados. Além disso, a estabilidade das micropartículas armazenadas em diferentes temperaturas foi avaliada. Com o objetivo de avaliar a liberação da capsaicina, as micropartículas foram submetidas ao ensaio de digestão utilizando os fluidos que simulam as fases oral, estomacal e intestinal.

A última etapa dos resultados experimentais desta tese é representada pelo ***Capítulo 7.*** O objetivo desse estudo foi avaliar o efeito da oleoresina de pimenta adicionada na dieta hiperlipídica sobre os efeitos adversos causados pela obesidade e sobre o desempenho dos sinais de saciedade, utilizando camundongos como modelo experimental.

Todos os principais resultados apresentados na forma de artigo foram discutidos no ***Capítulo 8.*** Na sequência, o ***Capítulo 9*** conclui de forma geral os resultados apresentados em cada capítulo referente aos dados experimentais, e o ***Capítulo 10*** apresenta todas as referências que compreende este trabalho.

Por fim, a tese apresenta os **Apêndices** contendo as atividades gerais e divulgação dos resultados desenvolvidas durante o doutorado, e os certificados da Comissão de Ética no Uso de Animais, enquanto nos **Anexos** contem os comprovantes de permissão das revistas para o uso dos artigos já publicados nesta tese.

1.4 REFERÊNCIAS

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CAPÍTULO 2

Microencapsulation of Capsicum and its derivatives compounds: A review

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MICROENCAPSULATION OF *CAPSICUM* AND ITS DERIVATIVES COMPOUNDS: A REVIEW

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Abstract

Capsicum and its derivatives are natural ingredients rich in pigments, flavor, and antioxidant compounds. These plants can substitute many of the synthetic compounds in the industry. However, their incorporation into food is limited because these compounds are pungent, prone to oxidation, and poorly soluble in the aqueous phase. Microencapsulation technologies such as emulsification and spray drying promise to encapsulate these compounds and increase their commercial application in food, pharmaceutical, and cosmetic products. Spice formulations are composed of encapsulating matrices able to entrap the compounds to promote better stability and deliver a high concentration in the intestinal phase. Carbohydrates constitute one of the most important classes of compounds usually employed as encapsulating matrices. They are, in general, biopolymers with low-cost, allergenic-free, and abundant, considered potential matrices to obtain spice compound formulations. This review summarizes recent studies regarding spice compounds, their health benefits as food ingredients, encapsulating systems using carbohydrates as matrices, and their digestibility and release studies.

Keywords: *spray drying, digestive's enzymes, static digestion, chili pepper, health, nutrition.*

Capsicum and Derivatives Products: General Aspects

The genus *Capsicum* belongs to the Solanaceae family and is a horticultural species important to the economics, medicine, and nutraceutical fields (Momo *et al*., 2022). It is used in culinary dishes as a flavoring agent, which can partially replace the salt in food (Pickersgill, 2003).

Literature shows approximately 90 genera and nearly 3,000 species of *Capsicum, which* are widely distributed (Moreb *et al*., 2020). For example, Brazil has cultivated around 5,000 hectares of *Capsicum*, while China and India have more than 1,000,000 hectares similarly cultivated (Pelvine, 2019). There are five *Capsicum* types: *Capsicum annuum*; *Capsicum baccatum*; *Capsicum chinense*; *Capsicum frutescens*, and *Capsicum pubescens*. However, two of these species are widely used, namely *C. annuum* and *C. frutescens* (Berke & Shieh, 2012).

Paprika, chili peppers and oleoresin are derivatives of *Capsicum*. Chili pepper is a pungent derivative from *Capsicum* species employed to produce spice oleoresins (Berke & Shieh, 2012). Oleoresin products are concentrated products with high content of spices in small dosages since one kilogram of dry peppers results in 600 kilograms of spice (Ganapathy, 2022). They are nonpolar, dark red, with a pungent liquid widely used in industry (Berke & Shieh, 2012; Uquiche *et al.,* 2022).

Oleoresins are a mixture of resins and essential oils (fatty acids and triglycerides) extracted from distinct spices by hydro-diffusion, maceration, supercritical fluids, and organic solvents. Their extraction efficiency and quality depend on the method, temperature, type, and amount of solvent (Rao *et al*., 2021). The extraction operation using a solvent is commonly employed for large-scale production in the industry (Ganapathy, 2022). This process is characterized by extraction using a solvent such as acetone, ethylene dichloride, hexane, or alcohol, followed by a distillation step (Moln, 2017; Pickersgill, 2003; Rascon, Beristain, Garcia, & Salgado, 2011).

However, oleoresin extraction using solvents presents some disadvantages related to the toxicity of solvent, degradation of compounds due to heat, and the use of more steps to remove the excess solvent (Aguiar *et al.* 2013). Supercritical fluid extraction (SFE) is an alternate technology that presents many advantages compared to conventional extraction. SFE processes are considered clean labels and present short extraction times, specificity, and easy scalability (Soldan et al., 2021; Uquiche, 2022; Aguiar *et al*., 2022).

In general, extracts from spice oleoresins such as paprika and chili have achieved market in the last few years due to flavor, safety, and longer shelf-life (Ganapathy, 2022). Concerning its usage, these by-products find application in beverages, processed meat, soups, sauces, and the base of seasons (Berke & Shieh, 2012; Sá Mendes *et al.*, 2020).

Composition of Capsicum and its derivatives

Capsicum annuum, one the five species of the *Capsicum* genus, is mainly composed of carbohydrates (47.23% to 67.93%), fibers (22.66%), lipids (2.26% to 15.75%), protein (10.91% to 15.22%), moisture (3.48% - 11.23%) and ashes (5.08% to 9.78%) (Hernández-Pérez; Gómez-García; Valverde & Paredes-López, 2020).

As for the micronutrients, *Capsicum* fruits are reported as excellent sources of riboflavin, thiamin, folate, and niacin. The presence of minerals such as zinc (0,26 mg), potassium (322 mg), calcium (14 mg), iron (1.03 mg), magnesium (23 mg), phosphorus (43 mg) and sodium (9 mg) are also significative (Dhamodharan; Vengaimaran; Sankaran, 2022).

Furthermore, the high amounts capsaicinoids, capsainoids, carotenoids, and phenolic compounds in *Capsicum* and its derivatives make them remarkable sources of antioxidant compounds (Moreb et al., 2020).

Among the bioactive compounds in *Capsicum*, capsaicinoids are responsible for the spicy taste. Capsaicinoids are alkaloids formed by vanillylamides of branched fatty acids with 9–11 carbons. Its biosynthesis occurs in the placenta's epidermal cells and white rib in the middle of the pepper, along with the development of the aromatic region by the forming of a hydrophobic side chain connected via an amide bond (Sá Mendes & Gonçalves, 2020). Over 80% of capsaicinoids content is made of capsaicin (8-methyl-N-vanillyl-6-none amid C) and dihydrocapsaicin (8-methyl-N-vanillylnonanamide: DHC), and 20% are composed by nordihydrocapsaicin (n-DHC), homocapsaicin (h-C) and homodihydrocapsaicin (h-DHC) (Naves et al., 2019). According to scientific literature, hot peppers extracts show amounts of capsaicin and dihydrocapsaicin ranging between 35.1 to 2945 µg/g and 16.8 to 1016 µg/g, respectively. Capsaicin ($C_{18}H_{27}NO_3$ - MM = 305.41 g/gmol) presents lipophilic properties and has been employed in many applications in the pharmaceutical and medical field (Waheed et al., 2021; Adaszek et al., 2017). Peppers and their products are spicy due to capsaicinoids content. For industry purposes, these products' pungency is determined by quantifying capsaicinoids or sensory evaluation, expressed as Scoville Heat Unit

(SHU) (Aguiar et al., 2022). *Capsicum* peppers are generally recognized as safe (GRAS) for consumption and a good source of phytochemicals (Rao et al., 2021).

Regarding the properties of *Capsicum*, a study correlated antioxidant activity with carotenoid content. Double bonds in the carotenoid structure can capture the free radicals, which means the ability of these compounds to catalyze lipid peroxidation reactions in fatty foods. The oxidative index of *Capsicum* oleoresin obtained by supercritical carbon dioxide ranged from 14.5 to 20.3 mmol. Kg^{-1} carbonyl/oleoresin (Unique et al., 2022). Table 1 presents the bioactive compounds and antioxidant activities of distinct peppers sources.

Table 1. Main bioactive compounds and antioxidant activity of *Capsicum* and its derivatives

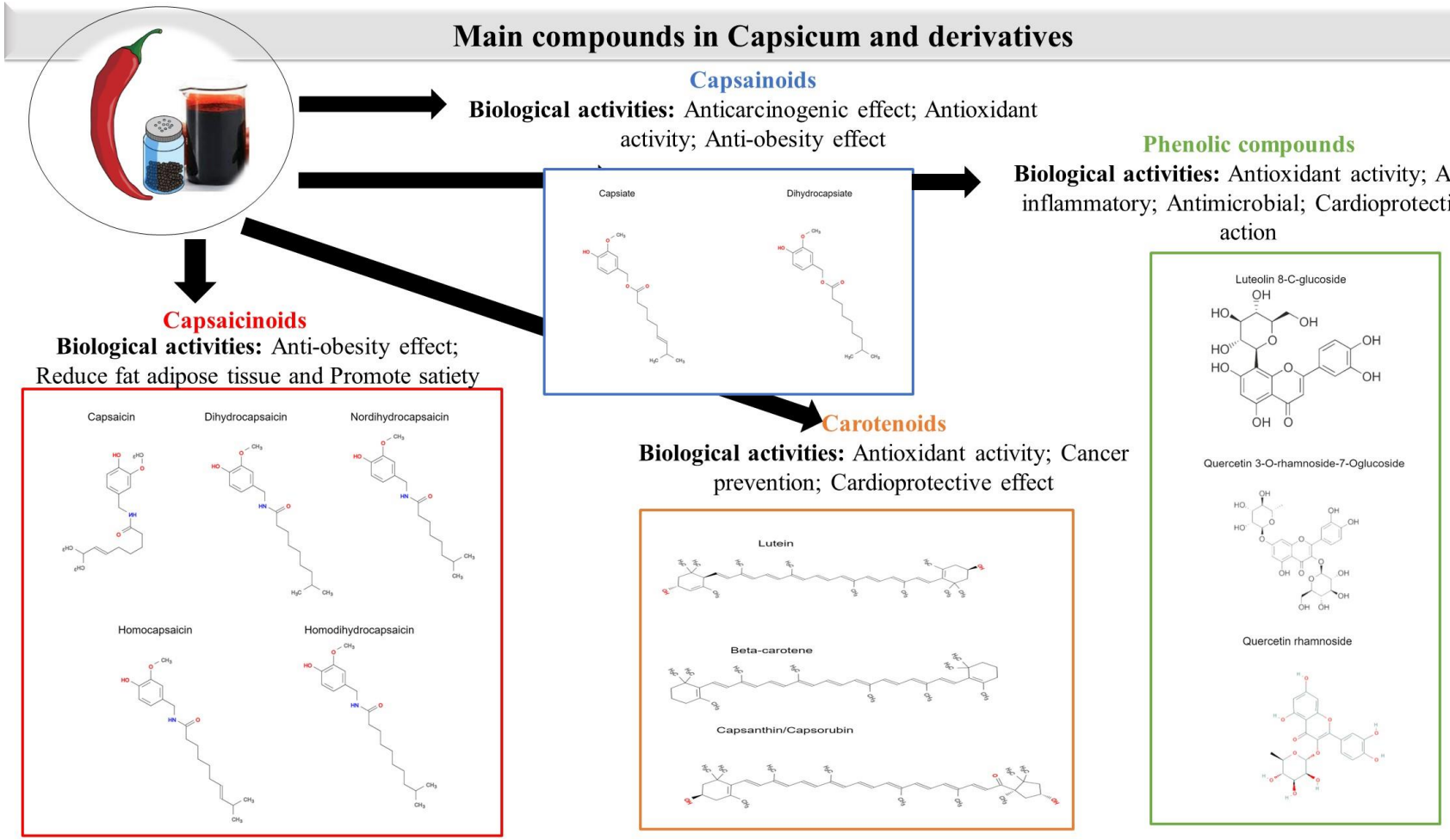
Source	Bioactive compounds	Antioxidant activity	Reference
Malagueta (*Capsicum frutescens)*	-Capsaicin (1020±86 µg/g fresh fruit) -Dihydrocapsaicin (496±61 µg/g fresh fruit) -Total phenolic compounds (36±2 mg GAE/g extract)	*	Aguiar *et al*. (2013)
Fresh red Pepper (*Capsicum*)	-Capsaicinoids (25.15 ± 0.84 (mg/l))	*	Dong *et al*. (2014)
***Capsicum* oleoresin**	-Carotenoids (99.47 ± 2.01- 338.29 ± 2.70 mg β-carotene equivalent per kg of dry weight) -Flavonoids (6541 ± 490- 20,576 ± 1325 mg catechin equivalent per kg of dry weight) -Total phenolic compounds (10,999 ± 125- 12,115 ± 141 (mg catechin equivalent per kg of dry weight))	*	Sricharoen *et al.* (2017)
Red peppers (cultivar Sarakura)	-Carotenoids (304 ±92 mg/g), -Capsanthin (146±35mg/g), b-carotene (9±3 mg/ g), provitamin A (103±44 mg RAE/100 g)	*	Agostini-Costa *et al*. (2017)
Biquinho pepper extract	-Capsiate 8.67 mg/g oleoresin -Rutin isomer (441 g/g extract) -Total phenolic compounds (11.3–11.9 mg GAE/g EBP)	-h-ORAC (68 ± 10 mol TE/g EBP -133 ± 42 mol TE/g EBP)	Aguiar *et al*. (2019)
Chili peppers	-Flavonoids (0.86 mg/g fresh weight-1.04 mg/g fresh weight)	-DPPH (0.50-0.77%) -ABTS (0.55-0.6%)	Mi *et al*. (2022)

* Data not presented

Among capsaicinoids, the capsinoids group is composed of capsiate and dihydrocapsiate, with an effect against obesity, in hunger control, and cancer prevention (Antonio; Wiedemanna & Junior; 2018). Other chemical compounds present in *Capsicum* and derivatives are carotenoids as lutein, beta-carotene, beta-cryptoxanthin, zeaxanthin, violaxanthin, capsanthin and capsorubin. These antioxidants are responsible for pigments of plants while its consupmtion is associated with a reduced risk of cancer and cardiovascular disease (Hernández-Pérez; Gómez-García; Valverde & Paredes-López, 2020). The main phenolic compounds present into chili peppers and derivatives are known as quercetin rhamnoside, quercetin 3-O-rhamnoside-7-Oglucoside, and luteolin 8-C-glucoside (Aguiar *et al.*, 2022). These constituents are high in antioxidant activity, present anti-inflammatory action, exhibit significant antimicrobial properties, and cardioprotective effects (Antonio; Wiedemanna & Junior; 2018; Dhamodharan; Vengaimaran; Sankaran, 2022).

The main compounds currently determined in *Capsicum* pepper fruits and their biological activity are presented in Figure 1.

Figure 1. *Capsicum* and derivatives' main compounds, their chemical structure, and biological activities



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Health benefits associated to Capsicum and its derivatives

Obesity is currently one of the major health problems of modern society, known as a public health issue. Nowadays, people have a challenging job routine; they exercise less and frequently do not have the availability to cook their own meals, hence consuming fast foods, or highly processed products with high contents of salt, and additives. Consequently, this increases the risk of developing insulin resistance, non-alcoholic fatty liver disease, elevated plasma triglycerides and cholesterol levels, and contributes to developing cardiovascular problems. All these diseases increase public health expenditure and contribute to the loss of quality of life (Luo, Peng & Li, 2011; Sandner et al., 2020).

Oleoresins, oils, and extracts from chili peppers are functional ingredients for their ability to help with weight management and protect against the metabolic consequences of obesity (Baboota *et al.*, 2014; Sandner *et al.*, 2020). Furthermore, chili pepper and its derivatives are responsible for health effects, like cardioprotective action, anti-inflammatory activity, and beneficial effects on the gastrointestinal system, and can also relieve pain and prevent cancer. In addition, they have thermogenic effects, promote anti-obesogenic action, and induce satiety (Ohyama *et al.*, 2016; Adaszek *et al.*, 2019; Cope, 2021; Zhang, 2021).

Capsaicinoid intake was able to reduce ad libitum energy intake by 309.9 kJ (74.0 kcal) $p<0.001$ during the meal (Whiting, Derbyshire & Tiwari, 2014). The antiobesogenic action of capsaicinoids from *Capsicum* sp. was also observed in a human clinical trial study, with daily consumption of 6mg for 12 weeks of capsaicinoids resulting in a decrease of abdominal adipose tissue when compared to the placebo group (Cope, 2021).

Among all capsaicinoids, capsaicin is considered the most important compound due to its multiple biological benefits (Ohyama *et al.*, 2016; Song *et al.*, 2017; Li, Yang & Lu, 2019). A study carried out by Shen *et al*. (2017) showed the action of the capsaicin compound and its relationship with the increase in the gut microbiota population. Mice fed a high-fat diet (High-fat) supplemented with capsaicin for 9 weeks reduced weight gain compared to the control group due to improved glucose homeostasis. Gut microbiota modulation and the increase in *the Akkermansia* population have been observed and associated with obesity prevention (Shen *et al.*, 2017). Lipid metabolism disorder was prevented by capsaicin, since this compound can regulate oxidative stress, decreasing the hepatic malonaldehyde content and increasing the glutathione peroxidase activity (Li, Yang & Lu, 2019).

Compounds from chili pepper can also stimulate appetite reduction (Hanse, Astrup & Sjödin, 2021). More specifically, there are two main orexigenic hormones responsible for body weight management. Firstly, leptin is a peptide hormone produced in adipose tissue that regulates food intake and energy expenditure (Feng *et al.*, 2014; Liu *et al.*, 2019). Secondly ghrelin, is a hormone responsible for stimulating hunger, weight gain, and growth hormone production (Rigamonti *et al*., 2018; Whiting, Derbyshire, Tiwari, et al., 2014). Liu et al. and Hanse, Astrup and Sjödin (2021) observed the modulation effect of chili pepper dietary intake in leptin and ghrelin body levels. Some literature reports regarding the effects of *Capsicum* and its derivatives compounon satiety and obesity control are summarized in Table 2.

Table 2. Evaluation of the effect of *Capsicum* and derivatives on anti-obesity parameters and hepatic metabolism

Experimental model	Treatment	Diet*	Period	Results	References
Mice (Swiss albino)	Capsaicin	Control, HFD fed and capsaicin (2 mg/kg, bw) + HFD	12 weeks	Increased expression of thermogenesis and promotes satiety effect	Baboota *et al.* (2014)
Mice (C57BL/6J)	Capsinoids	HFD supplemented with 0.3% (w/w) capsinoids,	8 weeks	Provoked thermogenic action	Ohyama *et al*. (2016)
Mice (C57BL/6J)	Capsaicin	Normal, low-capsaicin (0.01%), or high-capsaicin (0.02%) diet for 6 weeks	4 weeks	Inhibited the increase of fasting blood glucose and insulin levels	Song *et al*., (2017)
Rats	Capsaicinoids	Commercial solid diet + 3, 6, and 9 mg/kg·bw of capsaicinoids daily	4 weeks	Promoted hypoglycemic effect and inhibition of glucose absorption	Zhang *et al*. (2018)
Mice (C57BL/6J)	Capsaicin	Control diet; Beef-fat diet (standard chow containing 30% beef fa); Beef-fat diet plus capsaicin (0.001% capsaicin (0.9 mg/kg)).	12 weeks	Inhibited of obesity and dyslipidemia	Li, Yang & Lu (2019)
Guinea pigs	Capsaicin	Control, HFD (model); model + low-dose capsaicin (2.5 mg/kg); model + moderate-dose capsaicin (5 mg/kg); model + high-dose capsaicin (10 mg/kg), and model + simvastatin (1.5 mg/kg) (positive control)	14 weeks	Reduced hyperlipidemia and improvement of oxidative stress	Yang *et al.* (2019)
Mice (C57BL/6J)	Fermented *Capsicum*	Low-fat diet; HFD, HFD mixed with 0.05% EC (fresh *Capsicum*) (HFD-C); and another group was fed with HFD mixed with 0.05% EFC (fermented *Capsicum*) (HFD-FC)	12 weeks	Reduced levels of leptin and insulin and higher ghrelin levels	Liu *et al*. (2019)
Rats (Wistar)	Red chili (*Capsicum annum* L)	Normal diet (standard rodent chow) rats (NR) Group 2: High-fat diet rats (HFD) Group 3: HFD rats with CapF (Capsaicinoids encapsulated microbeads (CapF)) at 250 mg/kg b. wt. (HFD-C)	2 weeks	Improved anti-obesity effect	Joseph *et al*. (2021)
Mice (C57BL/6J)	*Capsicum* oleoresin microparticles	HFD, HFD + 0.022% capsaicin (CAP); HF+ 0.044% CAP; HF+ 0.0014% CAP; HF +0.0028%	4 weeks	Decreased weight gain	Anthero *et al*. (2022)

*HFD: High-fat diet; Control: normal diet; bw: body weight

Studies have used an *in vitro* assay to investigate compound activity due to its versatility, safety, and low cost compared to *in vivo* studies. Many bioactive compounds can inhibit enzyme activity. As is the case, low lipase activity reduces lipid absorption due to a delay in triglyceride hydrolysis, reducing fat storage and increasing weight loss (Zhang *et al*., 2014; Bort *et al*., 2019). The amylase inhibition activity retards carbohydrate absorption and decreases the rate of glucose on the plasm. The antidiabetic activity using bioassay by α-amylase showed that chili pepper extracts significantly inhibited the enzyme activity in the range of 20.61 ± 2.23% to 50.76 ± 1.46% (Sricharoen *et al.*, 2017).

Some relevant data regarding in vitro studies involving *Capsicum* and its derivatives are summarized in table 3.

Table 3. The effect caused by *Capsicum* and its derivatives on cell growth or enzymes activity.

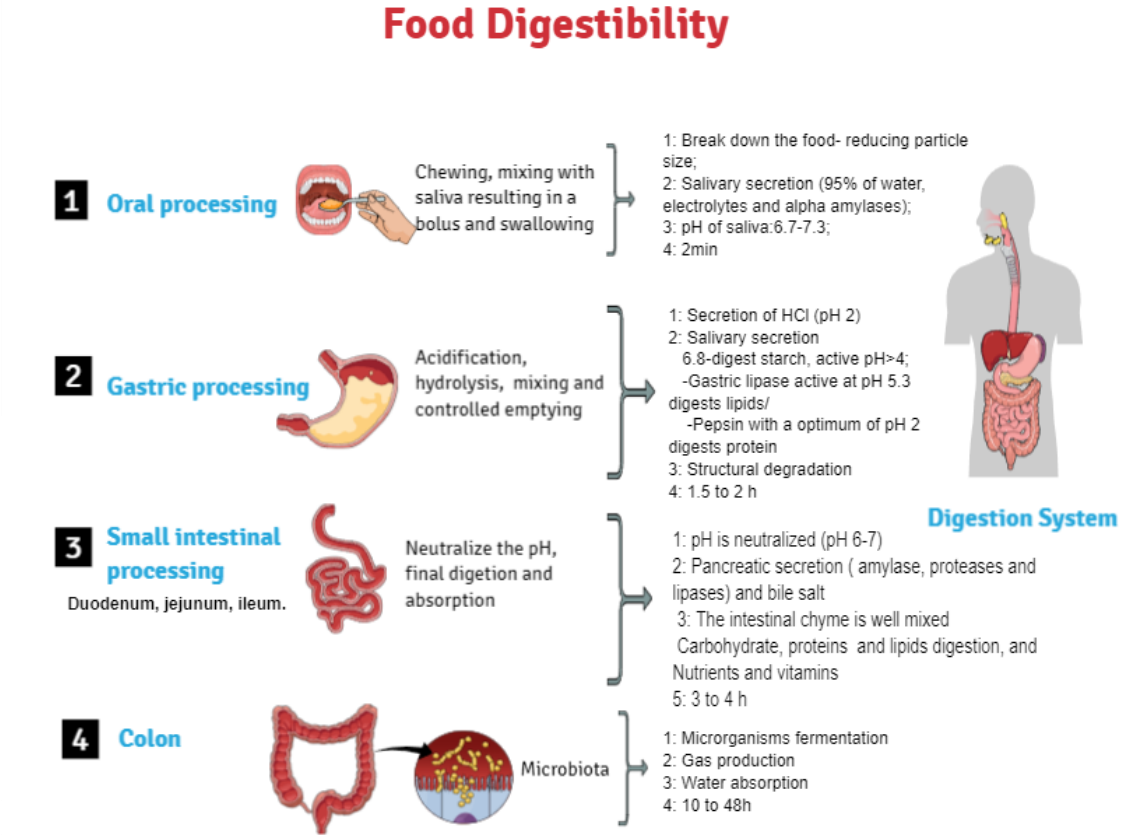
***In vitro* experimental model**	**Compound**	**Results**	**Reference**
3T3-L1 preadipocytes	Yellow *Capsicum* extract and capsaicin	The inhibitory action of cells grows, indicating that the compounds have obesity management potential	Zhang *et al.* (2014)
α- amylase	*Capsicum* oleoresin	Antidiabetic activity	Sricharoen *et al.* (2017)
HepG2 cells	Capsaicin	Decrease of neutral lipid content by 20% and 40%, respectively	Bort (2019)
Lipase	Capsaicin	Inhibitory action on lipase activity	Wu *et al.* (2022)

Digestibility, Bioaccessibility Studies for *Capsicum* and its Derivatives

Few studies have reported the release or digestibility of chili pepper and its derivatives within encapsulated matrices (Isaschar-Ovdat, Shani-Levi & Lesmes, 2021; Wu *et al*., 2022). In this sense, this topic intends to compile some of the more relevant published works regarding *Capsicum* pepper and its derivative's digestibility, which can be useful for the design microparticles loaded with these compounds for target delivery systems with functionality to health.

The human digestibility is composed of four main stages, namely (1) oral phase, (2) stomach phase, (3) intestinal phase in small intestine, and then (4) large intestinal phase. Figure 3 summarize this process.

Figure 3. Illustration of the human food digestion process



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In vitro digestibility is defined as a simulation without using a human system but with similar conditions close to the human body (Minekus *et al*., 2014). Protocols present different requirements regarding pH, time of digestion, type, and concentration of enzymes and bile (Dupont & Inra, 2016). Models formed by mono-compartmental systems are formed by the presence of a container, and the multi-compartmental digestion is characterized by various vessels able to simulate distinct steps of the digestive process. Additionally, models may present mechanical actions, changes in the flow, mixing, gut contractions and even variations of pH. They are known as a dynamic system, whereas *in vitro* system with fixed conditions is referred to as static model (Muttakin *et al*., 2019*).*

Static models are simulated in a single compartment, such as in Falcon tubes. This method is characterized by immersing the food in subsequent stages, separately, containing gastric and intestinal fluids, which modulate gastrointestinal digestion (Mat *et al*., 2017). These

fluids are composed of enzymes such as pepsin, pancreatin, peptidase, α-amylase, lipase, and mucin from human secretion, animals, or plant sources, and it also contains bile acid. In the literature, *in vitro* models have used enzymes from omnivorous animals, like pigs, rats, or human volunteers, and enzymes extracted from microorganisms (Mackie & Rigby, 2015). One of the advantages of static models is their low cost, which allows the analysis of various samples (Simões *et al.,* 2017).

On the other hand, dynamic models can reproduce physiological behavior using important parameters of human digestion (Dupont & Inra, 2016). The dynamic model is characterized by different compartments which present specific effects of fluid dynamics on digestion, showing or not a gastrointestinal fluid flow control. The oral stage, for example, presents mechanical movements able to break down particles. These movements promote the lubrication containing salivary enzymes responsible for converting the solid food into a bolus. Motility, mixing, mechanical forces, shear, and pressure against the gastric surface represent the gastric dynamic phase. We also have models that simulate wall motility, for example, segmentation and peristaltic movements, to better represent the chyme breakdown in the small intestine and, consequently, compound absorption rates (Muttakin *et al.,* 2019).

Oral, stomach, and intestinal phase using simulated fluids have been designed by INFOGEST model (Minekus *et al.,* 2014). One of the challenges for delivering *Capsicum* and its derivatives is its pungency, requiring avoidance of release in mouth and stomach and a greater release of these compounds into the intestine. For this reason, this section cited some examples of *in vitro* digestibility of *Capsicum* and its derivatives.

The first study investigated capsaicin nano complex composed of high amylose corn starch (HACS) submitted to a semi-dynamic *in vitro* adult following the INFOGEST protocol. Nanostructures containing HACS entrapped a high amount of capsaicin (99%). They presented more significant capsaicin digestion for adult and senior models into the intestinal phase than capsaicin non-encapsulated, increasing capsaicin bioaccessibility. Therefore, this formulation proved efficient regarding spice reduction, being a sound target delivery system (Isaschar-Ovdat *et al.,* 2021).

In sequence, the release of capsaicin encapsulated by water-in-oil high internal phase (HIPE) emulsions was studied. Capsaicin (CAP) was mixed with sodium alginate (SA), resulting in a water-dispersible phase, which was then mixed into different heated oil mixtures (6 g) in a

ratio of 4:1 through high-energy homogenizer. HIPE emulsion was assessed in the by simulated oral, gastric, and intestinal fluids following INFOGEST methodology (Minekus *et al*. 2014). Briefly, capsaicin nanoemulsion in a ratio of 1:1 was submitted to gastric step for 2h using the porcine pepsin (3.2 g/L), salts and pH adjusted to 2 under shaking at 37 °C. In sequence, samples from gastric phase were added into simulated intestinal fluid (SIF) composed of salt solution containing bile, lipase, and trypsin. Intestinal conditions were carried out at pH of 7.0, 100 rpm and at a temperature of 37 °C. In this study, capsaicin had a resistance under mouth condition, compared to the control (non-encapsulated capsaicin). In the gastric phase, there was a precipitation and sedimentation of capsaicin complexes due to low pH of stomach. Finally, they concluded that capsaicin into nanoemulsion presented a high delivery in large intestine because of emulsion structure and lipase activity (Wu *et al*. 2022).

In another study, *Capsicum* extract emulsifed with lecithin and fenugreek galactomannan and spray-dried was evaluated regarding the *in vitro* release, using in phosphate buffer (PBS) at pH 6.8 and in 0.1 M HCl at pH 2.0 for 24 h at 37 ± 0.5 ℃. Results evidenced the effectiveness of the fenugreek galactomannan structure as encapsulating material. The release of capsaicinoids was slow for pH assessed. After 12 h, only 40% of capsaicinoids was delivered at pH 2 whereas in pH 6.8 was observed an amount 39% at 8 h, 70% at 12h, and then the capsaicin was 90% released only after 24 h of experiment (Joseph *et al.,* 2021).

Thus, microencapsulation can target the spice compounds delivery through stabilisation or entrapment of the compound into the structure. Biopolymers, in general, offer a good system to improve the compounds bioavailability since they promote a better release in the intestinal phase.

Challenges Facing the Use of Pure Spice Oleoresins as a Food Additive

Spice oleoresins are becoming more popular once they impart unique flavors to foods. However, their application in food, comestical and pharmaceutical products still need to be improved because they are prone to suffer losses during processing when added directly to a food product (Tang, Zhang & Devahastin *et al.,* 2019). Other reasons that limit spice oleoresin application as a food ingredient are its high pungency, low solubility in water, and elevated content of pigments that are unstable to heat, oxygen, and light (Rao & Sowbhagya, 2017; Ganapathy, 2022).

Capsicum and its Derivatives microencapsulated by Spray Drying

One way to improve the use of spice oleoresins is to through the employment of the encapsulation technology (Tang, Zhang & Devahastin *et al.*, 2019). Encapsulation consists of covering the compound of interest within one or more coating layers or dispersing it into a polymeric matrix to protect the ingredient against environmental factors such as water, oxygen, heat, and light (Gharsallaoui *et al*., 2007). In addition, encapsulation can mask off-flavours, improve the delivery of drugs and bioactivity, and enhance the water dispersibility of liposoluble compounds (Sobel, Versic, Gaonkar, 2014). Particles/capsules/spheres obtained by encapsulation techniques are classified as macro (>5000 μm), micro (from 1.0 to 5000 μm), and nano (<0.1 μm) (Estevinho & Rocha, 2017).

Microencapsulation techniques applied to the food industry are traditionally classified into three different methods. First, physical processes include spray drying/cooling/congealing, supercritical fluids encapsulation, process with microfluidic devices, and spray coating. Secondly, there are chemical methods, also known as polymerization mechanisms. Thirdly, examples of physicochemical methods are complex coacervation, liposomes, micelles, emulsions, operations with nanostructured lipid matrices, solvent evaporation, and molecular inclusion (Comunian *et al.*, 2016).

Spray drying stands out as a very efficient dehydration method which is largely employed to produce food ingredients, chemical, cosmetical and pharmaceutical products. To provide more suitable characteristics to the infeed liquids used to produce the powders, it is commonly associated with emulsification methods.

Emulsification is an important process in the industry that, through high/low energy input, homogenizes two or more immiscible liquids, in which one of them is dispersed as small spherical drops in the other liquid (McClements & Jafari, 2018). Droplet size, surface charge, viscosity, retention of the compound of interest, and stability are related to oil concentration, emulsifier, and emulsification method (McClements, 2005).

Emulsions formed by the high-energy methods have been employed as vehicles for different spice oleoresins. For example, *Capsicum* oleoresin formed by emulsification through high-energy methods, such as micro-fluidization, Ultra-Turrax, and ultrasound, have resulted in droplets size varying from 500 nm to 18 μm, hence enabling an improvement of compound bioaccessibility, causing an anti-obesogenic effect on mice (Kim *et al*., 2014; Aguiar *et al*., 2016;

Akbas, Soyler & Oztop, 2018; Anthero *et al*., 2022). Cinnamon and paprika oleoresin emulsions produced by the rotor-stator presented small droplet size, low viscosity, and high antioxidant activity by FRAP and ORAC methods (Ferraz *et al.*, 2021), while *Capsicum* oleoresin emulsions showed good stability, orange color tones and high capsaicin content (Anthero *et al.*, 2022).

Spray drying is one of the most used techniques to produce powdered spice oleoresin (Juárez-Góiz *et al.*, 2018). It is recognized as an affordable, timesaving, continuous, and reproducible drying method. The products are usually fine powders of particles within the submicron-to-micron size range, generally showing a narrow distribution (Salama, 2020; Singh & Van den Mooter, 2016).

Though some variations might be found in the literature, the process can be briefly described as consisting of four steps: 1) preparation of the infeed solution, which may involve evaporation, homogenization, emulsification, or other mixture methods; 2) droplets formation through an atomizer; 3) water evaporation through the contact of the droplets with a hot gas stream and 4) particles recovery. Powder properties are affected by factors related to the infeed liquid (solid concentration, viscosity, chemical, and kinetic stability), process parameters (drying gas inlet and outlet temperatures, drying gas flow rate, infeed liquid rate, atomization pressure), and equipment design (drying chamber dimensions, drying gas flow direction, atomizer geometry) (Assadpour & Jafari, 2019; Paudel *et al*., 2013; Rezvankhah *et al*., 2020; Salama, 2020; Singh & Van den Mooter, 2016). In this sense, microparticles powders with different properties are obtained, like a spherical or polyhedral geometry, teeth concavities in the surface, smooth or rough structure, and surface with or without pores formation (Jafari, 2017; Wang *et al*.., 2017; Rybak *et al.,* 2020; Mendes *et al.,* 2020). Also, these powders can present low moisture, low hygroscopicity, and higher stability and are easily stored (Assadpour & Jafari, 2019).

The formulation of the infeed liquid in spray drying plays a relevant role in the production of food ingredients because, more than just acting as a vehicle or a coating material for a main core, the carrier materials are expected to develop enhanced functionalities, such as gastrointestinal tract resistance, delivery to targeted tissues, controlled release of the encapsulated material, etc. According to Hernandez Sanchez et al. (2015), the infeed liquid, with solids concentration ranging from 40 to 60 g/100 g liquid feed, must have a viscosity compatible with pumping, pulverization and drying. Furthermore, it must present a good drying ability (not too sticky) and is not supposed to react with the core material. For all those requirements, the association

between emulsification methods and spray drying is very frequently employed since the formation of emulsions usually provides infeed liquids with increased physical and oxidative stabilities.

Paprika oleoresin was transformed into powders with low water activity, high solubility in water, and great stability of total carotenoids and instrumental color (Anthero et al., 2021). Chili pepper oleoresin microparticles presented low moisture and high capsaicinoids content (Juárez-Góiz et al., 2018). Similarly, the encapsulation of Chili seed oil extract by spray drying showed to post-encapsulated by spray drying retarded the oxidation of the oil, henceoil hence and then avoiding the rancidavoiding ed rancid flavor of chili seed oil powder (Wang, Liu, Wen, Li, Wang & Ni., 2017).

Some of the main materials and combinations of materials that have been successfully employed to produce *Capsicum* and derivatives spray-dried microparticles, with special focus on food ingredients encapsulation, are described in Table 4.

Figure 4 illustrates the forms to encapsulate *Capsicum and its Derivatives*, and application possibilities.

Figure 4 Encapsulation and application of *Capsicum and its Derivatives*

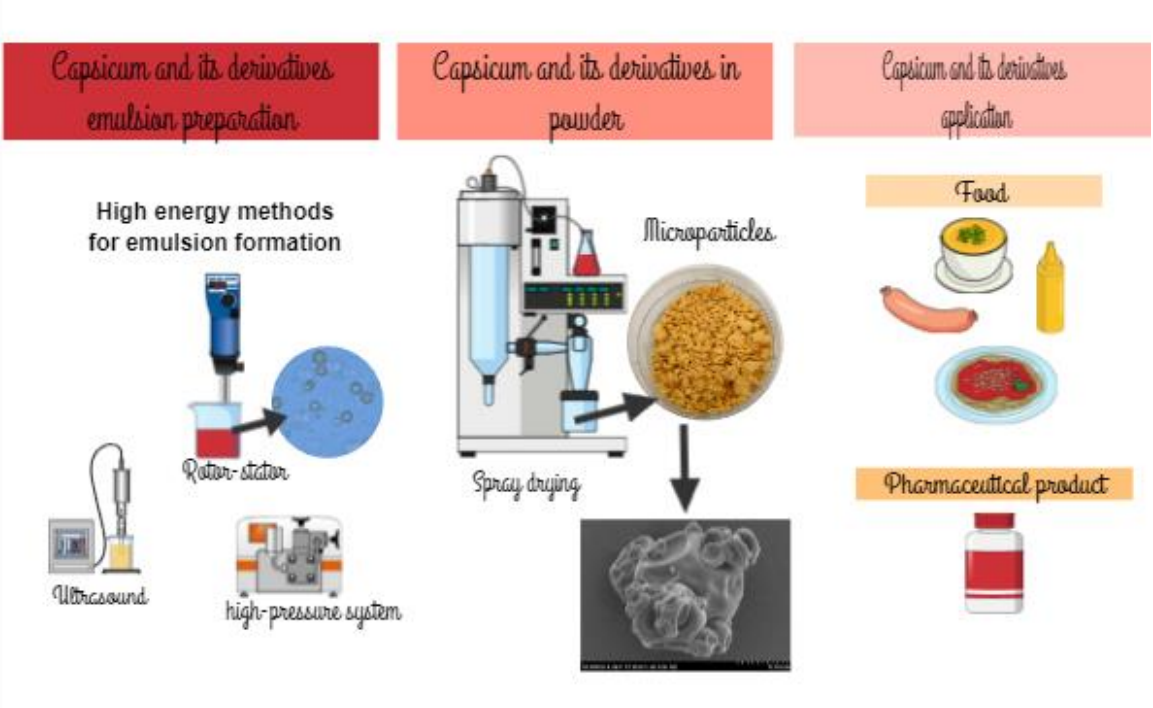

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Table 4. Recent studies about the *Capsicum* peppers and spice oleoresins microencapsulated by emulsification and spray drying techniques.

Microencapsulation technique	Core material	Encapsulating agent	Main results	Reference
Emulsification using a high-shear homogenizer followed by spray drying	Turmeric oleoresin	Gum arabic and maltodextrin	• The emulsion stability index varied from 0.81 to 0.96. • The size of spray-dried microparticles ranged from 301.85 to 413.99 nm. • The curcumin content of particles had an average of 77.2 to 98.61%. • The encapsulation efficiency of oleoresin into spray-dried microparticles varied from 68.10 to 96.43%.	Ipar, Singhal & Devarajan (2022).
Emulsification by rotor-stator	*Capsicum* oleoresin	Gum arabic, corn starch modified with OSA, and modified malt	• Emulsions present high capsaicin content for all formulations. • Good stability of emulsion during 4h for all formulations was observed. • Color parameters were affected by the type of encapsulating material. • All formulations showed pseudoplastic behavior.	Anthero *et al.* (2022)
Emulsification using a rotor-stator followed by spray drying	*Capsicum* oleoresin	Gum arabic, corn starch modified with OSA, and modified malt	• Microparticle moisture varied from 2.81 to 5.36 (g water/100 g powder). • The encapsulation efficiency of oleoresin presented an average of 68.5 up to 91.6%. • The solubility index varied from 74.55 to 93.92 %. • Particles had different size distributions and high antioxidant activity.	Anthero *et al.* (2022)
Emulsification by rotor-stator	Paprika and Cinnamon oleoresins	Whey protein isolate (WPI), gum arabic (GA), and maltodextrin (MD)	• Emulsions showed high antioxidant activity by FRAP and ORAC methods. • Droplet sizes ranged from 0.93 to 22.28 μm. • Carotenoid content varied from 3.67 to 5.09 mg carotenoids per g emulsion. • Emulsions presented Newtonian rheological behavior.	Ferraz *et al.* (2021)
Emulsification using a modified heavy-duty motor followed by spray drying	Black pepper oleoresin	Gum arabic (GA) and whey protein concentrate	• Microparticles formulated with GA presented a smooth surface with a few visible concavities. • Particle sizes ranged from 5 to 50 μm. • Whey protein as wall material presented a greater retention of piperine during storage.	Pineda *et al.* (2021)
Emulsification by high-pressure microfluidization	*Capsicum* oleoresin	Whey protein (WP), pea protein (PP), quillaja saponin (QS), and sunflower lecithin (SL)	• With the emulsifier concentration increase, the nanoemulsion droplet size achieved values from 134 and 103 nm at high pea and whey protein concentrations. • All nanoemulsions formulations were stable in relation to their capsaicin content, mean particle diameter, and zeta potential.	Aguiar *et al.* (2021)

Microencapsulation technique	Core material	Encapsulating agent	Main results*	Reference
Emulsification using a rotor-stator followed by spray drying	Paprika oleoresin	Gum arabic (GA), SNOW-FLAKE (SN), and starch modified with OSA	• Powder presented high encapsulation efficiency for paprika oleoresin achieving values up to 90%. • Particles presented bimodal distribution and irregular surfaces. • SN treatment presented higher carotenoid retention values over 45 days of storage at 25 and 35 °C.	Anthero *et al.* (2021)
Emulsification by rotor-stator followed by microfluizatior	*Capsicum* oleoresin	Soy lecithin and Sucrose monopalmitate	• Nanoemulsion had droplet sizes ranging from 39 up to 33 nm. • The encapsulation efficiency of oleoresin was 63 up to 71%. • Nanoemulsions formulated with lecithin showed antibacterial action by reduction of *E. coli* and *S. aureus* bacteria. • All formulations presented good antioxidant activities by the DPPH method.	Akbas, Soyler & Oztop (2019)
Emulsification by high-speed homogenizer and ultra-sonicator	Capsaicin	Oleic acid/Labrasol, Tween-20, and glycerol	• Stable oil-in-water nanoemulsions with capsaicin were obtained. • Particle size presented an average of 7.902 nm. • No toxicity effect on Neuro-2a cell lines was observed for capsaicin nanoemulsion.	Nigam, Gabrani & Dang (2019)
Emulsification using a rotor-stator followed by a high-pressure homogenizer	Capsaicin	Tocopheryl polyethylene glycol 1000 succinate (aqueous phase) Peanut oil (organic phase)	• Nanoemulsion was obtained with droplet sizes ranging from 100 to 200 nm. • Nanoemulsions were stable, with a small narrow size distribution. • Droplet size was affected by storage temperature and salt content.	Han, Zhang, Liu & Xiao (2019)
Emulsification using ultrasound and homogenization followed by spray drying	Paprika extract	Octenyl succinic anhydride (OSA)-modified partial waxy rice	• Particles obtained by spray drying presented a size of 9.82 µm. • Powders produced by spray drying with heat-stable color	No & Shin (2019)
Emulsification by rotor-stator followed by spray drying	Raw extract of jalapeño chili oleoresin	Maltodextrin and skimmed milk powder	• Microparticles presented pungency varying from 202,285 SHU to 322,235 SHU and high capsaicinoids level. • Microparticles showed density ranging from 0.297 g/ml to 0.5 g/ml, moisture maximum of 10 g/100g sample, and a lower value for dispersibility of 0.244.	Juarez-Goiz *et al.* (2018)
Emulsification by rotor-stator followed by spray drying	Chili seed oil	Starch sodium octenyl succinate (SSOS), soybean protein isolated (SPI), gelatin (GA), and maltodextrin (MA)	• An emulsion containing 10% wall material and 30% oil load showed low viscosity and high stability. • The diameter of spray-dried microparticles varied from 3 to 20 µm. • Encapsulation efficiency was up to 94.35%.	Wang *et al.* (2017)

SHU (Scoville Heat Unit) related to pungency of chili pepper.

Carbohydrates used in the Spray-Dried Microparticles Containing Capsicum and its Derivatives

Commercial and Innovative encapsulating materials starch-based

Native corn starch is a natural biopolymer mainly consisting of amylose and amylopectin. Both have D-glucose units as the basic structure: amylose presents linear long-chain molecules (200 – 10,000 units), with α-(1,4)-linked D-glucose units, while amylopectin is a highly branched molecule, where the D-glucose units are linked by α-(1,4) bonds in the backbones (10 – 140 units) and by α-(1,6) bonds in the branches (Pérez & Bertoft, 2010).

The employment of native starch in food formulations is advantageous since it is a food-grade ingredient, generally recognized as safe (GRAS), non-toxic, biodegradable, and biocompatible, not to mention its low cost in comparison to other ingredients (Chen *et al.*, 2015; Jiang *et al.*, 2019). Corn starch presents low hygroscopicity, which is desirable when producing spray-dried powders. Low hygroscopicity implies low water adsorption, which favors microbiological and physicochemical stability and increases the products shelf-life (Ann-Charlotte, 2004).

There are some limitations for the use of starch in spray drying microencapsulation: One of them is the high viscosity that might be reached by starch suspensions when they undergo gelatinization, even at low concentration levels. Should the infeed liquid undergo heating during its preparation, this would bring the risk of nozzle blockage during the atomization step (Gharsallaoui *et al.*, 2007). Secondly, if the spray-dried powder is meant to be employed as an ingredient in products that should undergo heating during processing, then it could cause undesired thickening. Decreasing the starch concentration to a minimum is a good alternative to overcome these drawbacks, but in this case, it should be combined with other ingredients, such as proteins, gums, carbohydrates, and others, to ensure a satisfactory yield in the spray-drying process (Jacobs, 2014).

Native starches may not be the best choice regarding the encapsulation of oily core materials, such as essential oils and flavors, as it presents low emulsifying ability caused by the linear and polar molecular structure (Zoben & Stephen, 2006). Because of such limitations, this type of starch is not very frequently used in the encapsulation of *Capsicum* and its derivatives, which frequently present oily characteristics and, if they are to be used, the combination with other compounds with surface-active properties, such as proteins and gums, should be made.

Acid or enzymatic hydrolysis of corn starch dispersions provide molecules of lower molecular mass, which may vary depending on their dextrose equivalency (DE). Increased DE means a higher degree of hydrolysis hence lower molecular mass and higher solubility compared to native starch. These hydrolysates are named maltodextrins when DE is lower than 20 and corn syrups for DE from 20 to 60. Apart from the molecular mass, these compounds differ in their functional, sensory, and technological properties. Maltodextrins are usually nonhygroscopic compounds capable of dispersions of increased viscosity with no perceptible sweetness. As DE is increased, corn syrups present higher hygroscopicity and sweetness, with less ability to form viscous dispersions because of their lower molecular mass (Bemiller & Whistler, 1996). Regarding encapsulation purposes, the hydrolysates represent an excellent alternative for some limitations of starch itself, especially concerning its solubility and viscosity. In addition, they are readily available and non-expensive products.

As an alternative to overcome the poor emulsifying ability of native starches, chemical modification of these compounds has been largely employed. With esterification or etherification reactions, chemical groups are attached to the hydroxyl moieties of the starch molecules. The Degree of Substitution (DS) is usually employed to express the average number of hydroxyl groups undergoing esterification/etherification. DS values are generally within the range 0.002 - 0.2 (Ann-Charlotte, 2004; Bemiller and Whistler, 1996).

As for spray drying encapsulation, the most frequently used modified starches are referred to as OSA starches. This modification is produced by the esterification reaction between 1-octenylsuccinic anhydride and the starch molecules, which occurs in aqueous medium under mild alkaline conditions. A significant effect regarding emulsifying properties is then reached, because the resulting esters present both hydrophilic and hydrophobic groups, conferring an amphiphilic character. These esters can concentrate at the interface of oil-in-water emulsions even if a low degree of substitution is used (Rutenberg, Solarek, 1984; Wurzburg, 1986). This reaction does not affect the biodegradability of the starch, and slow digestion and increased resistance to hydrolysis by digestive enzymes have been reported to this modified starch (Agama-Acevedo and Bello-Perez, 2017; Bemiller and Whistler, 1996; Dokić et al., 2012).

In the encapsulation of hydrophobic core materials, systems that frequently require the formation of emulsions to stabilize the active core, OSA-starches can act both as emulsifiers and stabilizing agents. The stabilizing effect is associated with the formation of a thick film on the oil

droplets surface, caused by the high molecular weight of OSA starch. This film can impair droplet mobility, hence avoiding coalescence (Dokić et al., 2012; Wang et al., 2015). OSA starches can be employed in combination with other encapsulating agents, which may increase the effectiveness of core material protection (Ann-Charlotte, 2004; Zhu, 2017). For instance, native and octenyl succinic anhydride (OSA) and succinylated (SUC) sorghum starches were employed to encapsulate nutmeg oleoresin. Formulations containing 100% of OSA or SUC encapsulating material achieved encapsulation efficiency over 84% (Arshad, Ali & Hasnain, 2018).

Alternatively, modification of starches can also be done using organic acids. These acids are considered GRAS food additives and are naturally present in foods. An example of this is stearic acid, a by-product obtained by the hydrolysis of triacylglycerols that has been used in the modification of starches as a good alternative for promoting physicochemical changes in the granules, thus improving its encapsulating properties (Alves, Viell, & Plata-Oviedo, 2015; Maphalla & Emmambux, 2016; Ocloo, Minnaar, & Emmambux, 2016).

Ultrasound processing can physically and chemically modify cereal starches. Experiments carried out with bath or probe ultrasound demonstrated a strong effect on pores formation, depressions and cracks because of the rapid water jets caused by the cavitation bubbles collapsing with different frequencies (15 to 211 kHz) and power ranging from 100 to 750 (W) and amplitude of 50 to 100% (Cui & Zhu, 2021). Cavitation occurs by collapse of the bubbles, producing a strong pressure and temperature gradient in the system (Cai et al., 2022). Cavitation reduces the air penetration inside starch granules, promoting the entering of water molecules and reagents into the internal channels. Ultrasound combined with inorganic or organic acid can result in a chemical modification as well, because the cavitation phenomenon contributes to enhance the chemical reactions, adding hydrophobic groups into starch chain (Zhang *et al*., 2020; Estivi *et al.*, 2022).

Khurshid et al. (2021) produced starches with dual modification: ultrasonication followed by acetylation, using acetic acid. Starches obtained presented low values for viscosity, swelling and digestibility, whereas solubility showed an increase after modification compared to native starch.

As an innovative spray drying carrier, Ferreira *et al.* (2019) evaluated the use of mango kernel starch. The authors assessed the effect of the drying parameters on the powder properties, as well as on the process yield. Results showed that controlling the relative humidity could positively

affect the process yield. Furthermore, spray-dried mango kernel starch revealed antioxidant and lipid oxidation properties.

Innovative encapsulating material was also obtained using annatto (*Bixa orellana* L.) seed starch. Authors performed the esterification of annatto (*Bixa orellana* L.) seed starch with octenyl succinic anhydride (OSA) employing a short processing time of ultrasound obtaining a new emulsifier with the ability to stabilize annatto seed oil-in-water emulsions (Silva *et al.* 2022). Anthero et al (2022a, b) produced *Capsicum* oleoresin in emulsion and powder using octenyl succinic anhydride corn starch, modified malt with stearic acid modified with ultrasound and obtained ingredients with high content of capsaicin.

Gum arabic as an encapsulating material

Gum arabic is a good choice as a wall material for hydrophobic compounds, due to its emulsifying properties and good solubility. Overall, gum arabic is a hydrocolloid found in a plant exudate from the Acacia family, which is comprised of monomers, such as galactose, arabinose rhamnose and glucuronic acid (Samborska et al., 2021). Although this polymer belongs to the group of carbohydrates, its structure contains protein fractions that are responsible for its emulsifying functionality, presenting appropriate core ratio ranging from 6% to 26% for spray-drying (Burnside, 2014). Due to its composition, the gums can inhibit flocculation and coalescence through electrostatic repulsions, in addition to developing a steric stabilizing layer in the emulsion (Williams, 2011).

Recent studies encapsulated spice oleoresins by spray drying using gum Arabic in combination with other wall materials like modified starches for paprika oleoresin (Anthero *et al.*, 2021) whey protein isolated for turmeric oleoresin (Lucas et al., 2020) and black pepper oleoresin (Pineda *et al*., 2021).

Microencapsulation of spice oleoresin/oil using modified starches and gum arabic: Formulation and Structure of spray-dried microparticles

OSA-modified corn starch and gum arabic alone or combined with other emulsifying agents, have been used as carriers for oil and oleoresins showing good, encapsulating property, as was reported studies have reported the microencapsulation of oleoresin from Nigella sativa, oleoresin from pepper black pepper, paprika oleoresin, annatto oil, non-aqueous pepper extract and oleoresin

from red pepper (Edris, Kalemba, Adamiec, & Pi, 2016; Guadarrama-lezama et al., 2012; Pérez-Alonso et al., 2008; Porras-Saavedra et al., 2018; Shaikh, Bhosale, & Singhal, 2006a; Silva, Gomes, Hubinger, Cunha, & Meireles, 2015).

In a recent work, Porras-Saavedra et al. (2021) evaluated the use of *Sechium edule* starch as encapsulating agent for cinnamon oleoresin. Even though *Sechium edule* starch was capable to form stable particles when used as the only carrier agent, the use of a ternary mixture of the starch, oleoresin with arabic gum provided a significant higher encapsulation efficiency, as well as increased retention of phenolic compounds. *Sechium edule* fruit may become a source of starch with potential application as encapsulating agent when combined with other wall materials.

Table 5 summarizes some studies regarding the effect of carbohydrates as carrier agents on microparticles loaded with spice oleoresin obtained by spray-drying. Overall, studies reveal that each material isolated or in combination can form particles with distinct characteristics regarding size and morphology.

Table 5 Effect of wall material on the formation of the structure of spray-dried microparticles containing spices oleoresins

CORE MATERIAL	WALL MATERIAL	FORMULATION	PARTICLE STRUCTURE	REFERENCE
Turmeric oleoresin	Modified starch (HiCap® 100): n-octenyl succinic anhydride-modified starch from waxy maize, maltodextrin DE 10, and gelatin	(A) (MG) Maltodextrin and gelatin (26:0.6). (B) (SG) Modified starch and gelatin (30:1).	• MG resulted in microparticles with rounded shapes, toothed surfaces, irregular depressions, and concavities, and then prone to form agglomerates. • SG formed microparticles with more spherical and smoother surfaces than MG. • SG produced microparticles with irregular depressions and concavities.	Malacrida, Ferreira & Nicolleti (2022)
Curcumin	Gum arabic, sodium alginate, modified chitosan	100 mL of wall material at 1% wall material (w/v) plus 2 mL of curcumin with 0.9% (w/v)	• All spray-dried particles presented regular and spherical shapes. • Microparticles formed by alginate and gum arabic showed a rough surface. • Particles produced by modified chitosan had a smooth surface.	Lucas *et al.* (2020)
Nutmeg oleoresin	Gum arabic, native, and modified (and octenyl succinylated) sorghum starches	Dispersions containing 10% (w/v) of oleoresin and 30% (w/w) of wall material. Gum arabic (GA) and sorghum starches (ST) (native (STN) and modified (STM)) were added in the ratio of 75:25, 50:50, and 25:75.	• Spray-dried particles showed a diameter in a range of 5–22 μm. • All formulations did not present apparent cracks, holes, and fractures on external surfaces, indicating low permeability to gases, enhanced protection, and retention of the core material. • With the increase of starch (STN/STM) on formulation occurred an improvement of smoothness on the surface particle.	Arshad, Ali & Hasnain (2018)
Ginger oil (GO)	Gum arabic (GA), maltodextrin (MD) and inulin (IN)	GO, and each biopolymer was 1:4 (w/w). Suspension contained 20% of the wall material. GA isolated, and combinations formed by GA:IN (1:1) and GA:MD (1:1).	• All formulations presented morphology with cracks and spherical shapes. • Inulin material, in combination with GA, resulted in particles with a more spherical shape and smaller size. • GA:MD produced higher particle size with concavities.	Fernandes *et al.* (2016)

Conclusion and perspectives

Capsicum-derived products such as oleoresin, oils, extracts, and their bioactive compounds have several health benefits with potential application as ingredients for the food and pharmaceutical industries. Through encapsulation technology, it is possible to enhance solubility, stability, and bioacessibility of these ingredients increasing their use in industries. The optimization of microparticle production using different source of carbohydrates as wall materials to obtain high encapsulation efficiency with low process and formulation cost is also another gap in the present knowledge. In addition, further research about the incorporation of these formulations into human and animal diet to understand the mechanisms of these wall materials in the release of compounds into systems are still needed.

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CAPÍTULO 3

BARLEY MALT ESTERIFICATION AFTER ULTRASOUND AND STEARIC ACID TREATMENT: CHARACTERIZATION AND USE AS STABILIZING AGENT IN OIL-IN-WATER EMULSIONS

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BARLEY MALT ESTERIFICATION AFTER ULTRASOUND AND STEARIC ACID TREATMENT: CHARACTERIZATION AND USE AS STABILIZING AGENT IN OIL-IN-WATER EMULSIONS

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Abstract

This work aimed to obtain novel materials from malt modification for using in oil/water emulsions. Malt was modified using stearic acid (2% w/w) in combination with different energy densities ($33.8x10^6$, $60\ x10^6$, $72.5\ x10^6$, and $107.5x10^6$ J/m³) from ultrasound probe resulting in four materials. These materials were characterized by degree of substitution (DS), amylose content, dextrose equivalent (DE), protein content, solubility, thermal properties, Fourier-transform infrared spectroscopy (FT-IR), crystallinity analysis by X-ray diffraction, and scanning electron microscopy. Emulsions containing 15% total solids (25% canola oil and 75% modified malt as stabilising agent) were prepared and characterized. Results showed that increasing energy densities produced different effects on malt properties due to an increase in DS, amylose content, and DE values. The sonication process in combination with stearic acid increased malt solubility and the gelatinization temperature of malt, caused modifications of starch crystallinity in the amorphous region and hydrolysis of starch granules, and promoted good stabilising properties for emulsions containing modified malt.

Keywords: FT-IR analysis; Energy density; X-ray diffraction; DSC analysis

Introduction

Malt is a byproduct of barley and an important ingredient for beer production. Malt is also source of nutrients to use in bakery products and soft drinks and is responsible for the sweet taste. This product is composed of proteins, dietary fibers, starch, amylolytic enzymes, and bioactive compounds (Leitao et al. 2012; Montanuci et al. 2016; Rittenauer et al. 2015). Among the phenolics, flavonoids comprise the main phenolic group since these compounds may produce therapeutic effects for various chronic diseases, thus contributing to glycemic index reduction, promotion of anti-inflammatory effects, and a decrease in oxidative stress (Leitao et al. 2012; López-martínez et al. 2017; Maetens et al. 2017; Nelson et al., 2016).

Barley malt (BM) is considered economically viable and safe and appears to be a promising alternative to use as a novel encapsulating material. However, native malt, flavonoids comprise as seen with other starch sources, presents high viscosity in solution, absence of hydrophobic groups, low resistance to the heat and shear, and low solubility in cold water. These features make malt modification necessary employing physical, chemical, or enzymatic techniques to expand and extend its use (BeMiller, 2019). Physical modification of malt by ultrasound is an excellent technology that can be considered a *green process* (safe, easy to operate, sustainable). Studies have shown that ultrasound can promote some changes in starch structure (Zhu, 2015). This technology can form pores and fissures in the starch granules, increase water solubility, and decrease viscosity due to the strong shear force, high temperature, and free radicals produced by cavitation during the process. Structural changes are dependent on operational conditions, such as temperature, time, and the frequency and intensity of ultrasound (BeMiller, 2017; Iida, Tuziuti, Yasui, Towata, & Kozuka, 2008; Zhu, 2015). Sonication at 40% of amplitude in relation to 750 W of power was shown to result in starches with techno-functional property alterations including degree of crystallinity, and an increase of granules size (Monroy et al., 2018). Ultrasound treatment was suitable for promoting a pregelatization of wheat starch (Abedi et al. 2019). Apart from, resistant pea starch was produced using the gelatinization followed by ultrasound assisted (You et al. 2019). However, the combination of ultrasound and a chemical treatment are still needed.

Chemical methods are also widely used to modify starch. For instance, an esterification reaction, characterized by using an inorganic or organic acid at alkaline pH, has been demonstrated. The ester group is added to the starch chain and promotes hydrophobic or hydrophilic properties (Wurzburg, 2006). Synthetic acids are examples of reagents used in

chemical modification. Palavencio, Penci and Ribotta (2019) studied the acetylation process using an acetic anhydride, hydrolysis with acetic acid, and esterification with octanoyl chloride for sorghum and tapioca starch, and they observed a strong influence on the pasting profile starches. Many acids such as propylene oxide, acetic anhydride, octenyl succinic anhydrides, and their derivatives are wide used in starches chemical modification, however, they can leave residues that promote off-taste in the product or cause adverse effects to consumers (Lui, 2005). Stearic acid is a by-product of triacylglycerol hydrolysis and can be used in the esterification reactions to impart hydrophobic characteristics to the starch. This reagent is a good alternative for starch modification because it is safe and presents low cost. Thus, when physical and chemical methods are combined, structural and chemical alterations are observed in the starch granules. A study showed that the combination of stearic acid/microwave resulted in an esterified cassava starch that could be used as a novel emulsifier agent and presented good stability in oleic oil emulsions (Alves, Viell, & Plata-Oviedo, 2015).

Many studies use ultrasound probes or stearic acid to modify the structures and physicochemical properties of different starches; however, the combination of the sonication–stearic acid process to induce the esterification reaction of modified barley malt has not yet been reported. This research focused on the evaluation of the safety-related effects of different ultrasound energy densities in combination with stearic acid on malt properties. The influence of the modification process on malt properties regarding degree of substitution, dextrose equivalent (DE), amylose content, protein percentage, solubility, microstructure, chemical structure (analyzed via FT-IR), glass and gelatinization temperatures, crystallinity, and their functionality as encapsulating agents in canola oil-in-water emulsions was evaluated.

Material and methods

Materials

Barley malt (BM) (Larger Pilsen) was obtained from Art Brew Company in Campinas, SP. The reagents used to modify the malt were stearic acid (95% purity) which was supplied by Dynamic Ltda (Indaiatuba, SP), sodium hydroxide (Vetec, 99% purity) obtained in Campinas, SP, and ethanol (Synth, PA) purchased in Campinas, SP. Canola oil (Vitaliv) was used as an oil model

and purchased at the local market (Campinas, Brazil). All the other reagents used in this work were of analytical grade.

Synthesis of esterified malt by stearic acid and ultrasound with different energy densities

Four dispersions were prepared separately containing thirty grams of milled BM dissolved in distilled water (120 mL) with the addition of 60 mL of NaOH (0.5 mol. L^{-1}). The modification process followed the methodology described by Alves, Viell, & Plata-Oviedo (2015) with minor modifications. The mixtures have been heated under agitation with a magnetic stirrer until they reached 60 ± 2 °C and formed a pre-gelatinized starch dispersion. Stearic acid (2% w/w) was then dispersed in a mixture of 10 mL of ethanol (99%) and 40 mL of NaOH (0.3 mol. L^{-1}) and immediately inserted into the pre-gelatinized starch. One-hundred eighty milliliters of distilled water were added to the mixture for a 400 mL total suspension, and pH was adjusted to 9.0 using NaOH (0.5 mol. L^{-1}). Then, all dispersions were placed in a jacketed Becker connected to a thermostatic bath, to keep the temperature at 35 °C during modification process, and the ultrasound probe with tip diameter of 19.1 mm was immersed into the liquid. Each dispersion was subjected to a specific level of power sonication (42, 72, 105, and 148 W) for 5 min; these levels represented 25%, 50%, 75%, and 100% of the power amplitude in relation to the nominal power of ultrasound (QSonica - Newtown, CT, USA).

After that, the pH of the dispersion was adjusted to 6.0 ± 0.02 using HCl (2.5 M). Finally, dispersions were washed with 800 mL of ethanol (90%). The dispersions were washed in a Buchner flask with a qualitative filter paper with a diameter of 15 cm and filtered by a vacuum pump to remove all unreacted fatty acids. In sequence, the washed material was dried at 45 °C for 4 h. This procedure resulted in four BM products: (1) BM25US; (2) BM50US; (3) BM75US; and (4) BM100US. All modifications were performed in duplicate.

Preliminary experiments were done to define the sonication time and ultrasound potency used. Modification malt replication was done randomly to eliminate any trend errors. All samples after modification were stored in a desiccator. After the modification process, these materials were characterized by physicochemical analyses and all experiments were carried out in triplicate, totalling six replicates.

Energy density calculation

Energy density (ED) of the malt modification process was determined according to Equation 1 (Jafari et al., 2007):

$$ED = \frac{P \times t_{son}}{V} \qquad \text{(Equation 1)}$$

in which ED ($J.m^{-3}$) is the energy density, P ($J.s^{-1}$) is the power delivered to the system by the ultrasound in each essay, t_{son} is sonication time(s), and V (m^3) is the volume of the dispersion containing the modified malt (0.0004 m^3).

Degree of substitution

Degree of substitution (DS) of modified malt was determined according to Smith (1967) with minor modifications. At first, the samples were subject to Soxhlet extraction using ethanol as solvent (99%, v/v) to eliminate unreacted fatty acids. The samples (1g, dry weight) were dissolved in 20 mL of distilled water and titrated with NaOH (0.1 mol. L^{-1}) up to a pH of 8.3. Ten milliliters of NaOH (0.472 mol. L^{-1}) were then added to the mixture and heated in a thermal bath at 70 °C for2 h. After 24 h incubation at 25 °C, the dispersions were titrated using HCl (0.3065 mol. L^{-1}) until the color changed. This method was applied to all the new products (BM25US, BM50US, BM75US, and BM100US), and pregelatinized malt was used as a blank sample. DS values were calculated using Equation 2:

$$DS = \frac{162 \times (V \times M)/W}{1 - [267.47 \times \frac{V \times M}{W}]} \quad \text{(Equation 2)}$$

in which V (L) is the volume of HCl used in the titration, M (mol/L) is the solution molarity, and W (g) is the sample weight in dry basis. The values of 162 and 267.47 represent the molar mass of the theoretical glucose unit (AUC) and the mass of the acyl group, respectively.

Amylose content

Amylose content of modified malt was determined following guidelines of the International Organization of Standardisation (1987). This method represents the colorimetric measurement of the iodine–amylose complex in an ultraviolet/visible (SQ 2800 UV/Vis)

spectrophotometer at 630 nm. The values were determined using Equation 3 in which 18.243 is the amylose content of the reference starch (corn starch) and A_1 and A_2 are the absorbances of the sample and reference starch, respectively.

$$\%\ Amylose = \frac{(18.432 \times\ A_1)}{A_2} \qquad \text{(Equation 3)}$$

Dextrose equivalent

To investigate the hydrolysis degree of malt starch, DE was determined using the method for reducing sugars as described by Whelan (1964) and Miller (1959). First, a calibration curve was prepared using glucose as the standard, and DE values were obtained based on this curve (Equation 4). Suspensions containing 1% malt were heated in a thermal bath at 80 °C until dispersion and cooled at 15 °C for 20 min. In sequence, 1 mL of this sample was added to 1 mL of dinitro-salicylic (DNS) acid reagent and heated at 100 °C for 5 min followed by cooling at 25 °C. An aliquot of 2 mL of DNS II reagent was added to the mixture and incubated for 5 min. To determine total reducing sugars, 25 mL of distilled water were added to the mixture, and the absorbance was measured at 540 nm using an UV/Vis spectrophotometer (SQ 2800 UV/Vis). The DE values were calculated using Equation 4:

$$DE = \frac{(abs - 0.0072)}{0.6627} x\ m \qquad \text{(Equation 4)}$$

in which DE represents the degree of starch hydrolysis, abs are the absorbance (nm) of the sample, and m (g) is mass weight.

Protein content

The protein content of samples (100 mg) was determined with a nitrogen and protein analyser (NDA, VELP, Italy). Total nitrogen was obtained by combustion (DUMAS), and its detection was done through a TCD (Thermal Conductivity Detector). Data acquisition was obtained in triplicate by DUMASoft™, 2.2.9 version.

Solubility

Solubility measurements were based on the method described in the patent from Kasica et al. (2001) with some modifications. Dispersions containing 0.5 g of native and modified malts and 30 mL of distilled water were stirred at 25 ºC for 30 min. After that step was completed, the samples were centrifuged (10,000 rpm, 15 min). An aliquot of 10 mL was put in a Petri dish and subject to drying at 105 ºC for 24 h. Solubility was then determined by the mass difference.

Fourier-transform spectroscopy

Chemical structures of BM and modified BM were analysed by Fourier-transform infrared spectroscopy (FTIR). Data were collected in the region of 4,000 to 400 cm^{-1} (IRPrestige-21, Shimadzu (Kyoto, Japan), and analysis of the data were performed by IRSolution software, version 1.60. Tablets were prepared in triplicate containing 100 mg of potassium bromide (KBr) and 1 mg of the oven dried sample (105 °C/48 h). These samples were then compacted in a manual hydraulic press.

Scanning electronic microscopy

Microstructure of the modified malt was analysed using the scanning electron microscopy ([SEM] LEO Electron Microscopy, model Leo 440i, Oxford, Cambridge, England). The samples were covered with gold at 200 A° using a Sputter Coater EMITECH, Model K450 (Kent, UK), and the analyses were performed under 2000x magnification with a voltage acceleration of 15.00 kV.

Crystallinity by X-ray diffraction

The selected X-ray diffractometer was Philips Analytical X-Ray, model X'Pert-MPD (Almelo, The Netherlands), using the Bragg-Bretano (θ: 2θ) with Kα geometry of copper of λ = 1.54056 Å. The X-ray source operated at 40 mA and 40 kV. X-ray diffraction data were collected for 15 min from 13° to 30 ° with a scanning step of 0.02 °. s^{-1}.

Thermal properties

Glass transition temperature and gelatinization events were determined using a differential exploration calorimeter (DSC-METTLER TOLEDO, DSC1, Schwerzenbach, Switzerland).

To determine the glass transition temperature, 6 mg of sample was added to an aluminum pan (40 μL capacity), and subject to a heating cycle of 25 to 160 °C at a rate of 10 °C.min^{-1} with a 5 min isotherm at 160 °C. After that, the sample was cooled from 160 to 25 °C at a rate of °C.min^{-1} with a 5 min isotherm at 25 °C, and finally, it was heated from 25 °C to 160 °C at a rate of 10 °C.min^{-1}. The analyses were conducted under an inert atmosphere of N_2 at a flow rate of 50 mL.min$^{-1.}$

To determine the gelatinization event, 2 mg of sample was weighed in an aluminium pan (40 μL) with the addition of 6 μL of distilled water. The pan was closed and left to rest at room temperature for 1 h to allow hydration of the malt starch granules to occur. Finally, the sample was heated from 30 to 100 °C at a rate of 10 °C.min^{-1} under an inert atmosphere of N_2 at a flow rate of 20 mL.min^{-1}.

Production of emulsion (O/W)

Native and modified malt (BM25US, BM50US, BM75US, and BM100US) were individually evaluated in emulsions using canola oil as a dispersing medium. The oil was selected based on the absence of lecithin, which is also used as an emulsifier agent. Emulsion (100 g) was prepared with 15% total solids in which 75% represents the encapsulating material and 25% the oil phase. Previously, the modified malts were hydrated for 12 h with stirring at room temperature. Thereafter, the canola oil and hydrated material were homogenized in a rotor-stator (15,000 rpm/10min) (Ultra-turrax IKA T18 Basic, Wilmington, USA). The solids concentration and process parameters were determined in preliminary tests.

Stability index by laser scanning turbidimetry

The emulsion (20 mL samples) was added to cylindrical glass tubes (Vial EPA colourless, 27.5x72.5 mm) and analysed in an optical scanning instrument Turbiscan ASG (Formulaction, France). The stability index was determined by turbiscan (TSI) at baseline and after 24 h of storage

since this time was enough for processing spray-dried emulsion on a laboratory scale. TSI values were obtained according to Equation 5 in which x_i is the mean backscatter per minute of measure, x_{bs} is the mean value, and n represents scanning numbers.

$$TSI = \sqrt{\frac{\sum_{i=1}^{n}(x_i - x_{bs})^2}{n-1}} \quad \text{(Equation 5)}$$

Droplet size distribution

Droplet size distribution was evaluated using a laser diffraction instrument, Mastersizer 2000 (Malvern Instruments Ltda, Malvern, United Kingdom) with distilled water as dispersant solvent for all emulsions and native barley malt dispersed in water (containing 15% of total solids). All samples were measured six times.

Optical microscopy

An aliquot of the emulsion and native barley malt (BM) dispersed in water was placed on a microscope slide for observation of the droplet morphology. The analysis was performed with an optical microscope (Axio Scope A1; Carl Zeiss AG, Germany) under a 40x magnification oil immersion lens for emulsions and 100x for BM dispersed in water (containing 15% total solids).

Rheological behaviour

Flow curves of the emulsions were determined with a controlled deformation rheometer AR1500ex (TA Instruments, Elstree, UK) using flat plate geometry (Ø = 40 mm and GAP of 400 µm). In this procedure, the samples were subject to a three-sequence stage consisting of up-down-up in the range of 0 to 300 s^{-1}. Apparent viscosity was then obtained for a shear rate of 300 s^{-1}. The shear rate was determined in relation to shear stress, and a power law (Equation 6) was used fit the data.

$$\sigma = K.(\gamma)^{\eta} \quad \text{(Equation 6)}$$

The equation parameters are represented by σ (shear rate [Pa]), K (consistency index [Pa.s^n]), γ (shear stress [s^{-1}]), and η (behaviour index [dimensionless number]).

Statistical analysis

The process of modification and emulsion production were performed in duplicate, and all the physicochemical results were obtained in triplicate. Analysis of variance (ANOVA) was performed using the Minitab 18® software with a confidence level of 95%. Differences between means values were compared using Tukey's test with 5% significance (p-value < 0.05).

Results and Discussion

The effect of energy density with acid treatment on the physical-chemical properties of modified malt

Degree of substitution (DS)

Table 1 presents the energy density (ED) values during the sonication process and physicochemical parameters, such as amylose and protein content, DE, and DS. Substitution occurs due to alkaline catalysis after a strong base was applied to the starches in the esterification reactions. This substitution involves the indirect reaction of starch with carboxylic anhydride to form a starch ester with elimination of carboxylate ions and a water molecule. Hence, an ester group is introduced into the starch chain (Wurzburg, 2006).

Table 1 Energy density used in modification process and physical-chemical properties of materials obtained

Treatments	Amplitude Power (%)	Energy (J)	Energy density (J/m^3)	Degree of substitution (DS) (%)	Amylose content (%)	Dextrose Equivalent (DE)	Protein (%)	Solubility (%)
BM	Nd.	Nd.	Nd.	$0.0012^{c}\pm0.0$	$19.3^{c}\pm1.0$	$2.95^{d}\pm0.02$	$7.23^{a}\pm0.03$	$15.2^{b}\pm0.3$
BM25US	25	45	$33.8x10^{6\ d}\pm0.0$	$0.016^{b}\pm0.006$	$21.6^{b}\pm0.6$	$3.34^{c}\pm0.02$	$4.81^{b}\pm0.10$	$62.1^{a}\pm4.7$
BM50US	50	81	$60.0\ x10^{6c}\pm1.3$	$0.034^{a}\pm0.008$	$21.7^{b}\pm0.0$	$3.36^{b}\pm0.13$	$4.36^{c}\pm0.02$	$62.7^{a}\pm1.3$
BM75US	75	97	$72.5\ x10^{6b}\pm0.8$	$0.033^{a}\pm0.005$	$47.5^{a}\pm0.0$	$4.01^{b}\pm0.12$	$4.26^{c}\pm0.06$	$64.9^{a}\pm1.4$
BM100US	100	144	$107.5\ x10^{6a}\pm1.7$	$0.043^{a}\pm0.008$	$48.2^{a}\pm0.5$	$4.21^{a}\pm0.05$	$4.23^{c}\pm0.08$	$62.3^{a}\pm2$

*BM: Native barley malt; BM25US: Barley malt modified with 25% of amplitude sonication in relation to nominal power; BM50US: Barley malt modified with 50% of amplitude sonication in relation to nominal power; BM75US: Barley malt modified with 75% of amplitude sonication in relation to nominal power; BM100US: Barley malt modified with 100% of amplitude sonication in relation to nominal power. Means followed by lower case in the same column do not differ significantly ($p < 0.05$) by the Tukey test.

A direct and proportional increase of DS with ED values can be seen. ED and DS values increased from $33.8x10^6 \pm 0.0$ to $107.7x10^6 \pm 1.7$ (J/m^3) and 0.016 ± 0.006 to 0.043 ± 0.008 (%), respectively, in relation to the amplitude increase for each treatment. The strong shear force and presence of free radicals caused by cavitation from the ultrasound process (Zhu, 2015) may facilitate the substitution of glucose in the starch chain with an acyl group from the stearic acid. Based on these outcomes, it appears to be possible to obtain malt with low esterification degree (due to hydrophobic group presence) during the short modification procedure (5 min). BeMiller (2019) stated that in etherification or esterification reactions, it is common to obtain a low DS in which the values may vary from 0.002% to 0.2%.

Some advantages for obtaining cereals, flours, or starches esterified with hydrophobic groups are associated with the enhancement of functional properties. For instance, these materials can be used as a fat replacements in foodstuffs and emulsifying agents in salad dressings and beverages (Eliasson, 2004). Thus, ultrasonic modification combined with stearic acid was shown to be efficient for chemical structure alterations in malt and can contribute to improving its emulsifier properties.

Amylose content of malts

Amylose content is shown on *Table 1*. Native malt showed approximately 19.3±1.0% of amylose, which is close to the amylose content obtained for barley starches, as well as, 23.9±0.9% (Jeon et al., 2003) and 28.7±0.4% (Emani et al. 2012). After evaluating the amylose content of modified malt (21.6%–48.2%), an increase in these values was identified with an increase in ED. In the same way, Chan et al. (2010) showed the effects of the ultrasonication process on the elevation of amylose degree for all modified starches (maize, potato, mung bean, and sago sources) using the same method for amylose content determination as used in this study. The authors verified that the ultrasonic process promoted an increase in the amylose content and a decrease in the degree of polymerization in relation to native starches (Chan et al. 2010). Conversely, other researchers reported a decrease in amylose contents of corn, wheat, and rice starches during ultrasound treatment (in a bath for 30 min at 20 °C and power of 170 W) in water or ethanol; however, these values were more pronounced when starch was subject to a water system due to the occurrence of depolymerization (Sujka & Jamroz, 2013).

The expected tendency is reduction of amylose content with a corresponding increase in applied energy. High energy density promotes an increase in the linear fragments responsible for forming complexes, which intensifies the blue staining. This phenomenon occurs due to amylopectin debranching and partial amylose hydrolysis into two smaller fragments, which results in higher complexation with iodine and an artificial increment of ‘amylose content’.

Dextrose equivalent

DE averages are presented in *Table 1*, in which the influence of energy density on malt hydrolysis can be noted. This energy promoted a slight elevation in reducing sugars, thus increasing the DE values. These values represent the hydrolysis degree of starch. Native starch or whole cereal does not contain reducing end chains; therefore, it presents 0 DE whereas D-glucose (reducing sugar) has 100 DE (BeMiller, 2019). Malt before modification presented a dextrose equivalent value of 2.95±0.02 DE. After ultrasonic modification, DE values increased due to the hydrolysis of the amylose chain. DE values found in this work for modified malt were around 3 and 4, which allowed classification of the materials (BM25US, BM50US, BM75US and BM100US) as yellow dextrins (BeMiller, 1996).

In addition, it is important to emphasize that materials containing elevated DEs present high hygroscopicity and solubility in water; meanwhile, low DE materials can form a stronger gel due to the presence of a long chain of linear fragments (BeMiller, 2019). Dextrin with low DE may be incorporated into the oil-in-water emulsion as demonstrated in the study by Matsuura et al. (2015). These researchers evaluated the effects of corn starch with different DE degrees (2, 10, and 25) as encapsulating agents on coconut oil emulsions followed by a spray drying process. This suggests that the modified malt obtained in this work can be used as an encapsulating agent.

Influence of modification process over protein content and malt solubility

The effects of energy density regarding malt protein content are also shown in *Table 1*. The modified malt underwent significant protein content reduction due to the selected ultrasound–stearic acid treatment. The decrease in protein content could be related to an increase in soluble proteins after modification in which its content was reduced during the washing step. The protein content of the native malt found in this research (7.23 g of protein/100 g) was lower than those obtained by Montanucci, Ribani, Jorge, & Jorge (2016) (9.67–10.9 g of protein/100g of malt). These distinct values can be explained by the type of malt used in each study.

Table 1 shows the solubility data of native and modified malts. Native malt is insoluble in water at 25 ºC, but when it is modified with acid reagent plus energy density, its solubility increased from 15% to ~62%. Presumably, the modification process caused a reduction of some starch granules size due to acid treatment plus the high energy used in the modification process. This reduction is a consequence of damage to the amylose region caused by collapse of cavitation bubbles during the ultrasound process. This phenomenon produces more linear low-weight fragments, apart from cleavage of amylopectin chain that results in a higher solubility index. Falsefi et al. (2019) also observed a similar behaviour for oat starch modified using ultrasound treatment and Amini et al. (2015) for sonicated corn starch.

Abedi et al. (2019) observed a lower value for increase in the solubility using sonication treatment for native wheat and tapioca starch using two ultrasound probes with tip diameters of 20 mm and 100 mm. This difference can be related to the use of stearic acid in the malt modification.

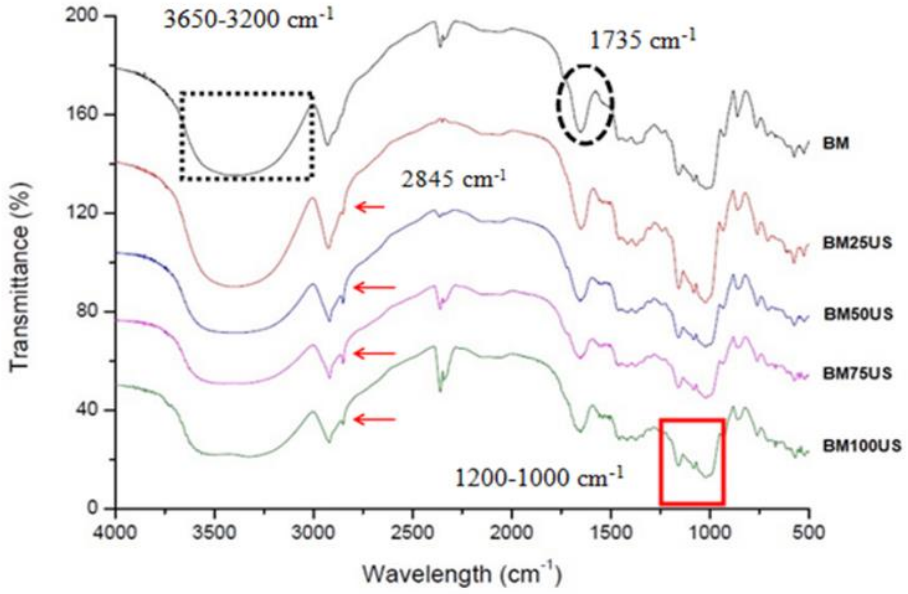
On the other hand, the insoluble solid content of 40% in modified malts are the fibers, insoluble proteins, and native granules of starch. There are some advantages for using these materials with complex composition as emulsifiers agents. Their partial solubilities in water can

contribute to easy dispersion, and the insoluble fraction presence can prevent the movement of oil droplets and increase the dispersion viscosity, thus stabilizing the emulsion.

FT-IR analysis

Figure 1 shows the structural changes in the malt samples and the differences between native and modified malt FT-IR spectra. Native malt presented a spectrum similar to native barley starch (Halal et al., 2015). The spectrum showed a broad peak between 3650 and 3200 cm^{-1}, which represents hydroxyl groups (O-H) of amylose and amylopectin molecules (Monroy et al., 2018). However, BM75US and BM100US samples presented a flatter peak when compared to the other two samples due to the alterations of amylose content during modification as explained n the "Amylose Content of Malts" *section.* Moreover, low-intensity peaks in the 2845 cm^{-1} and 1735 cm^{-1} regions were observed and corresponded to esters groups (COOH) inserted during the ultrasound–stearic acid reaction. These peaks are present in all modified malt spectra; however, the peak at 1735 cm^{-1} was more evident for BM50US, BM75US, and BM100US samples, which agrees with the results of degree of substitution presented in the *"Degree of Substitution (DS)*." The same peaks (2845 and 1735 cm^{-1}), in which the absorptions can be attributed to ester chains, were observed in the conventional modification of the esterification process of rice starch using lauric acid (García-Tejeda, Leal-Castañeda, Espinosa-Solis, & Barrera-Figueroa, 2018). Finally, absorption peaks for all samples in the range from 1200 to 1000 cm^{-1} (BM25US, BM50US, BM75US, and BM100US) were observed. These peaks and bands are characteristic of starches as reported in other FT-IR analysis of distinct starch sources (Arijaje & Wang, 2017; García-Tejeda et al., 2018; Majeed et al., 2017). Thus, the FT-IR spectra showed chemical and structural modifications in malt after processing.

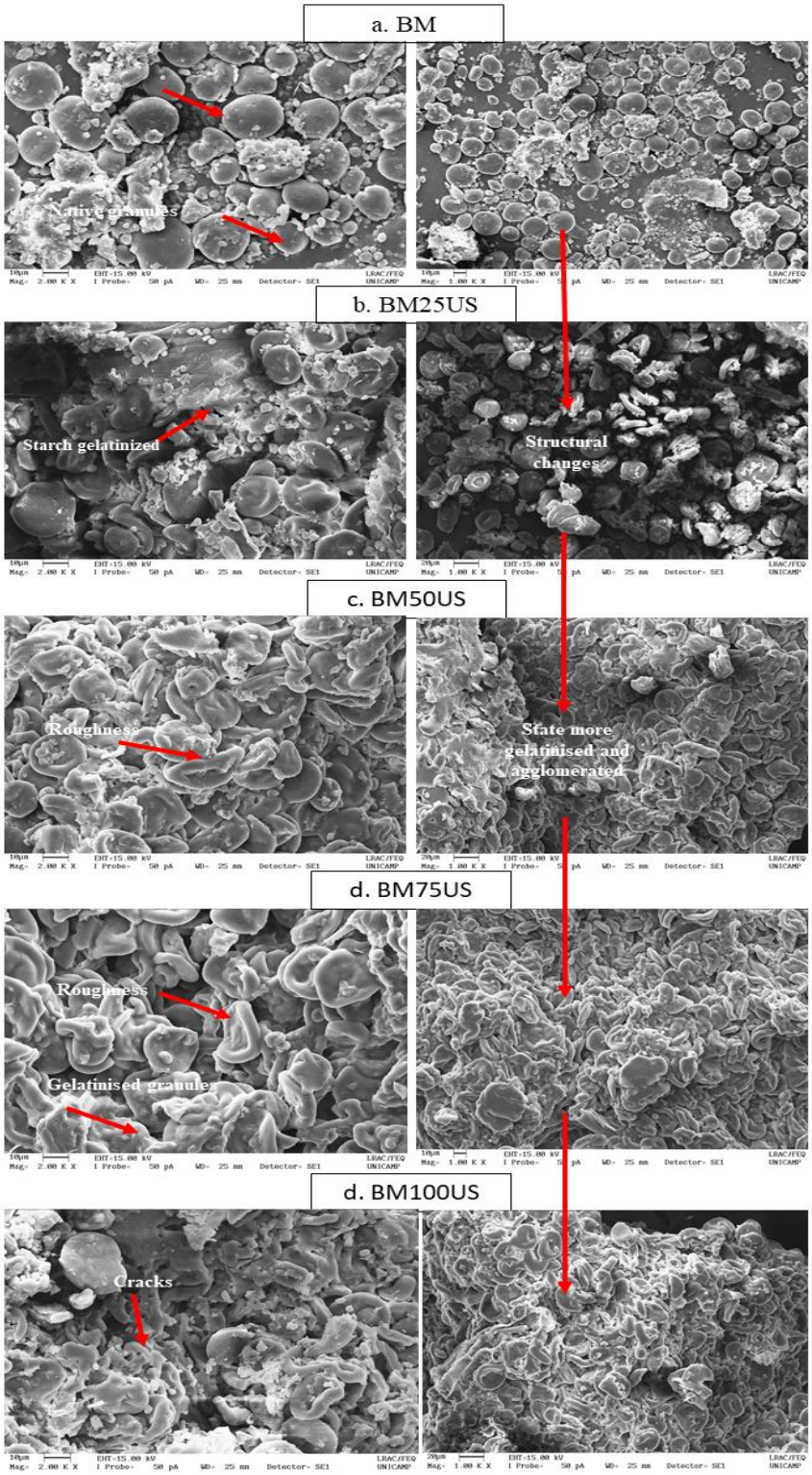
Fig. 1 Fourier transformed infrared spectroscopy (FTIR) of barley malt and modified barley malt



Microstructure characterisation by SEM

Figure 2 shows the microstructure of native and modified malts based on SEM. The native malt (Fig. 2a) granules presented lenticular shapes and large sizes. A similar microstructure and presence of fiber were found by Halal et al. (2015) in a study of barley starch. The differences between isolated barley starch and barley malt can be attributed to their composition, which contains β-glucans, starch, and protein as the main macronutrients (Montanuci et al., 2016).

Fig. 2 Scanning Electronic Microscopy (SEM) of barley malt and modified barley malts with approaches of 2.0 kx and 1.0 kx



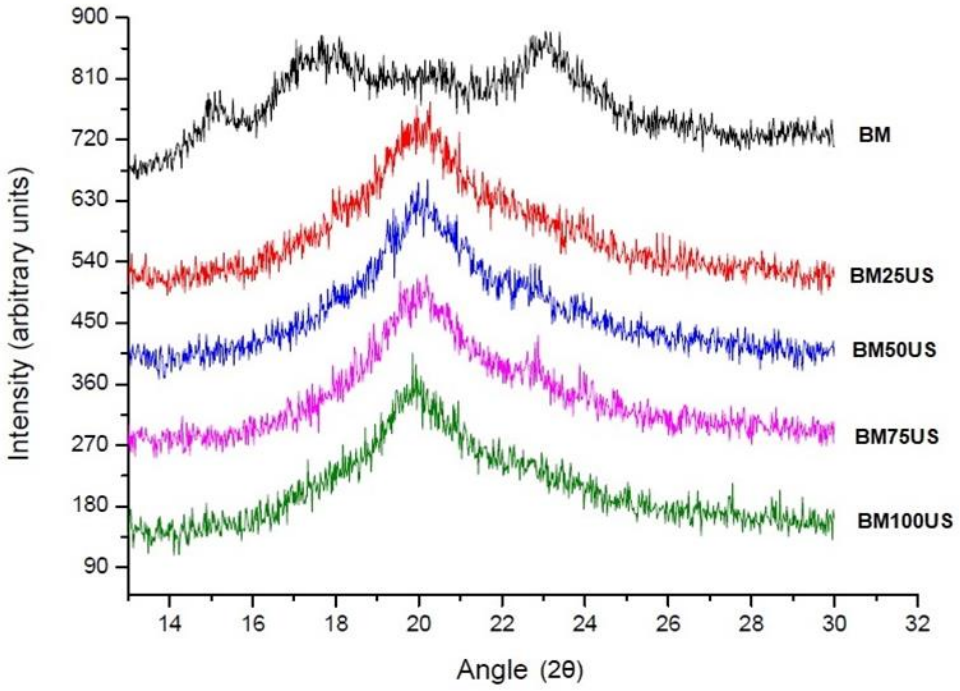
The BM25US, BM50US, BM75US, and BM100US samples (Fig. 2b-e) underwent some microstructural alterations. These granules presented similar appearance to red blood cells, visible cracks, and roughness. You et al., (2019) obtained resistant pea starch by ultrasound treatment with a rough surface and cracks. Authors noticed a strong modification caused by cavitation in the starch molecular chain. The microstructure alterations were expected since sonication can promote high-shear stress, causing damage to the granule structure (Sui & Kong, 2018). Other authors observed an increase in surface roughness in the granules of cassava starch after undergoing sonication; however, they did not notice alterations in their shape (Monroy, Rivero, and García, 2018). This result corroborates a study carried out by Sujka & Jamroz (2013) in which no changes in shape granules after modification by the ultrasound probe were observed. This study showed depressions on the surface of potato and wheat starch granules, which agrees with the results obtained for our BM25US, BM50US, BM75US, and BM100US samples. In research by Falsafi, Maghsoudlou, Aalami, Jafari, & Raeisi (2018), resistant starch was produced by ultrasound-crosslinking and presented cleft and pores on the granules' surfaces. These data may be associated with the different pH and reagents (sodium trimethophosphate/sodium tripolyphosphate 99:1, 5-15%) in the modification reaction. Some differences among reported studies can be associated with the types of materials, processing times, and pre-treatment before ultrasonic application.

X-ray diffraction

X-ray diffraction of starch can be represented by three distinct groups: (1) group A, which is associated with the peaks at 15.3°, 17.1°, 18.2°, and 23.5°; (2) group B, which is related to angle intensity at 5.6°, 14.4°, 17.2°, 22.2°, and 24°; and (3) finally, group C, which corresponds to the peaks at 5.6°, 15.3°, 17.3°, and 23.5° angles (Sajilata, Singhal, & Kulkarni, 2006). In addition, there is a region characterized by V-pattern that is associated with amylose-lipidic crystallinity and represented by peaks at the 12.6°, 13.2°, 19.4°, and 20.6° angle (Corradini et al., 2005).

Figure 3 illustrates the crystalline profile of native and modified malt samples in our study. According to X-ray analysis, native malt presented peaks at 15°, 18°, and 20° angles as represented by the A-type crystalline pattern. This profile is characteristic of cereal starches (Simsek et al., 2015).

Fig. 3 X-ray diffraction patterns of barley malt and modified barley malt by combination stearic acid and sonication



Analyzing the diffractograms of samples BM25US, BM50US, BM75US, and BM100US, only one peak could be observed in the 20°–21° region as represented by the V-type crystal structure; meanwhile, for native malt this structure was not visualized. This result is in accordance with the data provided by Liu et al. (2016) in which the presence of the same peak at 20° for starch samples was associated with a hydrophobic amylose–lipid interactions.

Therefore, sonication–stearic acid modification also influenced the malt structure caused by the applied energy density *(Figure 3)*. X-ray diffractograms revealed an increase in the V-type crystal structure and a reduction in the A-type crystal structure of all modified malts. Abedi et al. (2019) also verified a destruction of the A-pattern structure and a great reduction in the crystallinity degree after sonication procedure. The crystallinity reduction could be associated with the erosion of weak crystalline structures caused by intense energy of sonication procedure. The ultrasonic waves can

promote a destabilization of the lamellar array of starch granules affected mainly amylose region due to its linear chain (Falsafi et al., 2019; Flores-Silva et al. 2017; Zhu, Li, Chen, & Li, 2012).

The results found in this work regarding microstructural properties may be associated with the modification process by sonication–stearic acid; however, the modified malts did not present differences between them based on qualitative analysis as could be observed in in FTIR (the "FT-IR Analysis" *section*), and SEM images (the "MicrostructureCharacterization by SEM" *section*).

Thermal properties

Table 2 shows the thermal properties of native and modified malts. Native starch is composed by amorphous amylose and part of the crystalline amylopectin is a semi-crystalline material. Due to this composition, the glass transition temperature (T_g) of cereal, starches, and their derivatives are represented by a second-order event that shows a change from an amorphous phase to a vitreous state (Roos, 1995).

Table 2 Thermal properties of materials

Samples	Glass Transition Temperature (ºC)	Gelatinization Temperature (ºC)	Gelatinization Enthalpy (J/g)
BM	126.8	62.50	4.68
BM25US	118.8	67.93	0.06
BM50US	115.7	66.35	0.24
BM75US	113.2	66.19	0.2
BM100US	104.1	71.68	0.3

*BM: Native barley malt; BM25US: Barley malt modified with 25% of amplitude sonication in relation to nominal power; BM50US: Barley malt modified with 50% of amplitude sonication in relation to nominal power; BM75US: Barley malt modified with 75% of amplitude sonication in relation to nominal power; BM100US: Barley malt modified with 100% of amplitude sonication in relation to nominal power.

T_g is a phenomenon influenced by crystalline region. Different behaviours of modified malt were obtained by X-ray analysis. As shown in Table 2, the T_g values decreased with ultrasound energy density increments. For instance, native malt presented a Tg of 126.79 °C; meanwhile, BM100US had a glass transition of 104.09 °C. In a study with only barley starch, van Donkelaar et al. (2015) verified

a range of glass transition temperatures from 284 to 240 °C as defined by differential scanning calorimetry (DSC) analysis. This difference could be associated with the type of material and process to which materials were subjected. These researchers also reported that inflexible crystalline structure states promote the reduction of the mechanical effects of the Tg. Based on these processes, the damage to the crystalline region resulted in a reduction of T_g with a corresponding increase in applied power. In addition, glass transition values are associated with DE degree so that the increase in DE degree resulted in a reduction of glass transition temperature (Pycia et al., 2016), a behaviour also observed in this work.

Another important thermal property is the gelatinization temperature *(Table 2).* This property is an endothermic event and results from the heating and presence of water. The phenomenon is characterized by swelling of starch granules, loss of crystallinity, and reduction in water absorption into their structures (Eliasson, 2004).

Table 2 shows an increase in the gelatinization temperature after elevation of the energy densities in the modification process using stearic acid, which varied from 62.5 °C (native malt) to 71.68 °C (BM100US). The enthalpy (ΔH) values of the gelatinization event decreased from 4.68 to 0.06 J/g. According to the study of Falsafi et al. (2018) regarding the production of resistant starch, sonication treatment causes an increase in the thermal phase transition of starch; however, the gelatinization enthalpy decreased. This behaviour was also observed by Abedi et al., (2019) in study involving native wheat starch and native tapioca starch submitted to ultrasound treatment. Therefore, ultrasound process can promote loss of integrity in the crystalline fraction of the starch granule.

In a recent study involving native cassava and modified cassava starches after ultrasound treatment, no differences between these materials were noted, except for modified starch with 60% amplitude in relation to nominal power of 750 W (Monroy et al., 2018). In this case, no peaks were present due to the complete gelatinization during ultrasound process. The observed differences could be associated with the type of modification process chosen by these authors.

Thermal properties were affected by the split of the amylose chain and crystalline region because of the ultrasound-treatment they suffered corroborating with FTIR spectrum and X-ray diffraction results. It is important to emphasize that studies regarding glass transition and gelatinization temperature of modified malt by ultrasound–stearic acid process have not been yet reported in the literature.

Effects of modification process on malt emulsifying properties

Emulsion stability

Figure 4A shows images of emulsions at time 0 and 24 h after preparation. At the initial time, a stable emulsion for samples prepared with modified malt was observed, and after 24 h, a small separation was noticed in the dispersion. On the other hand, emulsion composed of native barley malt presented a phase separation after the homogenization.

Figure 4B presents the stability index values obtained by turbidimetry for all emulsions after 24 h of storage. When low values for TSI (<5) are observed, these emulsions are considered stable because the higher the TSI value the more unstable the emulsion is (Kang et al., 2011).

Fig. 4 Pictures of emulsions in the initial time (0) and after 24h of storage (a) and the graph containing TSI values for all emulsions prepared with native barley malt and modified barley malts

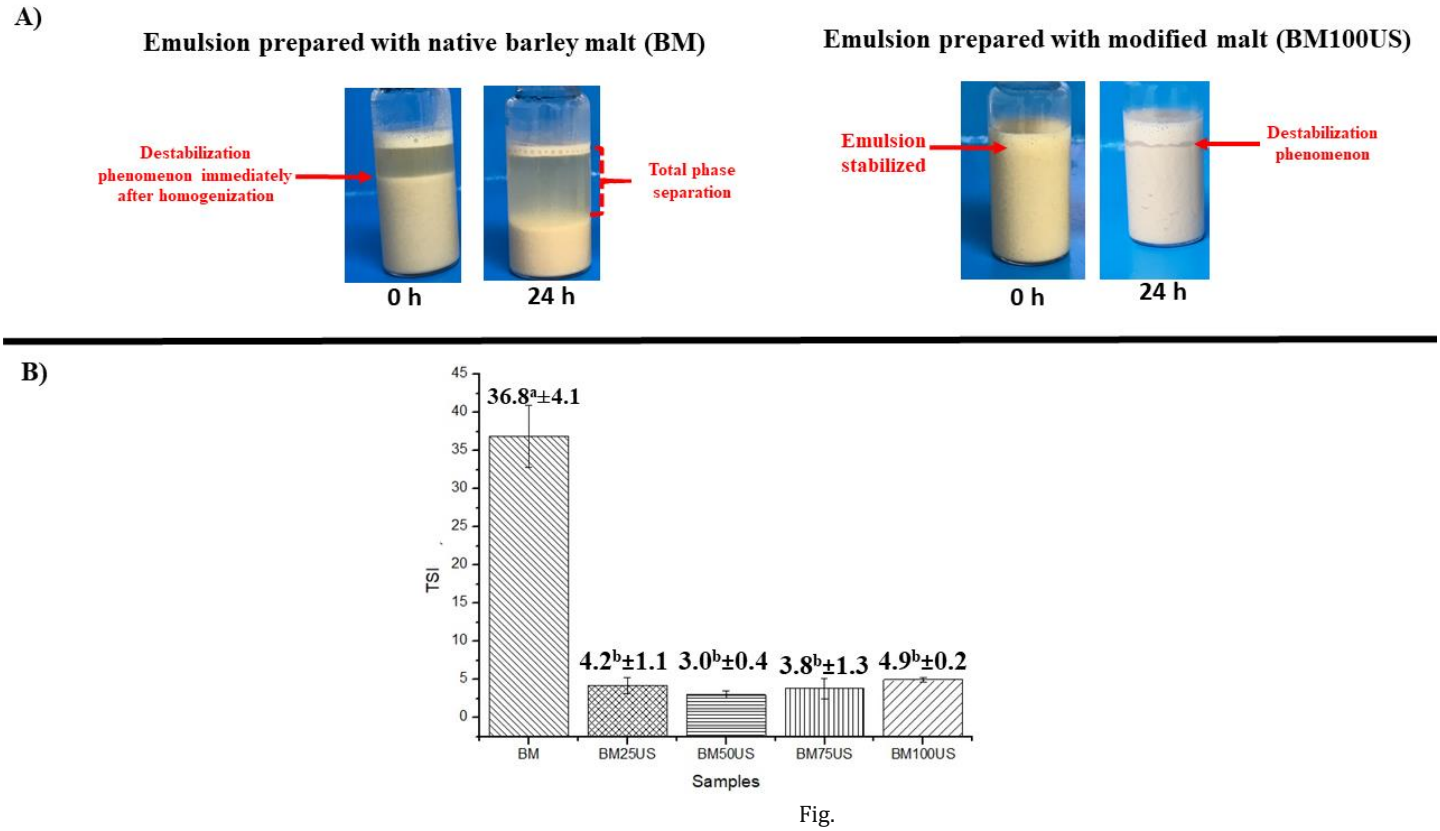


Fig.

The TSI values varied from 3.0 ± 0.4 to 4.9 ± 0.2 for emulsion with modified malt after a 24 h storage period at 25 °C; meanwhile, emulsions with the native malt had a stability index of 36.8 ± 4.2. In the emulsion prepared with native malt, a creaming event was observed, which is a gravitational phenomenon characterized by upward droplet displace due to their lower densities than in the continuous phase (McClements, 2007).

Regarding the stability index of native malt (36.8), a higher average was observed compared to other emulsions samples. This value revealed that no emulsion was formed due to the precipitation of the system immediately after homogenization. In this case, native malt was not capable of reducing surface tension between oil and water. Similar results were found by Simsek et al. (2015) for native potato, wheat, tapioca, and corn starches in emulsions using medium-chain triglyceride oil as a dispersing medium and processing conditions of 22,000 rpm for 30 sec by high-shear mixing. Leal-Castañeda et al. (2018) also obtained higher TSI values (ranging from 11.02 to 16.79) for native amaranth starch in Pickering emulsions containing 2%–6% starch in their formulations. For this reason, one can say that native starch has low emulsifying properties.

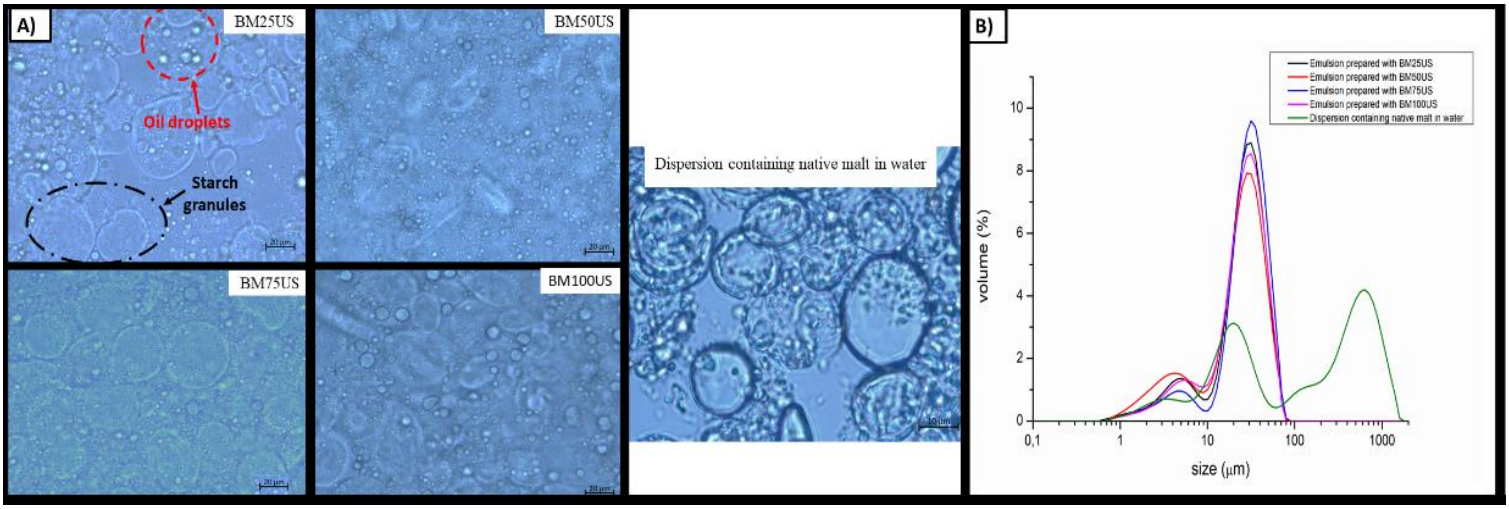
Emulsions prepared with modified malt obtained from different energy densities presented TSI values < 5 after 24 h storage at 25 °C, which did not differ significantly ($p < 0.05$) from the analyzed emulsions. These data showed that modification by ultrasound–stearic acid resulted in a novel material with emulsifying properties. The emulsifying property may be associated with the presence of an ester group in the malt starch chain, resulting in an increase of affinity between the malt and the oil phase. As reported by Fonseca-Florido et al. (2018), starches with hydrophobic groups presented greater absorption at the oil/water interface and improved stability for an extended period of time. The same result was observed for amaranth rice starch that was esterified by lauric acid. The authors obtained a TSI value of 5.12 after 24 h of preparation for an emulsion homogenized by Ultra-Turrax at 11,000 rpm for 1 min with 20% of modified starch and canola oil as a dispersing medium in a proportion of 7:3 (v/v) (water:oil) (García-Tejeda et al., 2018). In addition, the gelatinized malt starch granules can also promote steric stabilization for emulsions. A similar behaviour was found in stable emulsions using non-chemical modified gelatinized starch in the work of Kasprzak, Macnaughtan, Harding, Wilde, & Wolf, (2018).

Microstructure and droplet size distribution of emulsion stabilized by modified malts

The structure of the emulsion was obtained after size distribution and microscopy analyses. *Figure 5A* reveals that the droplets presented small size differences for all emulsions obtained by modified malts. The starch granule of malt surrounded the oil droplet, so we concluded that the smaller droplets were oil, and the bigger droplets were malt starch granules. This result can be compared with images of dispersion containing only native malt in water because this type of image emphasizes the size of whole starch. A similar phenomenon was also shown by Simsek et al., (2015). According to the authors, the starch granules with amphiphilic character may form a dense coating at the oil/water interface, resulting in emulsions with greater stability.

Figure 5B shows the bimodal distribution for all the analysed emulsions and the dispersion of native malt. The emulsions presented one small peak that was related to oil droplets and ranged from 1 to 10 µm, another peak that varied from 10 to 100 µm, corresponding to whole starch granules, and other insoluble materials present in the malt. On the other hand, polymodal behaviour was observed for the curve of dispersion composed by native malt in water. We can check three curves ranging from 1 to 10 µm, 10 to 100 µm, and 100 to 1000 µm. These peaks are characterized by different sizes of native starch granules and others by biopolymers in addition to insoluble proteins and fibers. The process reduced the size of starch as can be viewed in this graph (Figure 5B), but there are still whole starch granules and insoluble particles that contribute to emulsion stabilization.

Fig. 5 Microscopy images (a) and graph of size distribution (b) for canola oil in water emulsion composed by modified barley malt (40x) and dispersion containing barley malt.

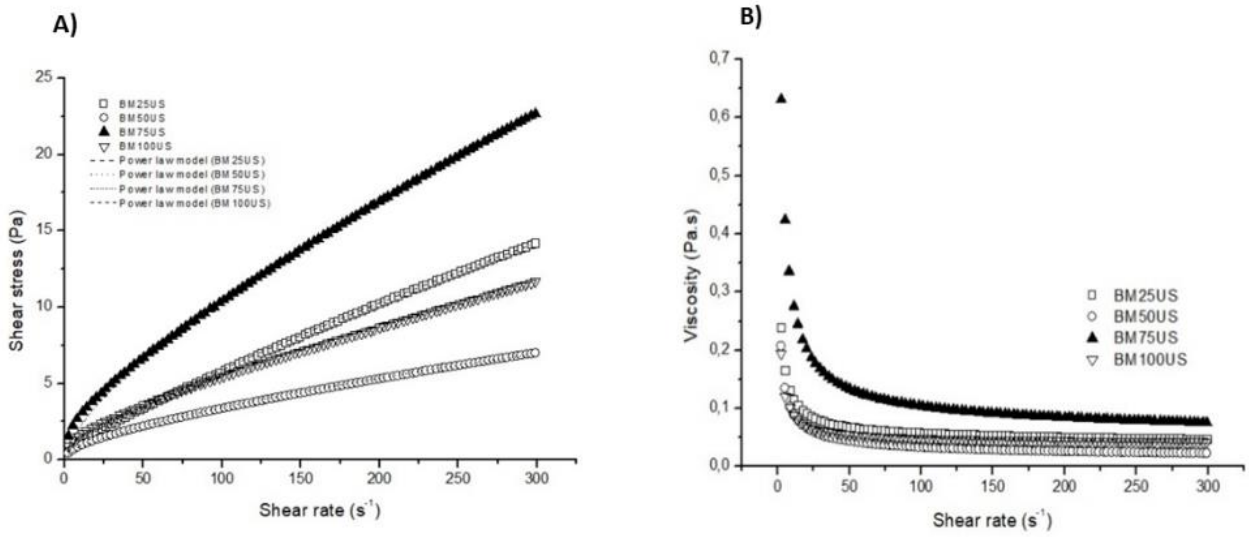


Rheological behaviour

A rheological study provides important parameters for the food industry, and the resulting data may be associated with droplet size distribution and the stability of emulsions.

Figure 6A and *6B* represent the rheological performance of the emulsions, and *Table 3* shows the results of the power law model. The fitting by power law model was satisfactory ($R^2 > 0.997$), and the flow showed pseudoplastic behaviour ($\eta < 1$) for all emulsions. The results in *Table 3* demonstrated an increase in the consistency index (0.148 to 0.449 $Pa.s.n^{-1}$); meanwhile the behaviour index has decreased from 0.800 to 0.645. Similar result was observed for Pequi oil emulsion using WPI, maltodextrin and inulin as an emulsifier agent (Oliveira et al. 2019).

Fig. 6 Rheological properties of emulsions stabilized by modified barley malts



In relation to viscosity values for the rate of 300 s^{-1}, *Table 3* shows low viscosities ranging from 0.023 to 0.069 Pas. Pickering emulsions produced with modified starches and whey protein isolated presented containing 8% (w/w) solids presented low values. According to authors this result for apparent viscosity is suitable for emulsions processing by spray drying due to lower energy expenditure (Comunian et al., 2019).

Table 3 Rheological behavior determined for canola oil-in-water emulsions by fitting the Power law to flow curves (shear rate x shear stress).

Emulsions	Parameters by fitting power law model			Apparent Viscosity in shear rate of 300 s^{-1} (Pa.s)
	k(Pa.s.n^{-1})	η	R^2	
BM25US	0.15^{b}±0.0	0.800^{a}±0.002	0.999	0.048^{c}±0.001
BM50US	0.17^{b}±0.03	0.645^{d}±0.003	0.997	0.023^{d}±0
BM75US	0.45^{a}±0.01	0.685^{b}±0.004	0.998	0.069^{a}±0.009
BM100US	0.26^{b}±0.07	0.663^{c}±0.005	0.997	0.043^{b}±0.001

*k (Pa.s.n^{-1}): consistency index, η: behavior index and R^2: determination coefficient by fitting power law model.BM25US: Emulsion composed by barley malt modified with 25% of amplitude sonication in relation to nominal power and canola oil as model oil; BM50US: Emulsion composed by barley malt modified with 50% of amplitude sonication in relation to nominal power canola oil as model oil; BM75US: Emulsion composed by barley malt modified with 75% of amplitude sonication in relation to nominal power canola oil as model oil; BM100US: Emulsion composed by barley malt modified with 100% of amplitude sonication in relation to nominal power canola oil as model oil. Means followed by lower case in the same column do not differ significantly ($p < 0.05$) by the Tukey test.

Conclusions

Results achieved in this study showed relevant information regarding malt modification for use as a novel encapsulating agent. The energy density used in ultrasound processing increased the degree of substitution, amylose content, solubility index, DE values, and the protein solubility. With an increase in energy density, there were changes in the malt microstructure, such as reduction of the crystalline region, increase in ester group content, and shape alterations. BM25US, BM50US, BM75US, and BM100US formulations added into the oil-in-water emulsions presented good emulsifying properties. The modification process using 25% of amplitude power and a power of 45W were then chosen for the continuation of this study since malt presented structural modifications, higher protein content, and good emulsifying properties at a low energy density (33.8 $x10^6$ J/m^3). Overall, modified malts esterified with stearic acid have high potential as replacer to fat in sauces and mayonnaise due to their viscosity, hydrophobic properties, and good ingredients for energy drinks because of their sugars content. In addition, modified malts could be applied as emulsifier agents for hydrophobic compounds.

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Compliance with Ethical Standards

Conflict of Interest The authors of this study state that there is no conflict of interest

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CAPÍTULO 4

PHYSICOCHEMICAL PROPERTIES OF CAPSICUM OLEORESIN EMULSIONS STABILIZED BY GUM ARABIC, OSA MODIFIED CORN STARCH AND MODIFIED MALT

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PHYSICOCHEMICAL PROPERTIES OF *CAPSICUM* OLEORESIN EMULSIONS STABILIZED BY GUM ARABIC, OSA MODIFIED CORN STARCH AND MODIFIED MALT

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Abstract

The objective of this study was to evaluate the individual and combined use of gum Arabic (GA), OSA-modified corn starch (EMCAP) and stearic acid-modified malt (MALT) as emulsifiers of *Capsicum* oleoresin emulsions. Emulsions were investigated regarding their morphological and physicochemical properties and capsaicin retention. The synergism between GA:MALT and EMCAP:MALT was able to reduce the interfacial tension values (<8.7 mN/m) and enabled the formation of kinetically stable emulsions (Turbiscan Stability Index < 4.4). Steric forces and increased viscosity also contributed to the stability of these emulsions. Also, MALT promoted an increase in droplet size due to the presence of whole starch granules. The impact of different formulations on the color parameters resulted in Chroma values ranging from 31.4±0.7 to 61.2±0 and hue angle between 42.1°±0 to 60.4°±0.1, characterized by orange and red tones. Our study found that the interaction of MALT with gum Arabic and EMCAP promoted an increase capsaicin retention and viscosity of *Capsicum's* oleoresin emulsion, showing the potential of these carbohydrates for further use as encapsulant materials in spray drying microencapsulation.

***Keywords**:* Capsaicin; Interfacial tension; Carbohydrates; Modified starch; Emulsion stability

Introduction

Capsicum oleoresin contains fatty acids, triglycerides, carotenoids and is a rich source of capsaicin, a chemical compound that is isolated from chili peppers. The underlying characteristics of *Capsicum* oleoresin are responsible for prevention of chronic diseases, such as diabetes, cardiovascular, cancer and obesity (Melgar-lalanne et al., 2016; Adaszek et al., 2019). However, there are certain challenges, such as strong pungency and low stability at varying temperature and pH, limiting the application of *Capsicum* oleoresin in food products (Melgar-Lalanne, Hernández-Álvarez, Jiménez-Fernández, & Azuara, 2016).

These limitations can be overcome by encapsulating the oil through emulsification. Oil in water (O/W) emulsion formation is characterized by dispersing oil droplets in the aqueous phase. Emulsions can improve the solubility of compounds and protect them from processing conditions. In addition, emulsification can mask the off-taste and improve the bio accessibility of hydrophobic compounds, further extending its application in food products (McClements, 2009).

Different methods have been used to encapsulate *Capsicum* oleoresin, such as high-pressure homogenization, ultrasonication (Akbas et al., 2018a), microfluidization (Akbas et al., 2019), and diffusion method (Surassmo et al., 2010). However, in food technology, *Capsicum* oleoresin emulsification by low energy methods remained still unexplored. For instance, rotor-stator mixer is a low-cost method, resulting in a dispersion that contains the core compound dissolved in the dispersed phase, being interesting to produce powder microparticles by spray drying (Gharsallaoui et al., 2007).

Associated with the emulsification method, the aqueous phase composition is also responsible for some aspects regarding emulsion stability, droplet size, encapsulation efficiency, and rheological properties. Polysaccharides play an important function as emulsifiers, stabilizers, gelling agents due to their physicochemical properties. For instance, modified starches and gum Arabic are commonly used in emulsion formulations (Comunian et al., 2020; Ribeiro et al. 2020; Matos et al., 2018; Kanakdande et al., 2007) due to their low-cost, overall safety when consumed and availability (Gaonkar et al., 2014). Gum Arabic has excellent emulsifying properties that account for a high encapsulation efficiency of natural hydrophobic pigments, flavors, oils, oleoresins and phytosterols (Dalgleish, 2004; Kanakdande et al., 2007; Ma et al., 2020; Premi & Sharma, 2017; Rascón et al., 2011). Starches modified by esterification with octenyl succinic anhydride (OSA), or stearic acid are also used as emulsifier agents attributable to its amphiphilic property, abundance, and non-allergenic nature. These starches show hydrophobic groups in their molecular structure chain that lead to lower gelatinization temperature,

resistance against enzymatic digestion and higher paste clarity (Altuna et al., 2018; Alves et al., 2015; Zhu, 2017).

Current research has undertaken developing new encapsulating agents from alternative sources, such as octenyl succinate modified quinoa starch (Li et al 2020) and β-glucan derived from barley (Salgado, Rodríguez-Rojo, & Cocero, 2017). In addition, modified barley malt could also be a novel and promising alternative as encapsulating material. Malt is produced in large scale around the world, being the main ingredient used in beer production, according to the Beer Institute (2019). Barley malt is of low-cost availability, rich in starch, fibers, proteins, and phenolic compounds. Moreover, literature reports have associated the malt intake with the reduction of glycemic index, decrease of oxidative stress and prevention of body against inflammation (Leitao et al., 2012; López-Martínez, Leyva-López, Gutiérrez-Grijalva, & Heredia, 2017; Maetens et al., 2017; Montanuci, Ribani, Jorge, & Jorge, 2016; Nelson et al., 2016).

Although there are studies in the literature that address the encapsulation of pepper oil with surfactants (Galvão, Vicente & Sobral, 2018), and alginate plus chitosan (Choi et al., 2011), the development of emulsions stabilized with cheap and available carbohydrates are still necessary. Previous work from our research group reported the good emulsifying properties of modified malt for corn oil emulsions due to its characteristics as higher solubility, presence of ester groups, and lower viscosity compared to native malt (Anthero et al. 2021). In this way, in order to design food emulsions to be further spray dried, this work evaluated the influence of stearic acid-modified malt (MALT), gum Arabic (GA) and OSA-modified corn starch, individually or in combination, to be used as emulsifiers of *Capsicum* oleoresin emulsions. The impact of different carbohydrates on the structure and physicochemical properties of the emulsions was evaluated by means of interfacial tension, emulsion stability, droplet size, morphology, zeta potential, rheological behavior, color parameters and capsaicin retention.

Material and Methods

Material

Capsicum oleoresin was purchased from Synthite Industries Ltd. (Kerala, India). Gum Arabic Gum Arabic Instantgum BA™ (GA) and octenyl succinic anhydride (OSA) corn starch (EMCAP^{TM}) were kindly donated by Nexira (São Paulo, Brazil) and Cargill (Campinas, Brazil), respectively. Malt (Lager Pilsen) was obtained from Art Brew Company (Campinas, Brazil). Barley malt was esterified by ultrasound (700W full power, 20kHz frequency - QSonica - Newton, CT, USA) with a probe of 19.1mm nominal diameter at 45 W for 5 min using 2% (w/w) of stearic acid (Dinâmica, 95%, São Paulo, Brazil). As result, the authors obtained a modified malt with a substitution degree of 0.016±0.006 %, dextrose equivalent of 3.34±0.02, protein content of 4.81±0.10, amylose content of 21.6% and solubility 62.1% ± 4.7(Anthero et al. 2021). Capsaicin standard was acquired from Cayman Chemical (Cayman Chemical, USA, >95%), and all the other reagents were of analytical grade.

Interfacial tension of biopolymers dispersions

The interfacial tension (mN/m) of the biopolymer dispersions was obtained using a tensiometer Tracker-S (Teclis, Longessaigne, France) by the pendant drop method. Assays were carried out with the formation of a drop of the six aqueous phases (GA, EMCAP, MALT, GA: MALT, EMCAP: MALT, or GA: EMCAP) composed by 14.25% (w/v) of solids with the proportion between the biopolymers of 1:1. In the oil phase, commercial canola oil was employed, and a syringe (3 mm diameter) was used forming drops with an oil volume of 10 μL. *Capsicum* oleoresin was not used since it has a dark color, which does not measure interfacial tension. For this reason, canola oil was used only for purposes of comparison among the different continuous phases. These analyses were performed in triplicate at 25 °C for 3,600 s.

Emulsion formation

Biopolymers (GA, EMCAP, and MALT) were previously dissolved in distilled water under magnetic stirring for 12 h. The materials as well as combinations of these materials (GA: EMCAP, GA: MALT, and EMCAP: MALT were used in emulsions composed of 15% of total solids (in which 5% was oleoresin and 95% represented emulsifier agent), with a proportion between biopolymers of 1:1, as can be seen in **Table 1.** All the six emulsions were prepared in duplicate by mixing *Capsicum*

oleoresin and aqueous phase using a rotor-stator (Silverson L5M-A Laboratory Mixer, Chesham, Buckinghamshire, UK) 5,000 rpm for 10 min, which resulted in twelve treatments.

Table 1. Emulsion formulation containing *Capsicum* oleoresin as oil phase

Treatments	Emulsifier material	Composition of oil and aqueous phase
GA	GA (gum Arabic)	100 g of emulsion containin
EMCAP	EMCAP (Octenyl succinic anhydride (OSA)-modified corn starch)	15% total solid (95% is emulsifier and 5% is *Capsic* oleoresin)
MALT	MALT (Stearic acid- modifi malt)	
GA:EMCAP	GA:EMCAP (1:1)*	
GA:MALT	GA:MALT (1:1)*	
EMCAP:MALT	EMCAP:MALT (1:1)*	

*(1:1) is proportion of materials blended, being 50% of each one.

Kinetic Stability Of Emulsions By Laser Scanning Turbidimetry

The stability of *Capsicum* oleoresin emulsions was analyzed by an optical scanning instrument Turbiscan ASG (Formulaction, l'Union, France). Twelve emulsions (20 mL) were placed in cylindrical glass tubes (Vial EPA colorless, 27.5x72.5 mm) immediately after preparation. The Turbiscan Stability Index (TSI) was measured simultaneously, and the values were obtained after 2 hours of emulsion preparation, which is the time enough to produce microparticles by laboratory scale spray drying. TSI values were calculated by Equation 1.

$$TSI = \sqrt{\frac{\sum_{i=1}^{n}(x_i - x_{bs})^2}{n-1}} \qquad (1)$$

Where x_i is mean backscatter per minute of measure, x_{bs} is a mean value, and n is scanning number. The measurements of delta backscattered light intensity were performed at 0, 1, 2, 3, 4, 5, 6 and 24 hours after preparing the emulsions and were calculated at different sample heights (mm) using a wavelength of 880 nm. All measurements were acquired in triplicate.

Emulsion droplet size and size distribution

The droplet size distribution of all emulsions was measured by laser diffraction using the Mastersizer 2000 (Malvern Instruments Ltda, Malvern, United Kingdom) equipment and distilled water was used as the dispersant solvent. The mean droplet diameter was expressed as the volume-surface mean diameter $D_{[3,2]}$ (Sauter mean diameter – Equation 2). The span value, which gives the width of size distribution, was calculated from Equation 3. All twelve samples were measured six times.

$$D_{3,2} = \frac{\sum n_i D_i^3}{\sum n_i D_i^2} \qquad (2)$$

$$Span = \frac{(d_{0.9} - d_{0.1})}{d_{0.5}} \quad (3)$$

Where $n_i$is the number of droplets, D_i is the diameter and $d_{0.1}$, $d_{0.5}$ and $d_{0.9}$ represent the diameter of accumulated distribution of 10, 50 and 90% of total droplets, respectively.

Optical microscopy

A morphology analysis was performed in triplicate on the twelve emulsions using an optical microscope (Axio Scope.A1, Carl Zeiss, Gottingen, Germany) coupled to an objective with 100x magnification. The software AxioVision Rel. 4.8 (Carl Zeiss, Germany) was used to obtain the images.

Z-potential

The zeta potential of the six biopolymer dispersions and twelve emulsions were measured using Zetasizer Nano ZS (Malvern Instruments, Worcestershire, United Kingdom). The samples were diluted into 0.1% (v/v) in Milli-Q water and the measurements were performed with six replicates.

Rheological behavior

All emulsions (twelve) were submitted to rheological analyses. Flow curves of the emulsions were determined in triplicate by a controlled stress rheometer AR1500ex (TA Instruments, Elstree, United Kingdom) using double concentric cylinders (GAP of 500µm). Data were acquired in a range between 0 and 300 $s^{-}1$ in a three-stage sequence: up-down-up. The third curve data was fitted to the power Law according to Equation 4. The apparent viscosity was obtained at a shear rate of 300 s^{-1}, similar shear rate reached by spray drying. The emulsions were performed at 25 ºC immediately after its preparation.

$$\sigma = K.(\gamma)^{\eta} \qquad (4)$$

Where σ is the shear stress (Pa), K is the consistency index ($Pa.s^{n}$), γ is the shear rate (s^{-1}) and η is the behavior index (dimensionless number).

Instrumental color

The instrumental color of the emulsions of *Capsicum* oleoresin was determined in triplicate by Ultra Scan Vis 1043 (Hunter Lab, Reston, United States of America) with the illuminant D65 and the observation angle of 10º (RESEX mode), and the results were obtained through Easy Match QC software (United States of America). Black and white standards were used for calibration. The measurements were determined according to the CiELAB scale: L* represents the lightness or darkness of the sample; a* (+a* = red and -a* = green) and b* (+b* = yellow and -b* = blue) express the color. Using a*, b* parameters, the hue angle (h°) and chroma (C) values of the samples were determined by the following equations:

$$h^{\circ} = arctan\left(\frac{b}{a}\right) \quad (5)$$

$$C = \sqrt{a^2 + b^2} \quad (6)$$

Capsaicin retention

Capsaicin retention (CR) was performed in triplicate using 25 mL of methanol solvent for extraction of capsaicin present in the emulsion (0.5 g) and for *Capsicum* oleoresin (CO) (0.005g) according to De Aguiar, Silva, Alves De Rezende, Barbero, & Martínez (2016) and De Freitas et al., (2018) with some adaptations. Samples were placed in an ultrasonic bath for 15 min and centrifuged for 15 minutes at 10,000 rpm. The supernatant was filtered with a PTFE (polytetrafluoroethylene) hydrophilic filter with a pore size of 0.45 μm and a diameter of 13 mm. Capsaicin content was obtained by HPLC (High-Performance Liquid Chromatography) (Dionex, UltiMate 3000 Standard LC, United States of America) using POROSHELL 120 EC-C18 column (100 mm × 4.6 mm, 2.7μm). The mobile phase contained a fraction of 85% of methanol and 15% of deionized water, and the analysis was performed at 25 ºC at 280 nm with a mean compound retention time of 1.6 min. The capsaicin standard was used to calculate the calibration curve (R^2 = 0.9992). Capsaicin retention (CR%) was calculated according to Equation 7.

$$CR\% = \frac{Capsaicin\ present\ in\ emulsion\ (g)}{Capsaicin\ present\ in\ CO\ added\ in\ emulsion\ (g)} x100 \quad (7)$$

Statistical analysis

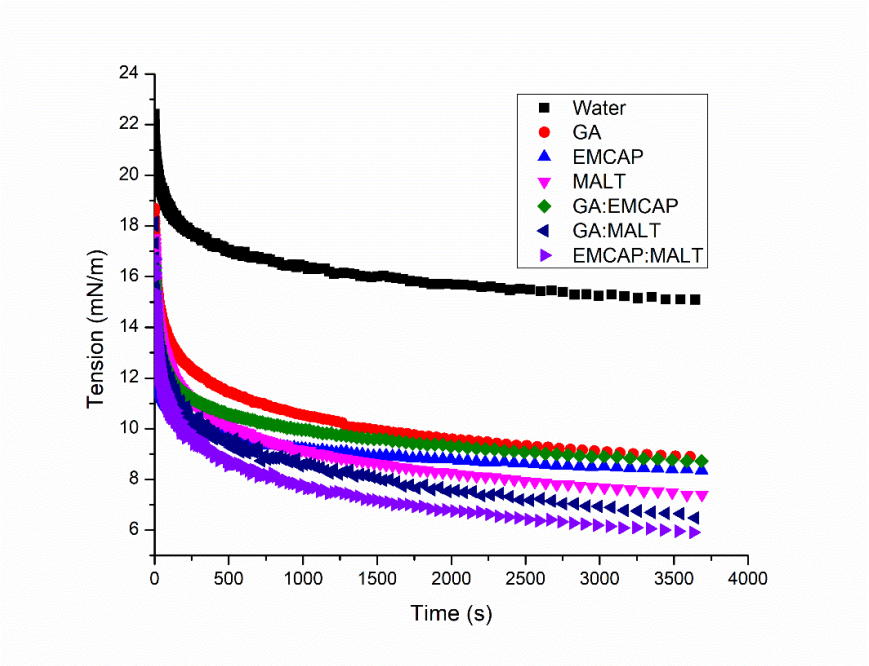
All assays were done in triplicate at least, and analysis of variance (ANOVA) of the average was performed using the Minitab 18® software at a 95% significance level. Differences between mean values were compared using Tukey's test at 5% significance (p-value < 0.05).

Results and Discussion

Impact of mixed biopolymers systems on interfacial tension

The initial and equilibrium interfacial tension between canola oil and the different biopolymers composition provided information about the effect of each component on the droplet interface, as can be seen in Fig.1. An aqueous phase in the absence of any carbohydrate presented a tension value of 16 mN/m since water does not present an affinity with the oil phase. Meanwhile, the adsorption of dispersions containing 14.25% of GA, EMCAP and GA: EMCAP promoted a reduction of contact area between oil and water molecules reaching low values of equilibrium interfacial tension (8.7 to 8.9 mN/m). These results can indicate that these materials have a good emulsifier property. Comunian et al. (2019) obtained final values for interfacial tension in the range of 4.21–9.97 mN/m in emulsions composed by protein: carbohydrate (WPI: GA) or only protein (WPI). These values are close to the data acquired in our research, showing that the combination of carbohydrates alone can produce stable systems. In another study, authors reached higher interfacial tension (17.04 mN/m) for gum Arabic dispersion phase (Flores-Andrade et al. 2020) probably due to the lower concentration (1-5%) of GA used in aqueous phase.

Fig. 1 Dynamic interfacial tension of the biopolymer dispersions. GA, gum Arabic; EMCAP, modifed corn starchOSA(EMCAP®); MALT, stearic acid-modifed malt; GA: EMCAP, GA: MALT, and EMCAP: MALT.



Concerning the novel encapsulating material (MALT), low values of interfacial tension were obtained, evidencing a good stabilizing property. The suspensions prepared with MALT presented interfacial tension value of 7.5 mN/m, while the mixtures GA: MALT and EMCAP: MALT had lower values, 6.5 and 6 mN/m, respectively, after 3,600 s of analysis. As a result, the combination of MALT with GA and EMCAP has improved its emulsifying properties. The ester group in its MALT chain contributed to reduce the tension between the aqueous phase and the oil layer due to the increase in lipophilic properties.

Starches modified with hydrophobic groups have been reached greater ability to adsorb at the air/water interface regarding the native starch (Kasprzak, Macnaughtan, Harding, Wilde, & Wolf , 2018; Prochaska et al., 2007, Tesch, Gerhards, Schubert, 2002). The interfacial tension (γ) of kudzu starches between soybean oil showed that octenyl succinic anhydride modified kudzu starch had a lower interfacial tension value than native material, and when the authors increased the kudzu starch concentration the γ decreased from 25.5 to 13.4 mN/m (Zhao et al., 2019). According to Kasprzak, Macnaughtan, Harding, Wilde, & Wolf (2018) aqueous phase containing gelatinized waxy rice starch only reached at 26.8±1.3 mN/m meanwhile the dispersions composed by OSA starch showed an lower interfacial tension at 18.2±0.1 mN/m. These results prove that starches containing hydrophobic groups into starch are interfacially active and able to decrease the interfacial tension.

Droplet size, size distribution and morphology

Table 2 shows significant differences in droplet size and span values for all treatments. The emulsion stabilized by GA, EMCAP and GA: EMCAP had mean droplet sizes ($D_{3,2}$) of 2.09±0.02, 1.66±0.01, and 3.21±1.49 µm, respectively, showing that the combination GA: EMCAP promoted an increase in $D_{3,2}$ values. Small droplet sizes also were observed by Chranioti & Tzia (2014) for fennel oleoresin emulsions produced with gum Arabic. However, emulsions prepared with modified malt alone had a droplet size of 18.0±0.3 µm, value higher towards formulations composed only with conventional materials. Incorporation of modified malt with GA and EMCAP increased droplet size values 16.1±0.2 and 17.7±0.4 µm respectively, indicating a strong interaction and aggregation between these carbohydrates. According to Anthero et al. (2021), this behavior also was associated with the present hydrophobic group and the large droplet size of malt. Moreover, some authors highlighted that starch molecules can affect the size and structure of the emulsion by starch swelling into the aqueous phases (Iqbal et al., 2019).

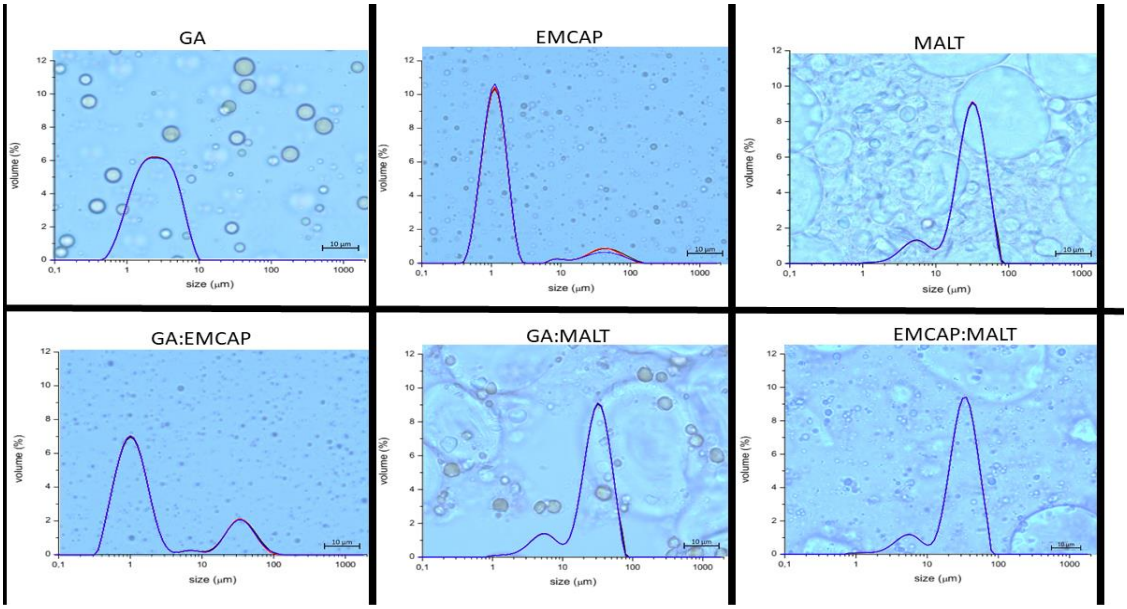
Table 2. TSI values, particle size (µm), polydispersity, pH and zeta potential (mV) of hydrated biopolymers and emulsions.

Treatments	TSI values (2h)	D_{32} (µm)	Polydispersity Span	pH of hydrated biopolymers	Zeta potential of hydrated bipolymers (mV)	pH of emulsion	Zeta potential of emulsion (mV)
GA	2.9^{a}±0.9	2.09^{d}±0.02	1.66^{a}±0.19	6.91±0.06	-26.1^{a}±1.5	4.11±0.03	-30.8^{f}±3.3
EMCAP	3.5^{a}±0.5	1.66^{d}±0.01	1.08^{d}±0.05	5.89±0.09	-10.9^{d}±1.7	4.73±0.03	-17.0^{bc}±1.4
MALT	1.8^{b}±0.1	18.0^{a}±0.3	1.50^{c}±0.01	6.38±0.12	-25.6^{a}±2.3	6.95±0.01	-26.1^{e}±0.9
GA:EMCAP	4.4^{a}±0.4	3.21^{d}±1.49	1.03^{d}±0.08	6.64±0.02	-22.9^{a}±1.3	4.65±0.0	-16.1^{cd}±0.5
GA:MALT	1.0^{c}±0.2	16.1^{b}±0.2	1.54^{bc}±0.0	6.62±0.06	-17.2^{c}±1.4	5.33±0.17	-12.9^{a}±0.6
EMCAP:MALT	0.8^{c}±0.1	17.7^{b}±0.4	1.50^{c}±0.01	5.95±0.51	-12.8^{d}±1.8	5.95±0.0	-20.5^{d}±0.8

Means followed by lower case in the same column do not differ significantly ($p < 0.05$) by the Tukey test.*GA: gum Arabic, EMCAP: modified corn starch-OSA(EMCAP®); MALT: Stearic acid modified malt; GA:EMCAP, GA:MALT and EMCAP:MALT

Figure 2 shows the droplet size distributions of all the emulsions. All formulations showed bimodal size distribution, except the sample prepared with gum Arabic alone. EMCAP and GA: EMCAP presented two modes of size, with one ranging from 0.5 to 5 µm and another smaller peak varying between 10 and 100 µm, which are characteristic values for foodstuff emulsions droplet size. In relation to the malt-based emulsions, a bimodal size distribution was also observed, with a peak ranging from 1 to 10 µm and another peak ranging from 10 to 100 µm, being the bigger one related to the malt starch granules. The microstructure (Fig. 2) corroborates the size distribution results, since populations with different sizes can be observed, highlighting the bimodal size distribution behavior. In addition, all the formulations composed by the modified malt presented large and small droplet sizes. Large particles refer to whole starch granules of malt meanwhile the smaller droplets represent oil droplets formed during emulsification.

Fig. 2 Optical morphology and droplets size distribution of the *Capsicum* oleoresin emulsions stabilized by different biopolymers. GA: Gum Arabic, EMCAP: modified corn starch-OSA(EMCAP®); MALT: Stearic acid modified malt; GA:EMCAP, GA:MALT and EMCAP:MALT



Emulsions with bimodal distributions may be interesting for applications such as flavoring agents in food products due to their different release times. Charles, Rosselin, Beck, & Guichard,

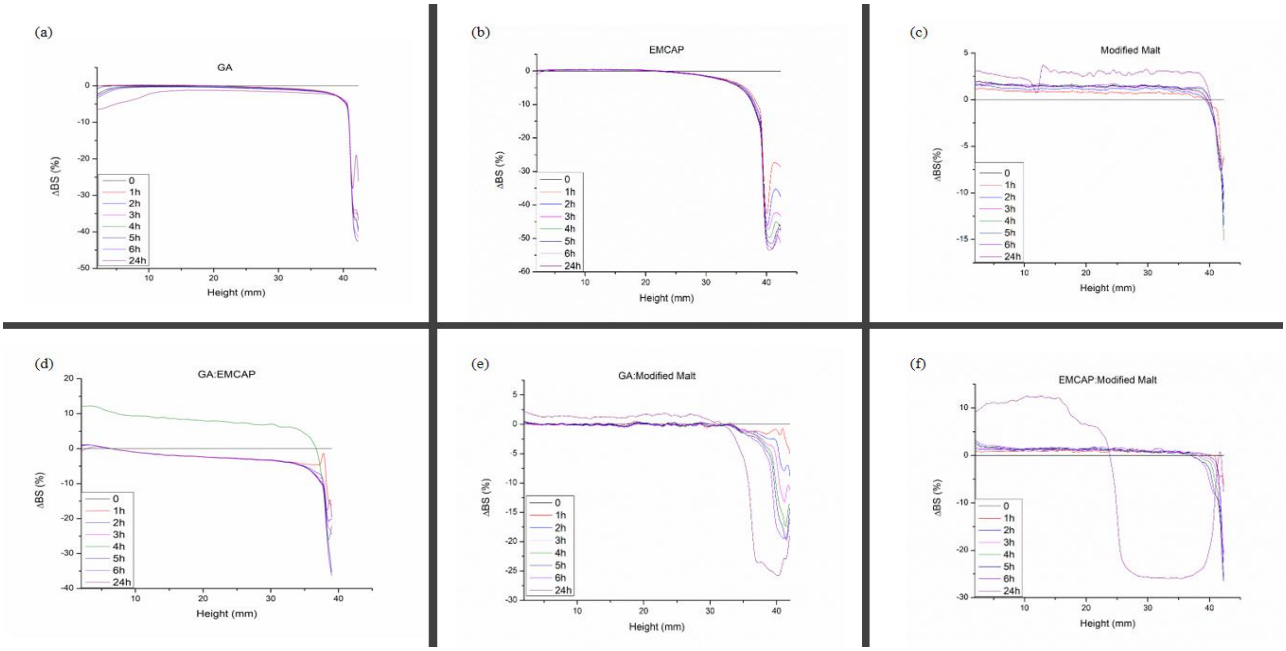
(2000) stated that the release of lipophilic compounds can be associated with the droplet size. Emulsions formed by larger droplets sizes can present an extended release of its encapsulated compounds during mastication, such phenomenon is known as a diffusion path- length.

Effect of different biopolymers on stability emulsions

The impact of three different biopolymers in their *Capsicum* oleoresin emulsions stability was investigated by Turbiscan Stability Index (TSI) (Table 2). TSI data at 2h for all emulsions ranged from 0.8 to 4.4, indicating a good kinetic stability (TSI< 5). Emulsions prepared with modified malt (GA: MALT and EMCAP: MALT) had the lowest TSI values. This result is probably associated to the reduced interfacial tension of these biopolymers, as can be seen in Fig. 1. The chemical structure of carbohydrates can explain this behavior. OSA-modified starch in combination with esterified malt, or with gum Arabic were able to increase the emulsion viscosity by structuring the aqueous phase and limiting the movement of the oil droplets. Due to its lipophilic property, gum Arabic and modified carbohydrates are used as encapsulating agents for oil, oleoresin, and flavors. They are able to interact with both water and oil phase, acting as oil–water interface stabilizers (Santiago et al., 2018; Wang et al., 2017). Previous work also noted a greater stability of canola oil emulsion by using lauric acid-modified rice starch because of hydrophobic groups present in the starch structure (García-Tejeda, Leal-Castañeda, Espinosa-Solis, & Barrera-Figueroa, 2018).

The kinetic stability of emulsions was determined by delta backscattering profile (ΔBS) (Fig. 3), before and after their preparation (0, 1, 2, 3, 4, 5, 6 and 24 h). The BS profile provided information about the instability mechanism on systems. The TSI values and delta backscattering allowed inference that emulsions prepared with modified malt alone (MALT) or mixed with other biopolymers (EMCAP: MALT and GA: MALT) are more prone to destabilization due to the increased droplet size of these emulsions and starch granule aggregation. Other explanation for that is associate with the migration of oil drops to the surface of dispersion due to their large size and low density or whole starch granules can sediment to their large density (Matos et al., 2018). The large starch granules, especially for emulsion composed by MALT, promoted a noise formation in the BS measurements.

Fig.3 Delta backscattering of all emulsions during 24h at 25 ºC.*(a) GA: Gum Arabic (b) EMCAP: modified corn starch-OSA(EMCAP®); (c) MALT: Stearic acid modified malt, (d) GA:EMCAP, (e) GA:MALT, (f) EMCAP:MALT



Two destabilization phenomena were observed in this study, such as sedimentation and coalescence, after 4 h of the emulsion preparation. Sedimentation was caused by a higher density of droplets resulting in a gravitational separation between phases, and then the coalescence phenomenon caused after two or more droplets merge and became a larger droplets forming a creaming layer (McClements, 2007).

Different from formulations containing MALT, the ΔBS profiles for GA and EMCAP emulsion showed a good stabilization, response of smaller droplet size of these emulsions. Nevertheless, all these formulations are feasible to be further spray dried since atomization process does not require stability for a long period.

Zeta potential values for aqueous phase and emulsions

Zeta potential (ζ-potential) values can be used to indicate the stability of emulsions by electrostatic forces. When the dispersion shows high values (positive or negative), they are not aggregating. On the other hand, low ζ-potential values indicate that attraction forces can surpass this repulsion, promoting gravitational phenomenon as coagulation or flocculation (McClements, 2007).

However, other factors should be considered to evaluate the stability of the system, such as the density difference between the phases (disperse and continuous) and the particle size (Tan, 2004).

The biopolymer suspensions presented different values of zeta potential according to their chemical characteristics, as can be seen in Table 2. GA presented a high negative zeta potential value of -26.1±1.5 mV at a pH of 6.91. Other dispersions containing MALT and GA: MALT presented high zeta potential values, indicating a high content of the electrolyte's presence in the system and the dissociated ions linked to the original colloid particles (Tan, 2004). In relation to suspensions containing EMCAP, a low negative zeta potential value was found, and when this polymer was blended with GA and MALT, a decrease of the ζ-potential value was obtained, compared to suspensions of GA or MALT alone. This result indicated that there were interactions between polymers EMCAP with MALT and GA with EMCAP, leading to a reduction of the zeta potential values.

The emulsion produced with GA presented the highest zeta potential (-30.8 mV), followed by the emulsions prepared with MALT (-26.1 mV) and MALT: EMCAP (-20.5 mV). This result corroborated data of zeta potential from stabilizers suspensions. Based on the ζ-potential data of biopolymers suspensions and emulsions, we can say that formulations with GA, MALT, EMCAP: MALT showed two stabilization mechanisms: electrostatic and steric; in this case, steric stabilization was dominant. Emulsions containing EMCAP, GA: EMCAP and GA: MALT with lower zeta potential values were characterized by steric stabilization. Thus, the ζ-potential is affected by the type of encapsulating material and combination between them. A previous study evaluated the effect of esterification of starch on zeta potential values and the authors concluded that the increase of starch substitution degree resulted in a more negative zeta potential value due to the high repulsion among starch molecules (Leal-Castañeda et al., 2018).

Emulsions rheological properties

Rheological behavior was evaluated to understand the effect of different biopolymers on *Capsicum* oleoresin emulsion composition. Table 3 shows the viscosity values and data fitted to a power-law model for all emulsions stabilized by different emulsifiers.

Table 3. Rheological parameters and viscosity values of the *Capsicum* oleoresin emulsions stabilized by different biopolymers.

Emulsions	Parameters by fitting power -law model			Apparent Viscosity in the shear rate at 300 s^{-1} (mPa.s)
	k(Pa.s.n^{-1})	n	R^2	
GA	0.0118	0.9889	0.9999	13.4^{d} ±1.9
EMCAP	0.0258	0.9771	0.9999	24.8^{b} ±7.3
MALT	0.0545	0.7857	0.9999	16^{c} ±0
GA:EMCAP	0.0139	0.9868	0.9999	13.8^{d} ±1.6
GA:MALT	0.0409	0.8330	0.9995	24.6^{b} ±7.5
EMCAP:MALT	0.0166	0.9866	0.9999	44.4^{a} ±4.9

Means followed by lowercase letters in the same column do not difer signifcantly ($p < 0.05$) by the Tukey.*k* (Pa.s. n^{-1}): consistency index, n: behavior index and R^2: determination coefficient by fitting power-law model. GA: gum Arabic; EMCAP: modified corn starch-OSA(EMCAP®); MALT: Stearic acid modified malt; GA:EMCAP, GA:MALT and EMCAP:MALT.

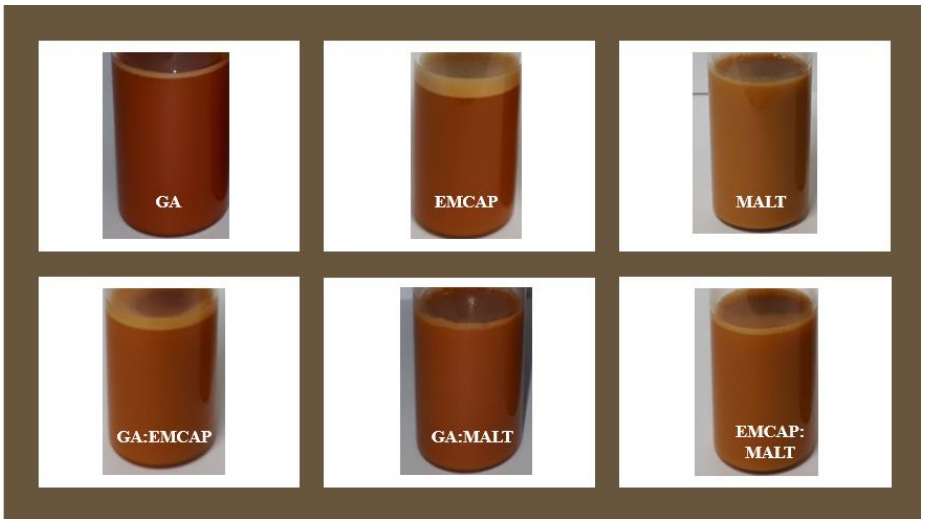
All emulsions showed pseudoplastic behavior (R^2~0.9999) (Table 3). Emulsions produced with MALT and GA: MALT dispersions presented higher values of consistency index and n<1 feature behavior of pseudoplastic fluids. Also, emulsion composition influenced viscosity data at a shear rate of 300 s^{-1}. The apparent viscosity varied from 13.4 ±1.9 to 44.4 ±4.9 mPa.s. When MALT was combined with gum Arabic or EMCAP, a viscosity value two-fold higher was observed compared to formulations containing the isolated materials. The higher viscosity was due to larger droplets in the formulations and because of the emulsifier properties of biopolymers. Valoppi et al. (2021) associated the increase of viscosity with the presence of insoluble materials since they can increase volume fraction or cause agglomeration by swelling. In addition, the carbohydrates present in the aqueous phase can interact with each other at the oil-water interface and alter the rheological properties, characterized by hydrophobic interactions (McClements & Jafari, 2018). This trend was also observed for non-Newtonian chili sauces containing modified starch/xanthan gum (Gamonpilas et al., 2011).

Influence of different carrier agents on color parameters

Color is a significant attribute of foodstuff, and it is related to the quality and physicochemical properties of the product, which influences the choices and preferences of consumers. *Capsicum* oleoresin is an ingredient rich in yellow, orange, and red pigments due to the presence of carotenoid compounds such as capsanthin, capsorubin, cryptoxanthin and zeaxanthin (Berke & Shieh, 2012).

The effect of carbohydrate on color parameters (L a*, b*, C and h) for each emulsion formulation can be seen by visual aspects in **Figure 4.** According to **Table 4** the luminosity values varied from 36.8±0.4 to 46.0±0.2 for all treatments. MALT, EMCAP and MALT: EMCAP treatments presented high values of luminosity due to the opacity of starch-based materials in the dispersion, whereas formulations containing gum Arabic had low L values.

Fig. 4 L* values and visual aspect of the *Capsicum* oleoresin emulsions produced with diferent biopolymers. GA, gum Arabic; EMCAP, modifed corn starch-OSA(EMCAP®); MALT, stearic acid-modifed malt; GA: EMCAP, GA:MALT, and EMCAP: MALT



Chromaticity (C) and hue angle (h°) varied according to a* and b* parameters, as described in **Table 4.** The systems containing two biopolymers (GA: EMCAP, GA: MALT and MALT: EMCAP) revealed an increase of C value, varying from 53.6±0.3 to 61.2±0, which is representative of the different saturation of orange color. Emulsions prepared with Arabic gum showed the lowest Chroma value that resulted in a system with color more saturated.

Table 4 Encapsulation efficiency of capsaicin and color parameters of the *Capsicum* oleoresin emulsions stabilized by different biopolymers.

Treatments	Capsaicin Retention (CR%)	Colour parameters				
		L*	a*	b*	C	h
GA	91.7^{a}±8	36.8^{d}±0.4	23.3^{f}±0.6	21.1^{g}±0.0	31.4^{f}±0.	42.1^{f}±0.0
EMCAP	71.1^{c}±2	43.3^{b}±0.0	27.5^{d}±0.0	30.4^{f}±0.8	41.0^{e}±0.	47.9^{e}±0.
MALT	94.4^{a}±0	46.0^{a}±0.2	24.1^{e}±0.4	42.4^{e}±0.5	48.8^{d}±0.	60.4^{a}±0.
GA:EMCAP	76.1^{c}±2	38.9^{c}±1.1	32.5^{a}±0.2	51.9^{a}±0.0	61.2^{a}±0.0	57.9^{c}±0.
GA:MALT	80.3^{b}±4.5	37.5^{d}±0.2	29.4^{b}±0	44.8^{d}±0.4	53.6^{c}±0.	56.7^{d}±0.
EMCAP:MALT	96.7^{a}±2.4	42.8^{b}±0.0	28.4^{c}±0.1	49.2^{b}±0.1	56.8^{b}±0.	59.9^{b}±0.

Means followed by lower case in the same column do not differ significantly ($p < 0.05$) by the Tukey test. *GA: gum Arabic, EMCAP: modified corn starch-OSA(EMCAP®); MALT: Stearic acid modified malt; GA:EMCAP, GA:MALT and EMCAP:MALT

For systems containing MALT, the parameters showed low a*, high b* and high hue angle. As a result, these emulsions had an orange-brown color, especially due to modified malt presence, material that has a light brown color in aqueous phase. In contrast to this, a reduction of h° value from 56.7°±0.2 to 59.9°±0.1 was observed for MALT:GA and MALT: EMCAP, in which the orange color was more intense, as shown in Figure 4.

A similar result was found by Surassmo, Min, Bejrapha, & Choi (2010) for *Capsicum* oleoresin encapsulation by poly-ε-caprolactone (PCL) using the emulsification-diffusion method. These different colors of capsaicin-loaded emulsions have the potential for their use in foodstuff as a colorant agent. For instance, some tones for specific target products, such as soups, meat products, spices, salad dressings and beverages were obtained through *Capsicum* oleoresin emulsification using different carbohydrates as emulsifier agents.

Capsaicin retention in *Capsicum* oleoresin emulsion systems

The high content of encapsulated compound depends on the nature of the encapsulating matrix, the method used for encapsulation, the total solid concentration of the system, the emulsion viscosity, the stabilizers interfacial tension, and the emulsion (O/W or W/O) stability (McClements & Jafari, 2018).

Table 4 provides the capsaicin content within the *Capsicum* oleoresin emulsion. *Capsaicin* retention (CR) varied from 71.1 to 96.7% and was significantly affected by the type of emulsifier. The emulsion prepared using MALT exhibited high CR (over 80%) and the same tendency was also observed for the formulation composed by GA. The possible reason for this result is associated with the nature of capsaicin. This chemical compound is an alkaloid of hydrophobic character; therefore, modified malt with substitution degree presented a higher affinity for the capsaicin compound. Systems composed by EMCAP and GA: EMCAP presented low values for capsaicin retention, 71.1±2 and 76.1±2%, respectively, corroborating the higher values obtained for the TSI, compared to the other emulsions (Table 2). Despite the emulsions presenting TSI values lower than 5% and their material having good interfacial properties, these systems possibly excluded out the bioactive compound due to the destabilization phenomenon.

The increased encapsulating efficiency of capsaicin was also observed by Akbas, Soyler, & Oztop, (2018). The authors obtained an 83% efficiency of capsaicin-loaded nanoemulsions using Tween 80 as an emulsifier. Interesting research conducted by De Aguiar et al. (2016) showed that capsaicin encapsulation efficiency may be affected by emulsifier agent and encapsulation method. They showed a retention of 30.73% of capsaicinoids for a formulation containing only Hi-Cap in a process of emulsification followed by supercritical fluid extraction of emulsions (SFEE). Hence, the use of modified MALT in combination with GA and EMCAP as emulsifiers might be a better alternative for capsaicin encapsulation since the retentions obtained in our research showed values higher than 80%. In this sense, the difference between these results could be associated with the encapsulation method and the emulsifier agent used.

Conclusions

The *Capsicum* oleoresin-in-water emulsion features had a strong influence by aqueous phase composition. Gum Arabic, OSA starch and modified malt into aqueous phase presented low interfacial tension, however a pronounced effect was observed when these emulsifiers were combined (GA: EMCAP, EMCAP: MALT, GA: MALT). MALT in combination with other materials had an influence on various properties, increasing the particles size and viscosity, improving capsaicin retention, and changing color parameters. Interestingly, all emulsions presented bimodal droplet size distribution, which is characteristic of flavor ingredients, except for the formulation composed of gum Arabic only. Formulation containing MALT or MALT combined with GA and EMCAP showed high capsaicin

retention and can be considered a potential source of this compound for application as colorant and flavor agent in food products. In addition, these emulsion systems can be successfully subjected to a further spray drying process for obtaining powder microparticles.

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Data Availability The authors confrm that the data supporting the fndings of this study are available within the article.

Declarations

Conflict of Interest The authors declare no competing interests.

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CAPÍTULO 5

CHARACTERIZATION OF CAPSICUM OLEORESIN MICROPARTICLES AND IN VIVO EVALUATION OF SHORT-TERM CAPSAICIN INTAKE

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CHARACTERIZATION OF *CAPSICUM* OLEORESIN MICROPARTICLES AND *IN VIVO* EVALUATION OF SHORT-TERM CAPSAICIN INTAKE

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Abstract

Gum arabic, modified corn starch (EMCAP), modified malt (MALT), either blended or isolated, were assessed as encapsulating agents for *Capsicum* oleoresin. *Capsicum* oleoresin microparticles were obtained by spray drying and analysed for physicochemical properties and *in vivo*. Obtained powders were adequate for storage, given their low water activity (<0.150), hygroscopicity (<11.43 g/100 g), moisture (<4.76%) and high glass transition temperature (<98.3 °C). FT-IR analysis concluded that carbohydrates matrices were loaded after spray drying, with peaks around 2850 cm^{-1} for aromatic compounds, and bands around 1760 cm^{-1}, pointing to the presence of capsaicin inside the microparticles. All formulations exhibited high antioxidant activity, low contact angles and great solubility in water. Any adverse effect was observed in the experimental assay, neither change on the level of hepatic aminotransferases. The intake of a High-Fat Diet (HFD) supplemented with *Capsicum* oleoresin microparticles decreased weight gain when compared to the HFD control.

Keywords: Capsaicin; Powder; Spray drying; FT-IR analysis; ORAC; FRAP; *In vivo* study.

Introduction

Obesity is a major concern now spanning every age group. It is an underlying factor for diabetes, cardiovascular diseases and various metabolic disorders arising due to a sedentary lifestyle and lack of proper nutrition and diet awareness.

Many compounds impact weight loss through the thermogenic effect. One such compound, capsaicin, has shown promising results in the treatment of obesity related chronic diseases (Adaszek et al., 2019): the intake of *Capsicum* oleoresin was related to reduction of adipose tissue and inflammation, by Lee et al. (2017), and by Rogers et al. (2018). Authors observed changes on multiple gene expression, activation of AMP-activated protein kinase and inhibition of glycerol-3-phosphate dehydrogenase in white adipose tissue.

Capsicum oleoresin is the primary source of capsaicin, while also rich in phenolic compounds with high antioxidant capacity. Capsaicin is a pungent substance widely used in cooking as a flavouring agent, with high viscosity and low solubility in water, while also vulnerable to temperature, light and oxygen availability, which all obstruct its direct applications into food products (Berke & Shieh, 2010).

Encapsulation techniques are used to overcome those drawbacks of lipophilic food ingredients, such as spray drying, widely used due to its low-cost and ease of implementation in mass production. Powdered microparticles containing capsaicin have already been produced from red chili oleoresin (Cruz-Olivares et al., 2008) and chili seed oil (Wang et al., 2017), with the spray dried hydrophobic compounds showing enhanced stability, solubility, and enhanced shelf-life.

Many natural and modified carbohydrates are employed as carrier agents for encapsulation. In spray drying, gum arabic is an good material for its film-forming, high emulsifying and thermal stability features (Santiago et al., 2018). Starch sources are also commonly used in emulsification as well for their low cost and practicality. starch sodium octenyl succinate (OSA) is composed of hydrophobic alkenyl and hydrophilic carboxyl groups, making it a strong emulsifier agent (Wang et al., 2017). New experiments with blends of biopolymers are of great interest, due to their possible improvements on the antioxidant activity, encapsulation efficiency and film formation (Boonlao et al., 2020; Comunian et al., 2020).

The methodology designed for this study aimed at improving the *Capsicum* oleoresin protection and evaluating its functional properties using a combination of carbohydrates as wall materials. *Capsicum* oleoresin microparticles were obtained by spray drying using gum arabic

(GA), octenyl succinic anhydride (OSA) corn starch (EMCAP), modified malt (MALT), either individually and blended (GA: EMCAP, GA: MALT, EMCAP:MALT). Further, microparticles were evaluated in terms of their physicochemical properties, morphology, thermal characteristics, capsaicin retention, encapsulation efficiency and antioxidant activities.

Although there is no evidence about the protective effect of *Capsicum* oleoresin in obesity, there is neither any certainty that microencapsulation by spray drying could modify its bioavailability and thus, impacts its biological response. Therefore, a preliminary study was carried out: C57BL/6J mice, fed for 28 days on a high-fat (HF) diet supplemented with different concentrations of microparticles (10 or 20 mg/ kg of BW) were employed, in order to assess the acceptability of the diet and its toxicological response.

Material and Methods

Material

Gum arabic and octenyl succinic anhydride (OSA)-modified starch (EMCAP^{TM}) were kindly donated by Nexira (São Paulo, Brazil), and Cargill (Campinas, Brazil), respectively. Modified malt (MALT) was obtained by ultrasound, (45 W/5 min) using stearic acid (2% w/w), in a previous work by Anthero et al. (2021). *Capsicum* oleoresin was purchased from Synthite Industries Ltd. (Kerala, India), the capsaicin standard was acquired from Cayman Chemical (Ann Arbor, USA, Purity > 95%) and corn oil (Liza, Cargill, Campinas, Brazil) was purchased from a local supermarket. Other standards as 2,4,6-Tris(2-Pyridyl)-S-Triazine (TPTZ) and Trolox (x (Hidroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were acquired from Sigma-Aldrich (St. Louis, USA). All chemicals were of analytical grade.

Microparticles powder: Production and characterization

The production of microparticles

The dispersion of the wall materials (gum arabic, OSA modified corn starch, and modified malt) and blends, containing 15% solids, was dissolved in distilled water under magnetic stirring overnight. Six formulations (5% oleoresin, 95% wall materials) were made, according to the description in the **Table 1**. Dispersions composed of aqueous (95%) and oil phase (5%) were produced using a Silverson Laboratory Mixer L5M-A (Buckinghamshire, UK) at 5,000 rpm or

7650x g (Allegra 25-R, Beckman Coulter Inc., São Bernardo do Campo, Brazil), for 10 min. Emulsions (500 g) were then submitted to the Mini Spray Dryer B-290 (Büchi, Flawil, Switzerland) using a two-fluid atomizer nozzle of 0.7 mm. The drying air flow rate was of 35 m³/h, with an inlet temperature of 160 ºC. Air pressure was kept at 0.06 Mpa, with an emulsion feed flow of 15 mL/min, while the outlet temperature varied from 75 ºC to 78 ºC. Spray-dried powders were stored in sealed flasks at -10 ºC.

Table 1 Microparticles formulation

Treatments	Wall material	Composition
GA	GA (Arabic gum)	100 g of emulsion containing: 15% total solid, in which 95% is wall material and 5% is *Capsicum* oleoresin
EMCAP	EMCAP (OSA- modified corn starch)	
MALT	Modified Malt	
GA:EMCAP	GA:EMCAP (1:1)	
GA:MALT	GA:MALT (1:1)	
EMCAP:MALT	EMCAP:MALT (1:1)	

Microparticles characterization

Moisture and water activity. The moisture of the particles was determined by the moisture determination balance (Shimadzu brand, model Moc63u, Kyoto, Japan). While drying at 105 °C, powder samples (2 g) were scaled, until their weight difference was of less than 1%. Water activity was measured using an Aqualab model 3TE digital hygrometer (Decagon, Pullman, USA) at 25 ºC, based on the chilled mirror dew point technique.

Hygroscopicity. Powders hygroscopicity was determined according to the methodology described by Cai & Corke (2000) with some modifications. Approximately 1 g of powder was placed in a desiccator containing saturated NaCl solution, at 25 ºC, until constant weight. After 7 days, the hygroscopicity was calculated and expressed as grams of moisture adsorbed on 100 g of dry solids.

Solubility measurement. Approximately 0.2 g of microparticles (in dry basis) were added to 20 mL of distilled water and kept under stirring for 5 minutes. The solution was centrifuged at 10,000 rpm or 15,300x g (Allegra 25-R, Beckman Coulter Inc., São Bernardo do Campo, Brazil), for 10 minutes. An aliquot of 5 mL of the supernatant was transferred to a pre-weighed petri dish and dried at 105 °C for 24 hours. Thus, solubility was calculated based on the weight difference.

Contact angle. The contact angle was determined to characterize the wettability of the powder. The effect of different biopolymers on the hydrophilicity of *Capsicum's* oleoresin microparticles was measured using the Track-S tensiometer (Teclis, Longessaigne, France). Tablets of 14 mm thickness were produced with 150 mg of the sample using 80 kN of hydraulic pressure force (Shimadzu Corporation, Kyoto, Japan). A drop of water with a volume of 10 µL was applied to the surface of the microparticle and the consequent reduction of the angle was measured for 30 seconds.

Particles mean diameter and size distribution and their microstructure. Particle size was measured by light scattering technique in the Mastersizer 2000 instrument (Malvern Instruments Ltda, Malvern, UK), using the SIROCCO optical unit with dry air dispersion. Readings were repeated six times and mean values of the volumetric diameter and the Span index were determined. The microstructure of the particles was analysed using the Scanning Electron Microscope (SEM) by LEO Electron Microscopy (Model Leo 440i, Cambridge, UK). The samples were covered with gold, and the analysis was performed in 2.00 KX magnification with a voltage acceleration of 15.00 kV.

Glass transition temperature. Glass transition temperature (Tg) was measured in triplicate by differential scanning calorimetry (DSC) analysis (model Q100, TA Instruments, New Castle, USA). Four milligrams of powder were placed into an aluminum pan and sealed. An empty pan was used as reference. The procedure consisted of cooling down to -70 ºC (10 ºC/min), keeping an isothermal for 1 min and, at last, heating up to 120 ºC (10 ºC/min), twice. Hence, the last running temperature was balanced to 25 ºC. Tg was obtained from the second curve using software Universal Analysis 3.9 (TA Instruments, USA) (Consoli et al., 2019).

FT-IR analysis. Chemical structures of wall materials, free *Capsicum* oleoresin, and microparticles were analysed by Infrared spectroscopy. The data were collected in the interval between 4,000 and 400 cm^{-1} (IRPrestige-21, Shimadzu, Kyoto, Japan) and the results were obtained by IRSolution software, version 1.60. Tablets were prepared in triplicate by manual hydraulic pressing, containing 100 mg of potassium bromide (KBr) and 1 mg of the oven-dried sample (105 °C/48 h).

Oleoresin retention. An amount of 15 mL of hexane was added to 1.5 g of powder and mixed in a vortex for 2 min. The blend was filtered through nº.1 filter paper and the collected powder was rinsed with 15 mL of hexane twice. Thus, the samples were subjected to hexane evaporation in a circulating air oven at 60 °C, until constant weight. The encapsulation efficiency of oleoresin (EE%) was calculated by the weight difference, according to Equation 1:

$$EE\ (\%) = \frac{(Total\ oil\ content - surface\ oil\ content)}{total\ oil\ content} x\ 100 \qquad (1)$$

Capsaicin content. A sample of 0.25 g of the obtained microparticles was mixed to 2.5 mL of Milli-Q water in the Falcon tube. Thus, it was homogenized for 1 min using a vortex stirrer AP56 (Phoenix Luferco, Araraquara-SP, Brazil). Thereafter, 7.5 mL of methanol were added to the sample, with immediate stirring for another 1 min. These samples were kept in an ultrasound bath for 15 min and then centrifuged for 15 min at 10,000 rpm or 15,300x g (Allegra 25-R, Beckman Coulter Inc., São Bernardo do Campo, Brazil), at 20 ºC. The supernatant was filtered through 0.45 µm polytetrafluoroethylene (PTFE) syringe filters to posterior High Performance Liquid Chromatography (HPLC) analysis.

The content of capsaicin was obtained using a HPLC of Agilent 1100 series (Agilent Technologies, Santa Clara, USA) equipped with a diode array detector (DAD G1315B). An aliquot (10 µL) of each sample was injected in a Poroshell 120 EC-C18 column 4.6x100 mm, 2.7 µm (Agilent, Santa Clara, USA). Capsaicin was determined by monitoring in 280 nm, at 25 ºC. The mobile phase was composed of a blend of methanol: water (85: 15, v/v), while the flow rate was of 1 $mL.min^{-1}$, as described by De Aguiar et al. (2016). A calibration curve (R^2 0.998) was determined using diluted capsaicin, in concentrations varying from 7.14 to 142.8 µg/L. Quantification was obtained by Chromeleon software 6.8 and the capsaicin concentration was expressed as mg capsaicin/g powder. Each extraction was performed in triplicate.

Antioxidant activity of microparticles by FRAP and ORAC methods.

a) FRAP

The FRAP reagent was produced by a mixture of acetate buffer (300 mmol L^{-1}) (pH 3.6), TPTZ (10 mmol), HCl solution (40 mmol L^{-1}) and $FeCl_3$ (20 mmol L^{-1}). After, FRAP was mixed to 0.01 mL of aqueous microparticle extract (MP) (0.5 of MP: 2.5 mL of deionized water: 7.5 mL of methanol). After 30 min at 37 ºC, the absorbance of the sample at 595 nm was determined in a microplate reader (NOVOstar, BMG Labtech®, Germany) (Benzie & Strain, 1996). The results were expressed in μmol of Trolox equivalent (TE)/g of microparticle using a calibration curve varying from 10 to 400 μmol Trolox/L (y= 0.000651x +0.000223) with an R^2= 0.999.

b) ORAC

Antioxidant activity was determined by the hydrophilic-ORAC method. The preparation of the samples and the determination of the antioxidant activity by ORAC (Oxygen Radical Absorbance Capacity Assay) were conducted by the methodology described by Ou et al. (2013). Approximately, 0.5 grams of microparticles were homogenized with 2.5 mL of deionized water and then, 7.5 mL of methanol were added for complete extraction of bioactive compounds. An aliquot (0.2 mL) of this solution was homogenized with fluorescein (5.7 μmol/L), as a target for free radical attack, and with 2,2'-azobis-amidino-propane dihydrochloride (24 mM) as a peroxyl radical generator, at 37 ° C. The Trolox (5 μM) was used as a standard control and readings were taken on a microplate reader (NOVOstar, BMG Labtech®, Germany) with the following fluorescent filters: 485 nm for the excitation wavelength and 520 nm for emission wave. ORAC values were obtained in μmol of Trolox equivalent/g using standard curves varying from 5 to 75 μmol Trolox equivalent/g, (y= 0.35x + 1.15) and an R^2 0.99.

Effect of microparticles on weight gain and acute toxicity in the diet of mice

For this assay, formulations containing modified malt and gum arabic as a carrier agent were chosen as a dietetic supplement for the pilot test. The formulation blend was selected at random. Purpose of this assay was to assess if the animals would accept a diet supplemented with the obtained microparticles and, thus, to evaluate the acute toxicity of the encapsulated compound regardless of the wall material. Only two formulations were chosen to experiment to reduce the number of animals required to experimental design. Thus, microparticles loaded with capsaicin

were made into two different solutions, while additional corn oil was mixed to the least concentrated of them, in order to keep the same solid content in the emulsions and to check for any effect of this type of oil on the acute toxicity and weight gain in mice.

Formulation preparation

The wall materials (modified malt and gum arabic) were previously dissolved in distilled water under magnetic agitation for 12 h. Two emulsions were prepared: one using only *Capsicum* oleoresin as oil phase (OC) and another one containing OC plus corn oil (1:1, w/w), named Formulation 1 (F1) and Formulation 2 (F2), respectively. Dispersions were produced according to section *2.2.1* and, thus, the powdered particles were ready for use.

Experimental design

The study was approved by the Institutional Animal Care and Use Committee (protocol #5154-1/2019, CEUA, UNICAMP, Brazil), thus following the institutional ethical and the Brazilian National Council for the Control of Animal Experimentation (CONCEA) guidelines. Old mice C57BL/6J (28 days) were kept on a 12-h light/dark cycle, at a temperature of 25 ± 2 °C, with free access to food and water. The mice were divided into five groups (n= 6), as follows: Control group or Diet 1: animals received high-fat diet only without any microparticles; Diet 2: a high-fat diet containing 10% microparticles; Diet 3: a high-fat diet containing 20% microparticles; Diet 4: a high-fat diet containing 10% microparticles with corn oil; and Diet 5: a high-fat diet containing 20% microparticles with corn oil. The diets were formulated **(Table 2)** following the American Institute of Nutrition, to preserving the animal's modified nutritional status (AIN-93M) to high-fat profiles (Reeves, 1997).

Table 2 Composition diet for feeding mice groups during 28 days of experimental assay.

Groups	Composition of the diet	*Capsaicin in the diet (%)
Diet 1	High fat	-
Diet 2	High fat + high-fat diet containing 10% of *Capsicum* oleoresin microparticles composed by MALT:GA	0.0022%
Diet 3	High fat + high-fat diet containing 20% of *Capsicum* oleoresin microparticles composed by MALT:GA	0.0044%
Diet 4	High fat high-fat diet containing 10% of *Capsicum* oleoresin microparticles composed by MALT:GA with corn oil	0.0014%
Diet 5	High fat high-fat diet containing 20% of *Capsicum* oleoresin microparticles composed by MALT:GA with corn oil	0.0028%

*Percentage of capsaicin present into diet in relation to 1 kg of high fat diet.

During the experimental period (28 days), food intake was monitored three times a week and body weight gain was measured once a week. At the end, after 6 h of fasting, the animals were euthanized; heart, kidney, spleen, mesenteric adipose tissue, and liver were removed and weighed; also, samples from the liver were placed into formalin solution for further histological analysis. Blood fasting glucose was measured using the G.Tech Lite apparatus (Infopia Co., Ltd, Gyeonggi-do, South Korea). The blood samples were collected in appropriate tubes, from which serum was obtained by centrifugation (10,000 rpm or 15,300x g for 15 minutes) using the Allegra 25-R (Beckman Coulter Inc., São Bernardo do Campo, Brazil), separated and thus immediately frozen at -80 °C.

Acute toxicity. The enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were identified in the serum of the animals by a Labtest commercial kit (ELISA, USA), following the manufacturer instructions.

Hepatic lipid oxidation. The liver sample was used to assessing Thiobarbituric acid reactive substances (TBARS), according to Ohkawa, Ohishi and Yagi (1979). A standard curve (1.25 – 50 nmol malondialdehyde (MDA)) was obtained using 1,1,3,3 - tetraethoxypropane. The measurement was determined at 532 nm and the results expressed as nmol MDA mg^{-1} tissue.

Histology essays. Liver samples were kept for 24 h at room temperature (24 ºC) in 10% buffered formalin, preserved in 70% ethanol and embedded in paraffin. Slices were cut into sections of 5 μm thickness and stained with hematoxylin and eosin for visualization of the hepatocyte morphology, as previously described (Flister et al. 2018). NAFLD activity score (NAS) (Kleiner et al. 2005) was used for the semiquantitative analysis of the three defined criteria of NASH: steatosis (0–3), ballooning (0–3) and lobular inflammation (0–2), using a Leica DMI 4000B microscope (Heerbrugg, Switzerland).

Statistical analysis

All physicochemical analyses were performed at least in triplicated, and the results were subjected to analysis of variance (ANOVA) using the Minitab 18®. In addition, the significant differences (p-value < 0.05) between the treatments were evaluated using Tukey's test.

Results and discussion

Physicochemical properties of microparticles: moisture content, water activity, hygroscopicity

Table 3 shows moisture and water activity (a_w) values for all particles obtained by spray drying. Capsaicin microparticles presented low values for moisture content and a_w, ranging from 2.81 ± 0.62 to 5.36 ± 0.21 and from 0.105 ± 0.006 to 0.150 ± 0.004, respectively. Low values for water activity can affect the microorganisms' growth and contribute to the low rate of degradation of the encapsulated compound. Similar results for a_w and moisture were also observed for spray-dried microparticles (Consoli et al., 2019; Comunian et al., 2020).

Table 3 Physicochemical analysis, size diameter, oleoresin encapsulation efficiency, capsaicin retention and antioxidant activity for microparticles

Measurements	**Treatments**					
	GA	EMCAP	MALT	GA:EMCAP	GA:MALT	EMCAP:MALT
Moisture (g water/100 g powder)	3.83^{bcd}±0.73	4.76^{ab}±0.49	4.48^{abc}±1.59	5.36^{a}±0.21	2.81^{d}±0.62	3.19^{cd}±0.63
a_w	0.139^{a}±0.032	0.145^{a}±0.024	0.113^{a}±0.016	0.150^{a}±0.004	0.105^{a}±0.006	0.128^{a}±0.034
Solubility (%)	93.92^{a}±1.85	94.50^{a}±4.42	74.55^{c}±2.30	83.16^{b}±2.58	82.44^{bc}±7.72	78.51^{bc}±3.54
Hygroscopicity (g of adsorbed moisture/100 g of sample)	10.00^{a} ±1.00	10.81^{a}±0.92	6.95^{b}±1.57	11.43^{a}±0.94	11.12^{a}±0.64	7.49^{b}±0.30
Contact angle Θ (°)	46.6^{b} ± 0.9	65.6^{a} ± 1.6	64.6^{a} ± 1.9	69.7^{a} ± 5.9	49.3^{b} ± 1.4	68.5^{a} ± 1.3
$D_{[4,3]}$ (µm)	22.5^{d} ± 1.2	21.7^{d} ± 0.7	186.7^{a} ± 0.3	14.7^{e} ± 0.5	37.9^{c} ± 2.5	44.0^{b} ± 1.0
SPAN (dimensionless)	0.6^{d} ± 0.2	1.7^{c} ± 0.1	6.3^{a} ± 0.3	2.3^{c} ± 0.3	4.3^{b} ± 0.2	6.9^{a} ± 0.3
*OEE (%)	84.4^{b} ± 2.7	91.6^{a} ± 3.1	68.5^{c} ± 2.3	89.1^{ab} ± 0.9	89.7^{ab} ± 2.2	90.7^{a} ± 1.1
*CR (mg capsaicin/ g powder microparticle)	3.49^{a} ± 0.2	3.33^{a} ± 0.2	2.97^{b} ± 0.3	2.35^{b} ± 0.7	3.48^{a} ± 0.1	3.46^{a} ± 0.2
ORAC (µmol TE/g microparticle)	89.4^{a} ± 9.7	69.7^{b} ± 7.5	61.7^{bc} ± 10.7	64.8^{b} ± 2.5	45.7^{c} ± 0.6	28.6^{d} ± 2.5
FRAP (µmol TE/g microparticle)	28.5^{c} ± 0.7	21.3^{d} ± 0.9	27.9^{c} ± 0.8	31.1^{c} ± 0.6	88.9^{a} ± 3.8	72.3^{b} ± 2.6

Means that do not share a letter are significantly different. **a_w:** Water activity; **OEE:** Oleoresin Encapsulation Efficiency; **CR:** Capsaicin retention. GA: Arabic gum, EMCAP: modified corn starch-OSA(EMCAP®); MALT: Stearic acid-modified malt; GA:EMCAP, GA:MALT and EMCAP:MALT.

Hygroscopicity values showed minimal differences among formulations (**Table 3**). Microparticles containing MALT as a carrier agent presented the lowest values for hygroscopicity (6.95 g water/100 g powder), while commercial materials, either blended or isolated (GA, EMCAP, GA: EMCAP, GA: MALT), presented the highest values.

Gum and modified starches present high hygroscopicity due to the presence of carboxylic groups, which increase water adsorption. Food powders obtained in this research showed a low

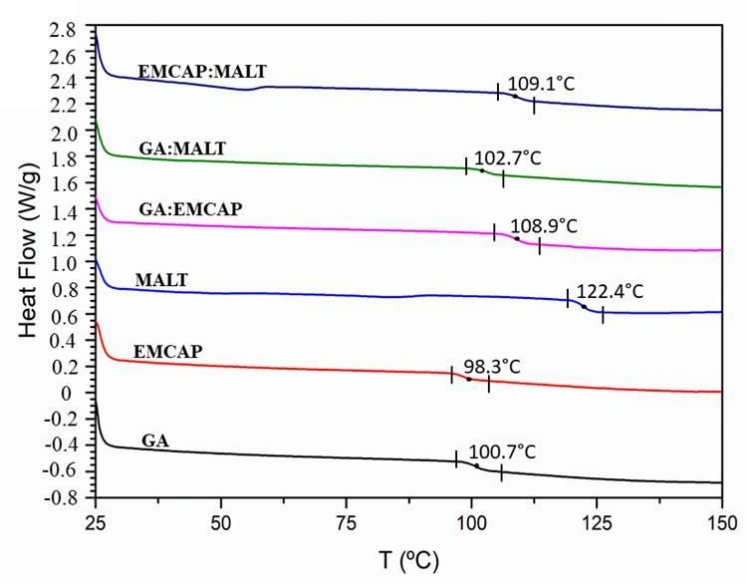
tendency to become liquid or hardened, making them suitable for storage and handling in food processing.

Glass transition temperature

Glass transition temperature (T_g) is an essential parameter for powdered products, especially in food containing carbohydrates. Particulate systems are glassy structure (solid) or a rubbery (liquid-like) state. The change from very thick glass to a rubbery state occurs at a given T_g, which is specific for each material. When particles are stored above the T_g, there occurs high mobility of molecules and thus the powder will change into a "gummy state". As a result, there is agglomeration, crystallization, loss of volatiles, structural transformations and increased rates for chemical and enzymatic changes on the food product (Bhandari & Howes, 1999).

In this research, *Capsicum* oleoresin microparticles presented different glass transition profiles, as shown in **Fig. 1.** Formulations containing only one wall material presented glass temperature values at 122.4 °C *(MALT), 100.7* °C (GA), and 98.3 °C (EMCAP).

Fig. 1. Microparticles glass transition temperature *GA: Arabic gum, EMCAP: modifed corn starch-OSA(EMCAP®); MALT: Stearic acid modifed malt; GA:EMCAP,GA:MALT and EMCAP:MALT



Powders formed by MALT show the highest T_g upon comparison to every other formulation. The difference in the composition of MALT concerning other pure wall materials explains this behaviour, for MALT contains fibers, sugars, granules of starch, and protein. Regarding EMCAP and GA microparticles, high values for water activity and low values for T_g were observed. The obtained results agree with the values obtained by Chen et al., (2019), which observed a glass transition temperature at 97.19 °C for beta-carotene microparticles encapsulated by OSA starch (corn starch esterified with octenyl succinic acid).

Blends of these wall materials (EMCAP: MALT, GA: MALT, and GA: EMCAP) resulted in a slight increase in T_g in comparison to formulations containing only isolated forms due to an interaction of biopolymers. Several other authors employed a distinct combination of wall materials on spray-dried particle formation and observed a similar effect on the glass transition temperature (Porras-Saavedra et al., 2018). According to Bhandari & Howes (1999), the addition of high molecular weight substances to solid mixture foods promotes an increase in T_g.

Contact angle

The contact angle is an indirect measure related to wettability, i.e., the water adsorption capacity of the particle surface. High values for contact angle imply low wettability, while low contact angle (<90°) imply a wetting behaviour (Wenzel et al., 1949). This is an important physical property for powdered foods because it is linked to the capacity of their re-constitution in water (Arshad et al., 2018).

Powders obtained by different carbohydrates resulted in contact angle values ranging from 46.6° ± 0.9 to 69.7° ± 5.9, as seen in **Table 3.** Treatments containing gum arabic only and gum arabic blended with modified malt showed statistically (p <0.05) lower contact angles compared to every other formulation. This result may be associated with the chemical structure of the gum arabic, for it is a hydrocolloid with highly branched polysaccharide chains, mainly acid arabinogalactans, which contribute to its anionic nature and greater affinity to water (Bemiller, 2019). Consoli et al. (2019) reported similar results (~46°) for resveratrol microparticles composed of Maillard-glycated conjugates (produced by sodium caseinate, maltodextrin and glucose syrup). Otherwise, for microparticles formulated by corn starch esterified with OSA (EMCAP) and malt esterified with stearic acid (MALT), or for their blends (EMCAP:MALT), the contact angles (°) were higher than those for formulations containing gum arabic. The results confirmed the impact on the hydrophobicity of hydrophobic groups of modified starches.

Solubility

The solubility is associated with the ability of the powder to disperse in water. An oil encapsulated by emulsion, followed by spray drying, displays high solubility in water, resulting in quick release of the encapsulated compound into the medium, which simplifies its application in water-based food products (Fernandes et al., 2016).

Post-encapsulated *Capsicum* oleoresin presented solubility indexes ranging from 74.5 ± 2.30 to 94.5 ± 4.42%, as shown in **Table 3.** The lowest value was found for a sample containing malt as a coating material, which could be related to the presence of insoluble solids. In contrast, commercial wall materials showed high solubility values due to their chemical structure.

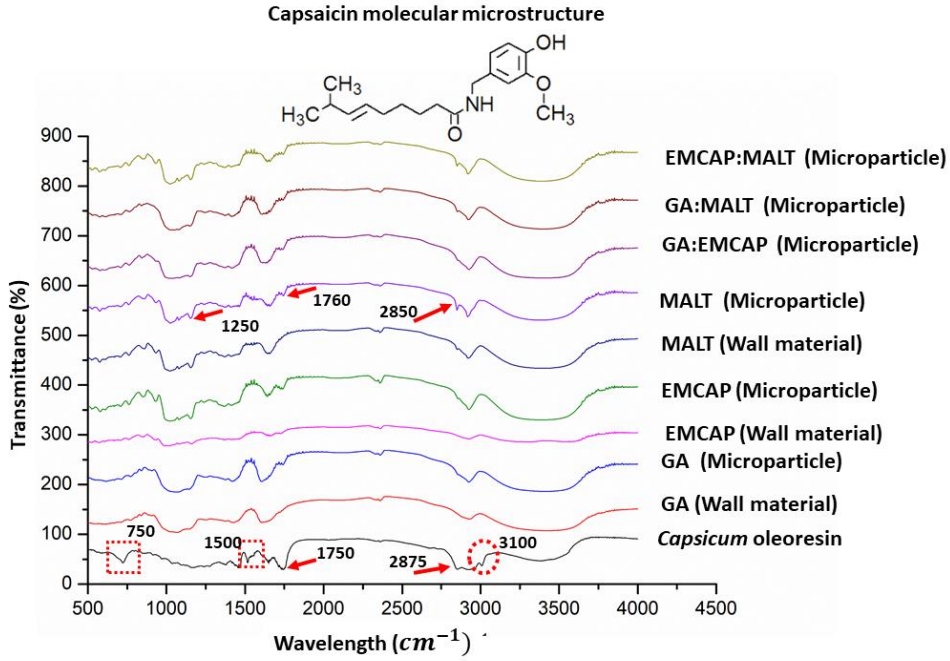
When the commercial materials (GA and EMCAP) were combined to MALT, there was a slight increase in the solubility index, due to a decrease in the concentration of insoluble solids. Overall, carbohydrates present high solubility in water. Similar results were found for ginger essential oil encapsulated by gum arabic, maltodextrin, and inulin, with values ranging from 81.4 to 84.6% for all blends (Fernandes et al., 2016 and for nutmeg oleoresin microencapsulated by gum arabic and modified sorghum starches-OSA, which showed a solubility of over 75% (Arshad et al., 2018).

Additionally, *Capsicum* oleoresin-loaded microparticles presented a good solubility in water due to low oil content. According to Mohammed et al. (2017), microparticles containing 5% oil phase presented an average solubility of 80%, also showing decreases in solubility for increases in the ratio of oil of microparticles.

Chemical structure by Fourier-transform infrared spectroscopy (FT-IR)

FT-IR is a qualitative and versatile technique that is able to identify organic groups in short time, with ease of sample preparation and accurate results. **Fig. 2** shows a molecular structure of capsaicin, FT-IR spectra for *Capsicum* oleoresin, OC microparticles and wall materials.

Fig. 2 Microparticles chemical structure *GA: Arabic gum, EMCAP: modifed corn starch-OSA(EMCAP®); MALT: Stearic acid modifed malt; GA:EMCAP, GA:MALT and EMCAP:MALT.



The spectrum of pure *Capsicum* oleoresin showed bands around 3100, 2875, 1750, 1500, 750 cm^{-1}, thus pointing to the presence of an aromatic ring, polar groups (hydroxyl, amide, and carbonyl groups), and a hydrophobic group (Freitas et al., 2018). Aromatic groups, represented in the 3000-3150 cm^{-1} spectral region, are the most important bands for this oleoresin. Meanwhile, the stretching vibrations of C-H groups in 2875 cm^{-1} region represent saturated aliphatic compounds (Nagavekar & Singhal, 2019).

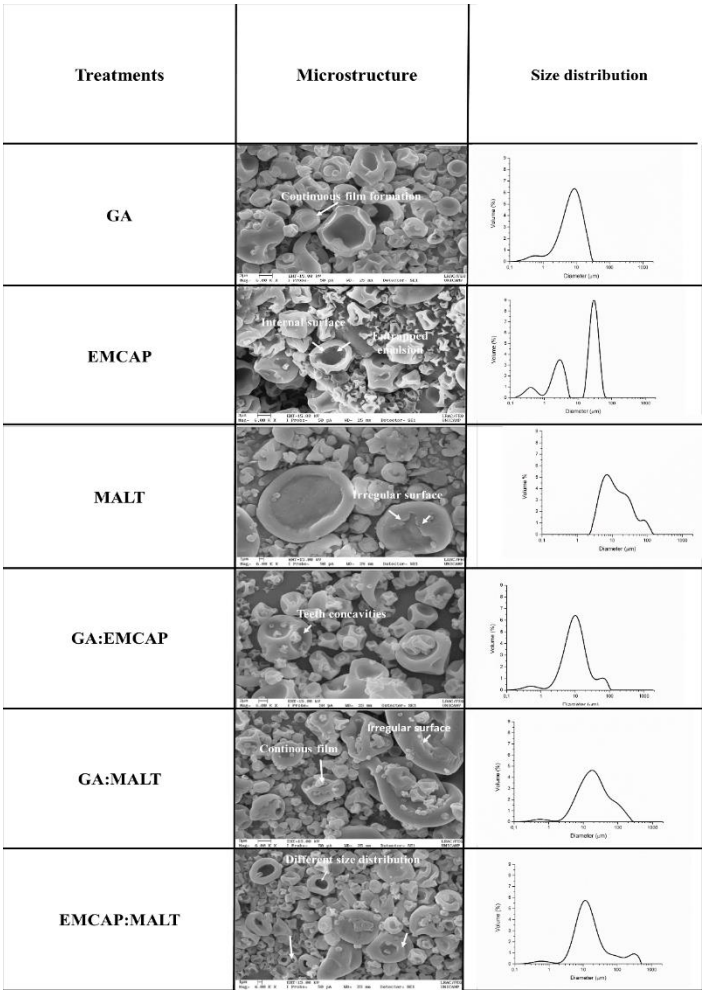
According to Techawinyutham et al. (2019), the signal peaks in 2854 to 2925 cm^{-1} relate to capsaicin (CAP), the main compound responsible for *Capsicum* oleoresin pungency. The peak at 1750 cm^{-1} corresponds to the stretching vibration of C=O (carbonyl group) and the one at 1510 cm^{-1} suggests flexion of N-H group. In addition, a band in the 750 cm^{-1} characterizes regional vibration of C-H and C-C, recognized as the aromatic bonds of the capsaicin phenyl ring. The

main band correlated to samples of pure wall materials (GA, EMCAP, and MALT) is placed around 995 cm^{-1}, comprehending a typical polysaccharide absorbing region (Binsi et al., 2017). At last, the loading profile of microparticles presented a band deformation of the carbonyl group and the hydroxyl group, implying that *Capsicum* oleoresin was likely bound to chemical groups of the wall materials. The same behaviour was reported by Freitas et al. (2018) for capsaicin-loaded albumin nanoparticles.

Microstructure of microparticles, size distribution and volumetric mean diameter

Scanning electron microscopy was used for assessment of the differences among *Capsicum* oleoresin microparticles made with various carbohydrates as wall materials. The microstructure of microparticles is seen in **Fig. 3.** Microparticles formulated by modified corn starch (EMCAP) or/and gum arabic show spherical geometry, teeth concavities in the surface and no pores. EMCAP particles also show small pits where the emulsion is likely located. In general, commercial wall materials (GA; EMCAP; GA: EMCAP) present good film forming properties, resulting in particles with smoother surfaces, as similarly described by Alcântara et al., 2019.

Fig. 3. Microparticles morphology and size distribution *GA: Arabic gum, EMCAP: modifed corn starch-OSA(EMCAP®); MALT: Stearic acid modifed malt; GA: EMCAP, GA:MALT and EMCAP:MALT



When modified malt is used as a carrier agent, due to its insoluble fraction, a blood cell structure and greater roughness in particle coating are seen. This effect decreased for MALT combined to commercial materials (GA: MALT; EMCAP: MALT) and it is associated to a low content of insoluble solids, which thus forms particles with smoother structures.

Different sizes were observed for all microparticles formulations, according to graphic data obtained from **Fig. 3**, in accordance with the micrograph images mentioned above. The formulation containing gum arabic only presented two peaks that ranged from 0.1 to 1 µm and a third and greater peak varying from 1 to 50 µm. Microparticles containing EMCAP in their formulation showed three peaks, the first ranging from 0.1 to 1 µm, the second from 1 to 10 µm and the third from 10 to 100 µm. The MALT exhibited a very large size range, with diameters

ranging from 5 to 120 µm when compared to other formulations. The high range of particles containing MALT are related mostly to the composition of malt. Modified malt is a complex material composed of insoluble solids, which all contribute to the formation of agglomerates.

The combination of GA: EMCAP materials resulted in particles with varying sizes and polymodal behaviour with a small peak between 0.1 to 1.5 µm, a second peak varying from 1.5 to 100 µm, typical of polysaccharides mixtures (Goëlo et al., 2020). On the other hand, the incorporation of MALT into formulation containing EMCAP or GA was observed a differential volume distribution with one short peak consisting of a small average size of particles, and another peak with a wide range of a large volume reaching values over 200 µm, which implies agglomeration of the powders.

Table 3 shows the mean volumetric diameter (D_{43}) and the SPAN values for all formulations. The treatment containing gum arabic only in its composition presented the lowest D_{43} (14.7 ± 0.5 µm), whereas MALT particles presented an average diameter of 186.7 ± 6.3 µm. The distribution graph of microparticles containing MALT evidenced the presence of small particles and microparticles with a considerable size, which contributed to obtaining a high D_{43} average value (186.7 ± 0.3). In addition, coating of microparticles by modified malt presented more agglomerates due to its rich composition in low molecular weight sugars.

Influence of different biopolymers on Capsicum oleoresin encapsulation efficiency and capsaicin retention

Microencapsulation efficiency implies the degree of entrapment of the emulsion by the wall material and quantify the amount of bioactive compound is encapsulated in the microparticle (Binsi et al., 2017, Goëlo et al., 2020). The encapsulation efficiency (OEE) of *Capsicum* oleoresin varied from 68.5% to 91.6%, pointing the influence of carbohydrates on the oleoresin retention (**Table 3**). Data showed higher OEE values for GA and EMCAP in relation to other treatments. These results are related to the strong emulsifying property of these materials and to their ability to form continuous films on the microparticle. As a result, the development of a continuous film entailed a stable system with better entrapment of the emulsion, reaching high values to oleoresin retention. In respect of microparticles composed by MALT, a low oleoresin efficiency encapsulation of 68.5% was observed. Possible reason for this trend can be associated with big

droplet size of emulsions and surface imperfections of microparticles (Jafari et al., 2008; Link et al., 2019).

Commercial materials in combination with modified malt (GA: MALT; EMCAP: MALT) promoted a significant increase (p <0.05) in the *Capsicum* oleoresin encapsulation efficiency. This improvement was associated to smoother particle covering, synergy between the hydrophobic groups of wall materials and better rearrangement of the carbohydrates, which all generated a strong emulsion entrapment.

A similar trend for OEE (from 84.2 ± 1.5% to 96.2 ± 0.2%) was reported for *Nigella sativa* L. oleoresin microparticles produced by *spray drying* using gum arabic and maltodextrin (1: 1) as wall materials. The authors associated the high encapsulation efficiency and the strong capacity to form a continuous film to gum arabic and the low viscosity of the solution to maltodextrin (Edris et al., 2016).

Among the compounds present in *Capsicum* oleoresin, the capsaicin (*trans*-8-methyl-N-vanillyl-6-nonenamide) has proved several health benefits, being a compound of great interest by scientific research. That way, the purpose of the *Capsicum* oleoresin encapsulation was also to produce microparticles carried with capsaicin with potential application as a functional ingredient. Thus, microparticles with high capsaicin content were obtained even using distinct carbohydrates matrixes, as is shown in **Table 3.** The obtained values were within the range from 2.35 to 3.49 mg capsaicin/g microparticles, i.e., the encapsulation capsaicin retention could be associated to *Capsicum* oleoresin entrapment into particle, and the efficiency of spray drying method to encapsulate both *Capsicum* oleoresin and capsaicin. Other examples of microparticles rich in hydrophobic ingredients produced by spray drying were found in the scientific literature, such as chia oil (Alcântara et al. 2019), astaxanthin (Boonlao et al. 2020), and resveratrol (Consoli et al. 2019).

Antioxidant activity of microparticles

ORAC and FRAP methods showed that all microparticles rich in capsaicin and various other bioactive compounds had high antioxidant capacity (**Table 3**). However, significant differences among formulations were observed. GA presented the highest value, by ORAC, for antioxidant activity (AA), of 89.4 ± 9.7 (µmol TE/ g microparticle); meanwhile, the mixtures GA:

EMCAP; GA: MALT; EMCAP: MALT showed an antagonistic effect, thus resulting in a decreased antioxidant activity. The same trend was reported previously in the literature. Higher values for AA by ORAC were obtained for propolis microparticles carried by gum arabic than those for the formulation containing maltodextrin as an encapsulating material (Andrade et al., 2018).

Otherwise, FRAP analysis resulted in values ranging from 21.3 to 88.9 µmol TE/g microparticles, which also implied a synergic behaviour between mixtures of wall materials. Blends composed by GA: MALT and EMCAP: MALT exhibited higher values for AA (88.9 ± 3.8, and 72.3 ± 2.6 µmol TE/ g of microparticles, respectively).

These outcomes are due to the content of oleoresin in the microparticle, the antioxidant capacity of oleoresin compounds and each of the distinct wall materials used. Andrade et al. (2018) concluded that the reaction mechanism used in ORAC analysis has high reactivity to aromatic substances and compounds that do not act as electron donors. In contrast, FRAP measures the ability of the sample to donate electrons; therefore, its strength may be correlated to the phenolic substances that form the product.

AA values for *Capsicum* microparticles are in accordance with the results in the literature. So, California red peppers presented averages of 14.78 µmol TE/g of pepper by the ORAC test and 79.62 µmol of eq. $FeSO_4$/ g of pepper by FRAP analysis.

Overall, spices such as peppers and their by-products are known as good antioxidant agents regarding oils and fats, and hence their powerful off-taste reduction from oxidative degradation. Spices are of adequate employment by the food industry since they are natural ingredients that can, thus, be applied to clean label products.

Effect of capsaicin microparticles consumption on in vivo experimental model

Various studies using different experimental models showed that capsaicin and *Capsicum* oleoresin are not toxic. However, the bioaccessibility and bioavailability of capsaicin are modified after the microencapsulation of *Capsicum* oleoresin (El Abbassi et al., 2016). Thus, the present study aimed at evaluating the effects of diets supplemented with *Capsicum* oleoresin either encapsulated with corn oil or not, in terms of food intake, body weight gain and acute toxicity parameters.

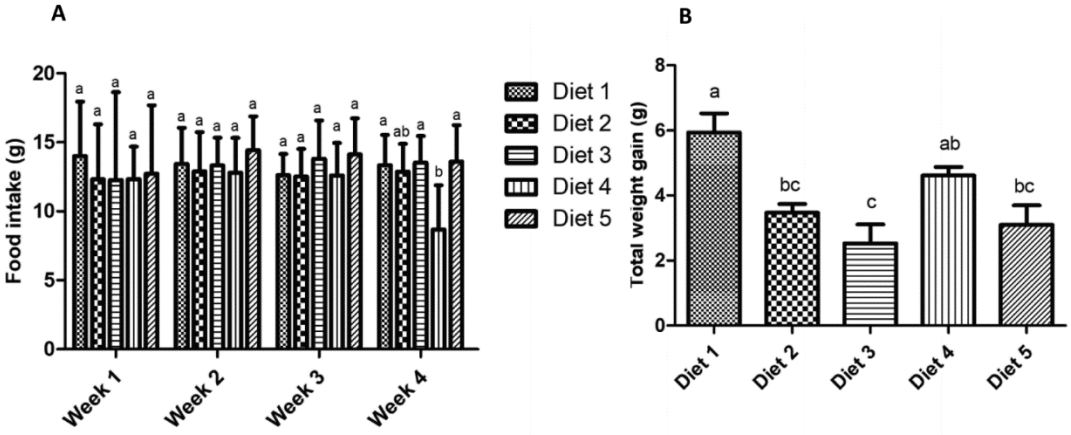
Each experimental group received different diets, i.e., with different percentages of capsaicin, representing 0.0022% of diet 2, 0.0044% of diet 3, 0.0014% of diet 4 and 0.0028% of diet 5, as detailed in **Table 2**.

The consumption was assessed weekly, for 28 days, as shown in **Fig. 4A**. No significant difference in intake was observed for diets, except for group 4, which showed a reduction in dietary consumption in the fourth week. Thus, the addition of capsaicin-rich microparticles did not affect the animals' dietary intake, which could become an issue due to the pungency of the compound.

Fig. 4B shows the total weight gain of the animals during the four experimental weeks. Overall, the addition of the capsaicin microparticles in the animals' diet promoted smaller weight gains, dose-dependent, in animals upon comparison to the control group (diet 1). Only group 4, which contains the lowest capsaicin content, does not show a statistical difference concerning weight gain when compared to the control group.

The consumption of a high-fat diet (high-fat and high-calorie) is an experimental model widely used to induce obesity in experimental models. The difference in dietary and caloric intake was not observed among groups. *Capsicum* oleoresin promoted less weight gain and provoked changes in metabolic or thermogenic activity, as previously reported in the literature (Ohyama et al., 2016). That way, the high-fat diets with a capsaicin content ranging from 0.0022-0.0044% (diets 2, 3, and 5) may contribute to a lower weight gain in animals. A similar result was observed in a study by Tan et al. (2014), who attended a reduction in body weight gain in Sprague Dawley rats with 30 mg capsaicin/kg during a five-week experiment. The authors observed that the intake of microencapsulated capsaicin had a significant effect on body weight reduction when compared to the consumption of non-encapsulated capsaicin. In another study authors also reported that food intake from a high-fat diet supplemented with *Capsicum's* oleoresin nanoemulsions was able to reduce the final body weight of Sprague Dawley rats, decrease the total adipose tissue and reduce the level of plasma triglycerides (Kim et al., 2014)

Fig. 4. A) Food Intake per week. B) Total weight gain by the mice after 28 days.



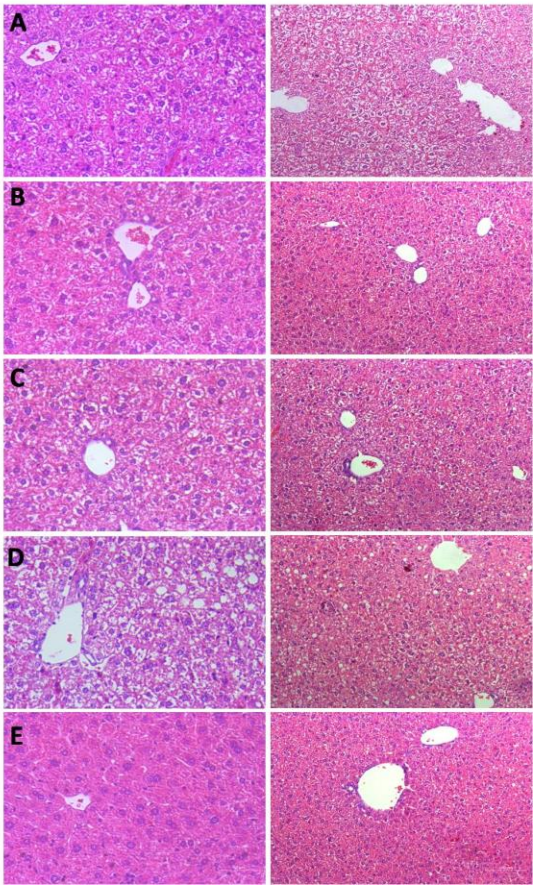
Diet 1: Control group which the animals received high-fat diet without microparticles of OC
Diet 2: High-fat diet containing 10% of microparticles of OC
Diet 3: High-fat diet containing 20% of microparticles of OC
Diet 4: High-fat diet containing 10% of microparticles of OC added of corn oil
Diet 5: High-fat diet containing 20% of microparticles of OC added of corn oil.

No impact of different diets on the tissue mass of the animals (liver, mesenteric adipose tissue, spleen, heart) or fasting blood glucose, with no difference observed among the groups (Supplementary Material – Table S1). AST and ALT analysis show if different diets caused any toxicity in animals, since increases in plasma levels of cytosolic aminotransferase enzymes indicate cellular liver damage. In addition, increases in the AST/ ALT ratio are often associated to liver damage (non-alcoholic steatohepatitis) (Sorbi et al., 1999). The AST and ALT levels were high only in the control group and for diet 4. The AST/ ALT ratio was high only in diet 4 **(Supplementary Material – Table S1)** which corroborates with the lower protection of these diets on the gain body weight.

Lipid oxidation results did not show statistically significant differences among groups regarding the level of hepatic TBARS, which values varied from 57.7 ± 6.2 to 59.2 ± 5.8 nmol MDA/ mg of tissue **(Supplementary Material – Table S1).** However, statistical differences were observed in the histological evaluation of the liver samples of the animals. The liver sections stained with HE showed that 28 days of ingestion of HFD induced dramatic accumulation of fat in the liver (hepatic steatosis), as well as the formation of cell ballooning, which is indicative of reversible liver damage, despite the absence of inflammatory infiltrates in the samples **Fig. 5.** Diet 5 (**Fig.5E),** containing *Capsicum* oleoresin and corn oil, promoted a better liver protection

concerning fat accumulation and cellular ballooning than other diets (diet 1, 2,3, and 4). Future studies are necessary to investigating if this protective effect is associated to an improvement of the bioavailability of capsaicin by the addition of medium-chain oil. Clegg et al. (2013) observed that the ingestion of a meal containing chili or pepper, sources of capsaicin, associated to medium-chain triglycerides increased energy expenditure and thermogenesis in healthy individuals when compared to the consumption of a meal containing chili or pepper associated to sunflower oil.

Fig. 5 Hepatic histological analysis



	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Steatosis	2.5 ± 0.83^{a}	$1.83 \pm 1.16^{a, b}$	$1.67 \pm 1.03^{a, b}$	$1.83 \pm 1.16^{a, b}$	0.33 ± 0.51^{b}
Balloning	1.33 ± 0.51^{a}	$1.17 \pm 0.40^{a, b}$	$0.50 \pm 0.54^{b, c}$	1.33 ± 0.51^{a}	0.17 ± 0.40^{c}
Inflamation	0.17 ± 0.40^{a}	0.33 ± 0.51^{a}	0.00 ± 0.00^{a}	0.17 ± 0.40^{a}	0.17 ± 0.40^{a}

Sections of liver samples stained with H&E for visualization of hepatocytes morphologies and score for steatosis, ballooning and inflammation assessed in mice fed a (A) high-fat diet without microparticles of OC (Diet 1,*n*= 6), (B) a high-fat diet containing 10% of microparticles of OC (Diet 2, *n*= 6), (C) a high-fat diet containing 20% of microparticles of OC (Diet 3, *n*= 6), (D) High-fat diet containing 10% of microparticles of OC added of corn oil (Diet 4, *n*= 6) and (E) a high-fat diet containing 20% of microparticles of OC added of corn oil (Diet 5, *n*= 6) for 21 days from weaning. Magnification: 200x and 400x. Values represent mean x`± S.D. (*Means that do not share a letter are significantly different).

Therefore, this research reached the objective of producing microparticles with functional benefits, rich in capsaicin. Total weight gain of the animals and the histology of the hepatic tissue showed a positive dose-dependent protective effect of *Capsicum's* oleoresin against the damage caused by obesity, induced by a high-fat and high-calorie diet. However, additional studies focusing on metabolic pathways and cell signalling can clarify the related mechanisms.

Conclusions

In this study, all carbohydrates affected the powder properties related to wettability, particle size, morphology, antioxidant activity and capsaicin retention. Regardless of the formulation, these materials proved to be great carriers' agents for capsaicin, thus turning these powders into potentially appealing ingredients. All formulations presented high contact angle, great solubility in water, low water activity and hygroscopicity. FT-IR analysis showed that capsaicin was successfully bound to the structure of the carbohydrates. Along with these results, animal assays using a high-fat diet (HFD) supplemented with microparticles rich in capsaicin evidenced that the consumption of the latter promoted a decrease in body weight gain compared to the HFD control. This outcome points to a promising employment of *Capsicum* oleoresin microparticles to controlling obesity and nonalcoholic steatohepatitis. Future studies on the *Capsicum* oleoresin microencapsulation, using different techniques, under various conditions and with distinct carrier materials are necessary, though, to improving the encapsulation efficiency and antioxidant activity. Assessing capsaicin microparticles regarding their gastrointestinal release and transport mechanism may also be helpful to assign satiety.

Declaration of Competing Interest

The authors declare that they have no known competing fnancial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary Material

Table S1. Effect of a diet supplemented with capsaicin on average values of tissue, fasting glucose, weight gain, and lipid oxidation of liver after four-week experimental essay.

	Groups				
Measurements	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**
Liver (g)	$0.996^{a} \pm 0.056$	$0.991^{ab} \pm 0.122$	$0.858^{ab} \pm 0.061$	$0.893^{ab} \pm 0.074$	$0.805^{b} \pm 0.108$
Heart (g)	$0.125^{a} \pm 0.013$	$0.120^{a} \pm 0.014$	$0.121^{a} \pm 0.016$	$0.121^{a} \pm 0.013$	$0.113^{a} \pm 0.008$
Mesenteric adipose tissue (g)	$0.625^{a} \pm 0.307$	$0.586^{a} \pm 0.219$	$0.553^{a} \pm 0.213$	$0.843^{a} \pm 0.253$	$0.606^{a} \pm 0.239$
Spleen (g)	$0.058^{a} \pm 0.021$	$0.068^{a} \pm 0.011$	$0.050^{a} \pm 0.008$	$0.065^{a} \pm 0.021$	$0.070^{a} \pm 0.022$
Kidneys (g)	$0.293^{a} \pm 0.032$	$0.253^{a} \pm 0.054$	$0.253^{a} \pm 0.033$	$0.270^{a} \pm 0.018$	$0.258^{a} \pm 0.025$
Blood glucose (mg/dL)	$254.6^{a} \pm 20.9$	$225.6^{a} \pm 28.5$	$262.5^{a} \pm 38.7$	$267.0^{a} \pm 58.0$	$214.0^{a} \pm 25.0$
Total body weight (g)	$5.93^{a} \pm 1.44$	$3.46^{bc} \pm 0.66$	$2.53^{c} \pm 1.41$	$4.61^{ab} \pm 0.63$	$3.10^{bc} \pm 1.46$
Tbars (nmol MDA/mg of tissue)	$59.2^{a} \pm 5.8$	$58.9^{a} \pm 3.4$	$57.9^{a} \pm 3.4$	$57.7^{a} \pm 6.2$	$55.0^{a} \pm x`x`3.0$
AST[#](U/L)	7.3	4.4	3.5	13.2	4.8
ALT[#] (U/L)	39.1	38.9	27.1	40.4	27.9
AST/ALT[#]	0.18	0.11	0.13	0.33	0.17

*Means that do not share a letter in the same line are significantly different. #It was not possible to calculate the statistical differences among the groups because some replicates were lost during the analysis.
Diet 1: Control group which the animals received high-fat diet without microparticles of OC;
Diet 2: High-fat diet containing 10% of microparticles of OC;
Diet 3: High-fat diet containing 20% of microparticles of OC;
Diet 4: High-fat diet containing 10% of microparticles of OC added of corn oil; Diet 5: High-fat diet containing 20% of microparticles of OC added of corn

CAPÍTULO 6

CAPSICUM OLEORESIN LOADED SPRAY-DRIED MICROPARTICLES: STABILITY, CYTOTOXICITY, AND IN VITRO DIGESTIBILITY STUDIES

Manuscript submitted in Food Structure

CAPSICUM OLEORESIN LOADED SPRAY-DRIED MICROPARTICLES: STABILITY, CYTOTOXICITY, AND *IN VITRO* DIGESTIBILITY STUDIES

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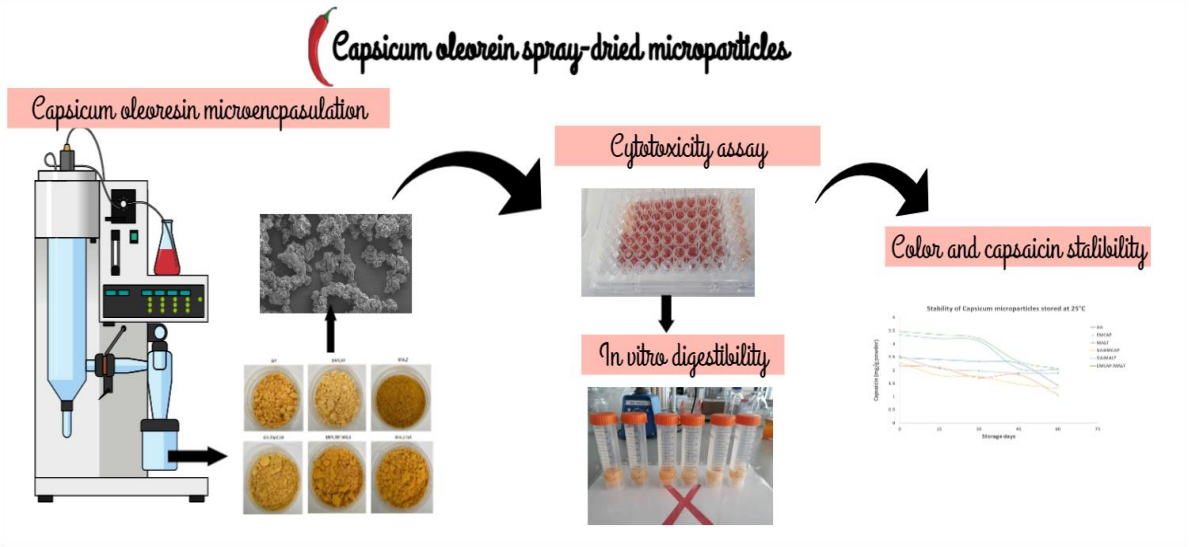
Graphic abstract



Abstract

Capsicum oleoresin is a source of capsaicin, which has many health-promoting benefits such as anti-obesity, thermogenesis, and inducer satiety. However, studies have shown capsaicin to have toxic effects. The main aim of this work was to formulate microparticles of *Capsicum* oleoresin and evaluate their cytotoxicity, *in vitro* digestibility, and controlled release of capsaicin. Arabic gum (GA), OSA-modified corn starch (EMCAP), modified malt (MALT), and their ratio 1:1 combination (GA: EMCAP; EMCAP: MALT; MALT: GA) were used as wall materials to formulate six different oil-in-water emulsions containing 15% solids (5% *Capsicum* oleoresin and 95% wall material). These formulations were homogenized, and spray dried. The encapsulation efficiency of each formulation was determined by Ultra Performance Liquid Chromatography (UPLC). The formulated microparticles, individual microparticle components, and *Capsicum* oleoresin were tested for cytotoxicity on human intestinal epithelial Caco-2 and HepG2 liver cells. Furthermore, the INFOGEST static *in vitro* simulation assessed the digestion of microparticles using UPLC to obtain % capsaicin release. EMCAP: MALT, MALT: GA and GA presented highest encapsulation efficiency of capsaicin reaching values of approximately 90%. *In vitro* cytotoxicity studies of all treatments with five concentrations ranging from 5 - 100µg/ml showed no significant cytotoxicity after exposure times of 4 hours on Caco-2 and 72 hours on HepG2. MALT: GA formulation presented the best controlled release. Microparticles suffered higher degradation of color and capsaicin during storage at 45°C than 25°C. Results showed that MALT: GA microparticles started with 7.41% of release in the oral phase after 2 min of digestion, reaching after 2 hours in the simulated gastric fluid a cumaltive release of 25 %, followed by 78.62 % of capsaicin release after 4 hours in intestinal fluid. This result indicated that MALT: GA was able to concentrate release in the intestinal phase (%), probably due to the low solubility of the particles and synergy between wall materials. In conclusion, the MALT: GA microparticles are the potential delivery formulation of capsaicin.

Keywords: spray-drying, microparticles, cytotoxicity, color, digestibility, stability, capsaicin.

1. Introduction

Interest in *Capsicum* oleoresin application has increased due to its benefits to human health (Adaszek *et al*., 2019). Studies have shown that compounds from *Capsicum* oleoresin, like capsaicin, can promote weight loss, reduce lipid tissue, and prevent diabetes disease (Song *et al*., 2017; Joseph *et al.,* 2021; Anthero *et al*., 2022). All these benefits are associated with *Capsicum* oleoresin composition, such as bioactive compounds, antioxidant activity, red pigments, and flavor compounds (Berke & Shieh, 2012). Such ingredients' characteristics make them desirable for use in food and pharmaceutical products (Aguiar *et al.,* 2022).

The oral administration and product application of neat *Capsicum* oleoresin are limited due to its pungency and fat-soluble nature. Moreover, it may exhibit some toxicity to healthy cells, and the pharmacokinetic profile of capsaicin can be low due to toxic metabolites and short half-life (Freitas *et al.,* 2018; Rollyson *et al*., 2014). In this sense, microencapsulation may be an alternative to mask the pungent flavor, protect its compounds and extend its application to food products and oral administration.

Spray drying is an important encapsulation method in the industry because it is simple, fast, and low-cost (Gharsallaoui *et al*., 2007). *Capsicum* oleoresin is encapsulated in an emulsion-based food structure formed through high-energy mixing methods such as rotor-stator, microfluidizer, and ultrasound (Aguiar *et al*., 2016; Aguiar *et al.,* 2021; Zhang *et al*., 2022). Subsequently, this emulsion is atomized and dried in a spray dryer, forming microparticles with very specific properties, such as size, structure, encapsulation efficiency, solubility, and thermal properties (Assadpour & Jafari, 2019; Jafari *et al.*, 2023).

The physical and functional properties of the powder are influenced by the wall material polymer-based used. Furthermore, the ratio of wall material to core material influences the properties of the powder as well as its ability to successfully function as an encapsulation system. Depending on these characteristics, microparticles can present resistance to the gastrointestinal tract, delivery to target tissues, controlled release of the encapsulated active (Vilstrup, 200; Assadpour & Jafari, 2019). Gums and modified starches are wall materials widely studied in the oleoresin encapsulation system (Arshad, Ali & Hasnain, 2018; Porras-Saavedra *et al.,* 2018; Porras-Saavedra et al., 2021). These materials have emulsifying properties, are low cost, are considered Generally Recognized as Safe (*GRAS*), and their use alone or in combination with other materials can increase the protective effect of the core material (Williams, 2011; Zhu, 2017).

The effect of different wall materials on the release of capsaicin into *Capsicum* oleoresin formulation is also worthy of investigation since the interactions between food matrices and the bioactive compound can influence its bio-accessibility (Lucas-González, Viuda-Martos, Pérez-Alvarez, & Fernández-López, 2018). Unfortunately, there are no reports in the literature evaluating the impact of different carbohydrates as wall materials on *Capsicum* oleoresin digestibility, cytotoxicity, and stability. The release of *Capsicum* oleoresin after administration is an exciting approach to determining the profile of absorption of the bioactive compound in the human gastrointestinal tract.

In this work, formulations of *Capsicum* oleoresin microparticles composed of gum arabic and octenyl succinic anhydride (OSA)-modified starch (EMCAP) and modified malt were characterized, *in vitro* digestibility and cytotoxicity were evaluated for the development of an oral delivery formulation containing capsaicin.

2. Material and methods

2.1 Material

Gum Arabic and Octenyl succinic anhydride (OSA)-modified starch (EMCAP^{TM}) were kindly donated by Nexira (São Paulo, Brazil), and Cargill (Campinas, Brazil), respectively. Barley malt was esterified using a combination of ultrasound (45 W/5 min) plus stearic acid (2% w/w), resulting in a modified malt which was characterized by Anthero et al. (2021). *Capsicum* oleoresin was used as an oil phase, and it was purchased from Synthite Industries Ltd. (Kerala, India). Capsaicin standard was acquired from Cayman Chemical (Cayman Chemical, United States of America. Purity>95%). Other reagents were of analytical grade**.**

2.2 Microparticles production

The dispersions of the wall materials (gum Arabic, OSA modified corn starch, and modified malt) and blends containing 15% solids were previously dissolved in distilled water under magnetic agitation for 12h. Six formulations containing 15% solids (5% oleoresin, and 95% wall material) were performed as described in Table 1. Dispersions composed by aqueous (95%) and oil phase (5%) were homogenized by rotor-stator (Silverson L5M-A Laboratory Mixer, Chesham, Buckinghamshire, United Kingdom) at 5000 rpm for 10 min. Emulsions (500 g) were submitted to a Mini Spray Dryer B-290 (Büchi, Flawil, Switzerland) using a double liquid atomizer nozzle of 0.7 mm. The drying air flow rate was 35 (m^3/h) with an inlet temperature of 160 ºC. Compressor air pressure was kept at 0.06 Mpa with emulsion feed flow of 15 ml/min, and the outlet temperature varied from 75 ºC to 78ºC. Spray-dried powders were stored in sealed flasks for further analysis.

2.3 Compound's profile and capsaicin determination

a) LC-QToF

The phenolic compound profile and capsaicin content of *Capsicum* oleoresin and *Capsicum* oleoresin microparticles were determined by a Liquid Chromatography with quadrupole Time of Flight Mass Spectrometry (LC-QToF) (Agilent 6545 QToF/LCMS, Agilent Technologies, United States). For sample preparation, microparticles or oleoresin were solubilized in methanol to reach a concentration of 10 ppm. The chromatographic separation was obtained using a Zorbax Eclipse Plus C18 1.8 m, 2.1 mm i.d., 100 mm column (Agilent Technologies, United States) and the mobile phase was composed by acidified methanol (0.1% formic acid) (phase B) and acidified water (0.1% formic acid) (phase A). The methodology used was the same employed by Aguiar *et al.* (2019), which consisted of a gradient method: 0–28 min, 3–100% B; 28–30 min, 100-3% B, using a flow rate of 0.5 ml/min at 40 ◦C with a volume injection of 1 µl. The mass spectrometer conditions were as follows: drying gas at 290 ◦C, drying gas flow of 11 L/min, nebulizer at 0.31 MPa, sheath gas at 350 ◦C, sheath gas flow of 12 L/min, VCap 3000, fragmentor 100V, OCT 1 RF Vpp 750V and different collision energy (N_2) using a mass varying from 100 to 1500 m/z, positive ionisation mode. Integration and data analysis were then obtained using the Mass Hunter Workstation software (Agilent Technologies, United States).

b) UHPLC

To determine capsaicin in the samples, 0.2 g of microparticles were solubilized in 0.75 ml of methanol and 0.25 ml of distilled water, and 0.005 g of *Capsicum* oleoresin was diluted in 10 ml of methanol. Samples were mixed for 1 min by vortex, placed in an ultrasonic bath during 15 min and centrifuged for 15 minutes at 10,000 rpm. The supernatant was filtered with a PTFE (polytetrafluoroethylene) hydrophilic filter with a pore size of 0.45 μm and a diameter of 13 mm. Capsaicin content was obtained by Ultra-High Performance Liquid Chromatography with Refractive Index and Diode Array Detection (UHPLC RID, DAD), 1290 Infinity II High-Throughput System, Agilent Technologies, United States) using POROSHELL 120 EC-C18 column (100 mm × 4.6 mm, 2.7μm). The mobile phase consisted of 85% methanol and 15% deionized water (Aguiar *et al.* 2019). The column was stabilized at 25 ºC and capsaicin detected by a wavelength of 280 nm with a mean retention time of 1.5 min. The capsaicin standard (Cayman Chemical, USA, >95%) was used to calculate the calibration curve ($R^2 = 0.9993$).

2.4 Microstructure by scanning electron microscope (SEM)

The images of the sample surface were observed by obtaining scanning electron microscope (SEM) (Hitachi SU 6600 FESEM, Japan) images. The materials were dropped onto carbon tabs and coated with a 6 mm thick Au/Pd coating, and morphology was then performed using a range voltage between 1-4 kV with approaches from x1k to x6k.

2.5 Chemical structure of the Capsicum oleoresin microparticles by RAMAN spectroscopy

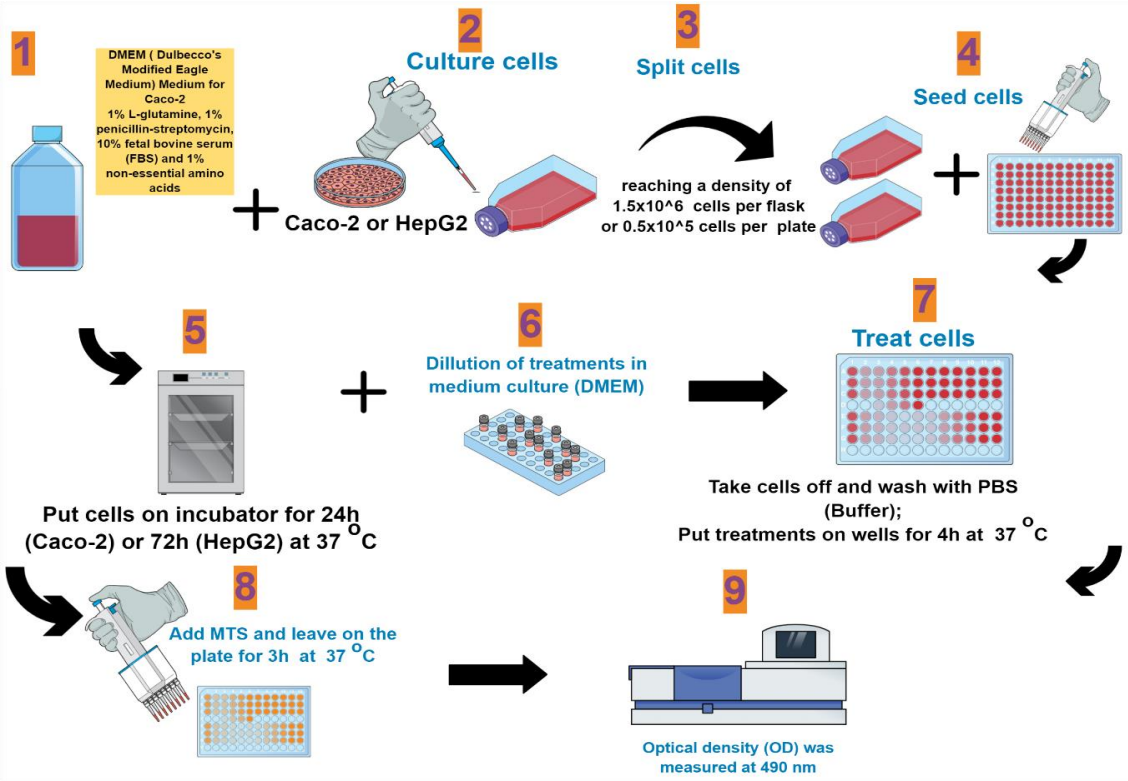
All samples were subjected to chemical analysis by Raman spectroscopy (Horiba Jobin Yvon LabRAM HR 800, France) equipped with a 785 nm laser. The best signals were achieved with x50 objectives, 10 seconds accumulation, and x5 acquisitions.

2.6 Cytotoxicity assessment

Caco-2 and HepG2 cells were seeded on a 96 well plate at a cell density of 1×10^4 cells/well in DMEM and EMEM, respectively. Both cells were incubated for 24 h at 37 °C in a humidified incubator with 5% CO_2 Test samples were incubated on Caco-2 cells for 4 h to mimic intestinal exposure and HepG2 cells for 72 h to mimic liver exposure (Khalid et al., 2022). Test samples included microparticles at concentrations ranging from 5 - 100 μg of microparticles or oleoresin/ml in Dimethyl sulfoxide (DMSO). The maximum concentration of DMSO in the well

was 0.1% and as such 0.1% of DMSO was used as a vehicle control (VC). Triton X-100™ (0.05 %) was used as a positive control, Caco-2 cells with no treatment as a negative control. After exposure, treatments were removed, the cells washed and MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazo-lium added. Optical density (OD) was measured at 490 nm. Each value presented was normalised to untreated control and calculated from three separate experiments, each of which included six replicates.

Figure 1 Toxicity assay steps illustrated



2.7 Color and capsaicin content of microparticles during the storage

Capsicum oleoresin microparticles and neat *Capsicum* oleoresin were stored in sealed pot for 60 days at 25 e 45 °C at a relative humidity of 36.2± 3%, and then capsaicin content and color measurements were analyzed at 0, 15, 30, 45 and 60 days. Capsaicin content into microparticles was quantified by HPLC equipment following the same methodology and calibration curve already published in our previous work (Anthero *et al.*, 2022). Instrumental color was determined

according to methodology mentioned in another previous research (Anthero *et al*., 2021), which samples were measured by Ultra Scan Vis 1043 (Hunter Lab, Reston, USA) using a D65 illuminant and 10º observer (RESEX mode). L* (from white to black), a* (from green to red), and b* (from blue to yellow) parameters were recorded using Easy Match QC software and used for calculation of hue angle (*h*°) and chroma (*C*) values, according to Equations 1 and 2:

$$h^{\circ} = arctan\left(\frac{b}{a}\right) \quad (1)$$

$$C = \sqrt{a^2 + b^2} \quad (2)$$

h° value is the tonality in cylindrical coordinates with red purple at an angle of 0°, yellow at 90°, bluish green at 180°, and blue at 270°. The *C* value means the intensity or purity of the tone, commonly referred as color saturation of the samples, which varies from dull (low value) to vivid colors (high value). Curves for Lightness (L), Hue angle (h) and Chroma (C) were plotted.

2.8 In vitro digestibility of capsaicin into Capsicum oleoresin spray-dried particles

The behaviour of the microparticles composed by GA, EMCAP, MALT, GA: EMCAP, EMCAP: MALT and GA: MALT, was assessed in simulated gastrointestinal conditions using three different fluids: simulated salivary fluid (SSF), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF), also of the digestive enzymes. The analyses followed the INFOGEST static *in vitro* simulation of gastrointestinal food digestion (Brodkorb *et al.*, 2019).

In summary, this protocol divides the digestion procedure into three steps of the digestion procedure: preparation, digestion procedure, and sample treatment with its characterization analysis. Enzyme activity and bile assays were conducted to confirm experiment viability. The oral phase was represented by the addition of the particles into Salivary Simulated Fluid (SSF) at 1:1 ratio, followed by the addition of salivary α-amylase solution (75 U/ml). This blend was stirred for 2 min and adjusted to pH 7. After that, the gastric phase started when the samples were added to Simulated Gastric Fluid (1:1) with the pepsin (2000 U/ml), and calcium chloride, the pH adjusted to 3, and the mixture subjected to static digestion under stirring at 50 rpm for 120 min, at 37 ˚C. After 120 min of gastric digestion, Simulated Intestinal Fluid (SIF) containing lipase solution (60 U/ml), calcium chloride, and bile extract were added to the sample, and samples were kept under stirring for 120 min at pH 7, at 37 ˚C. Digestion experiments were carried out in

triplicate for each formulation, over four hours. Aliquots were collected at initial time and 2, 120, and 240 mins. The capsaicin content was quantified by Ultra-Heigh Performance Liquid Chromatography (UHPLC) according to the methodology described above in 2.3 b, using standard capsaicin, and then the release profile was calculated by Equation 3.

$$Capsaicin_{rel} = \frac{Amount\ of\ capsaicin\ released\ at\ time\ (t)}{\text{Concentration of loaded capsaicin}} \times 100 \quad (3)$$

2.9 Statical analysis

Analysis of variance (ANOVA) was performed using the GraphPad® Prism-9 software with a confidence level of 95 and 99%. Differences between means values were compared using Tukey's test with 5% significance (p-value < 0.05). For citotoxicity assay differences between control and treatments were compared using One-Way ANOVA with Dunnetts's multiple comparison test *P<0.05, *P< 0.01

3. Results and discussion

3.1 Characterization physicochemical of the materials

Capsicum oleoresin is a viscous liquid extracted of *Capsicum* species that contains fatty acids, triglycerides, carotenoids, and capsaicinoids (Berke & Shieh, 2012; Melgar-lalanne *et al*., 2016; Adaszek *et al.*, 2019). In this research, powders of *Capsicum* oleoresin were obtained using distinct carbohydrates such as gum Arabic, OSA-modified corn starch, and modified malt. Thus, we produced and characterized low-cost formulations with a potential application in pharmaceutical and food products regarding their physicochemical properties.

Table 1 shows the impact of each formulation on encapsulation efficiency of capsaicin (EE). The treatment presented a capsaicin content ranging from 63.6±2.38 to 90.5± 0.96%. GA: EMCAP formulation presented the lowest EE possibly attributable to the loss of the compound during the formation of the particles, resulting in a lower entrapment than other formulations or through the oxidation of capsaicin after atomization (Zhang, Zhang, Chen, & McClements, 2016).

Similar results were found for capsaicin encapsulation efficiency (EE) ranging from 63.4 to 98.3% of capsaicin-loaded albumin nanoparticles (Freitas et al., 2019). Surassmo *et al.* (2010) also reached great values of capsaicin EE (81.32 to 92.98%) for *Capsicum* oleoresin nanocapsules obtained through emulsion-diffusion followed by freeze-dried method, such values were influenced by the increase of the surfactant in formulation.

An effect of the interaction between carbohydrates on the retention of capsaicin retention was observed. Gum arabic or OSA-corn modified starch in combination with modified malt increased the capsaicin encapsulation. The interation between wall materials: whey protein (WP) and OSA-corn modified starch (OS) on capsaicin microencapsulation and the combination of WP:OS in ratio of 10:0, 9:1, and 7:3 resulting in high encapsulation efficiency of capsaicin achieving values over 90% was reported by Zhang *et al.*, (2020). It is likely that the interaction among these biopolymers probably improved film-forming capability and emulsifying properties.

Table 1. Microparticles formulation and encapsulation efficiency

Treatments	***Wall material***	***Encapsulation efficiency of Capsaicin (EE)***	***Composition***
GA	GA (gum arabic)	90.5^{a}±0.96	100g of emulsion containing: 15% total solids, which 95% wall material and 5% *Capsicum* oleoresin
EMCAP	EMCAP (OSA-modified corn starch)	86.3^{ab}±6.09	
MALT	Modified Malt	77.4^{b}±1.1	
GA:EMCAP	GA:EMCAP (1:1)	63.6^{c}±2.38	
GA:MALT	GA:MALT (1:1)	90.2^{ab}±9.01	
EMCAP:MALT	EMCAP:MALT (1:1)	90.4^{a}±2.60	

Means followed by lower case in the same column do not differ significantly ($p < 0.05$) by the Tukey test.

Regarding compound's profile, LC-QToF identified capsainoid, capsaicinoids and organic compounds present in *Capsicum* oleoresin and the microencapsulated *Capsicum* oleoresin. The ToF-MS is a high-resolution method that provides accurate mass measurements, and elemental composition information.

The detected capsainoid and capsaicinoids are shown in Table 2. Capsi-amide, capsaicin and dihydrocapasaicin were observed either in *Capsicum* oleoresin or *Capsicum* oleoresin microparticles as the main group of chemical compounds. These compounds were detected in the fragmentation fraction (m/z) at 270.2783 (capsi-amide), 306.2065 (capsaicin), 308.2224 (dihydrocapsaicin), and their peaks are presented in Figure 2. These data are in accordance with a study of Liu *at al*., (2016), which identified these three compounds in chili peppers in the same ion fragmentation fraction. A study of Aguiar *et al.* (2015) also identified capsaicin and dihydrocapsaicin by liquid chromatography coupled to mass spectrometry in several Brazilian peppers within a concentration of capsaicin from 88±9 to 4457±393 μg/g, and dihydrocapsaicin

from 10.7± 0.8 to 2358±202 μg/g. Capsaicin and dihydrocapsaicin are the main capsaicinoids responsible for pungency of chili peppers with high antioxidant activity (Materska & Perucka, 2005), meanwhile the capsi-amide belongs to the capsainoid family which is known as a very strong basic compound present in a bigger concentration in yellow bell peppers, red bell peppers, and pepper (*C. annuum*) (Takahashi et al., 1980; Liu *at al.*, 2016).

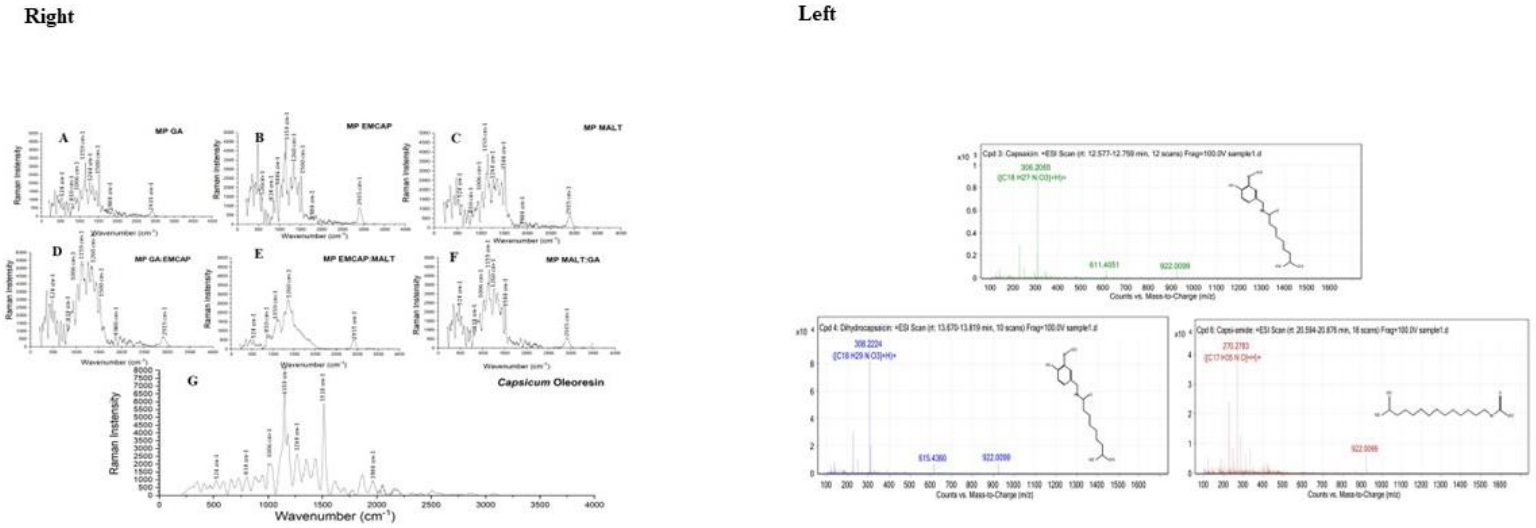
Other organic compounds were also found in *Capsicum* free oleoresin (CO), as shown in Table 2. The monosaccharide called erythrose and the second was the pyrogallo, known as pyrogallic acid, which is responsible by the flavour of product were identified in the samples. Other components were detected by Q-Tof, such as the p-anisaldehyde known as an aromatic compound, and the hispidulin, a bioactive flavonoid. Further, 7-hydroxycoumarinyl-γLinolenate, a linoleic acid ester presents in oils and oleoresins, and the solanocapsine, an antibacterial alkaloid found in some fruits and plants were also identified. From six organic compounds present in *Capsicum* oleoresin only two of them were found in post-encapsulated *Capsicum* oleoresin. The absence of erythrose, p-anisaldehyde, hispidulin, and 7-hydroxycoumarinyl-γLinolenate in microparticle powders could be associated to a low concentration of oleoresin (5%) in the formulations, making it difficult to detect them by mass spectroscopy analysis or to a preferential affinity of the polymers used for the two compounds.

Table 2. Compound's profile in *Capsicum* oleoresin non-encapsulated and encapsulated

***Capsicum* Oleoresin**			***Capsicum* Oleoresin microparticles: GA, EMCAP, MALT, GA:EMCAP, EMCAP:MALT,GA:MALT**		
Bioactive compound	**Fragments (m/z exp.)**	**Formula**	**Bioactive compound**	**Fragments (m/z exp.)**	**Formula**
Capsainoid & Capsaicinoids			***Capsainoid & Capsaicinoids***		
Capsi-amide	270.2783	C17 H35 N O	Capsi-amide	270.2783	C17 H35 N O
Capsaicin	306.2065	C18 H27 N O3	Capsaicin	306.2065	C18 H27 N O3
Dihydrocapsaicin	308.2224	C18 H29 N O3	*Dihydrocapsaicin*	308.2224	C18 H29 N O3
Organic metabolites			***Organic metabolites***		
Erythrose	121.0501	C4 H8 O4			
Pyragallol	126.0327	C6H6O3	*Pyragallol*	126.0327	C6H6O3
p-anisaldehyde	137.589	C8 H8 O2			
Hispidulin	299.0556	C16 H12 O6			
7-hydroxycoumarinyl-γLinolenate	423.2543	C27 H34 O4			
Solanocapsine	453.3452	C27 H46 N2 O2	*Solanocapsine*	453.3452	C27 H46 N2 O2

Gum Arabic (GA), OSA-corn modified starch (EMCAP) and modified malt (MALT).

Figure 2 Capsaicinoids mass spectrum in the *Capsicum* oleoresin **(Right)** and *RAMAN Spectroscopy* **(Left)** of the **A-** Microparticles GA, **B-** Microparticles EMCAP, **C-**Microparticles MALT, **D-**Microparticles GA:EMCAP, **E-**Microparticles EMCAP:MALT, **F-**Microparticles MALT GA, **G-***Capsicum* oleoresin



MP:Microparticles. Gum Arabic (GA), OSA-corn modified starch (EMCAP) and modified malt (MALT).

3.2 Analyses of the materials structure by means of Raman spectroscopy and scanning electron microscopy.

Few studies have analysed samples containing capsaicin through Raman spectroscopy. That way, this analysis was very important to detect the main compounds of the samples source of capsaicinoids.

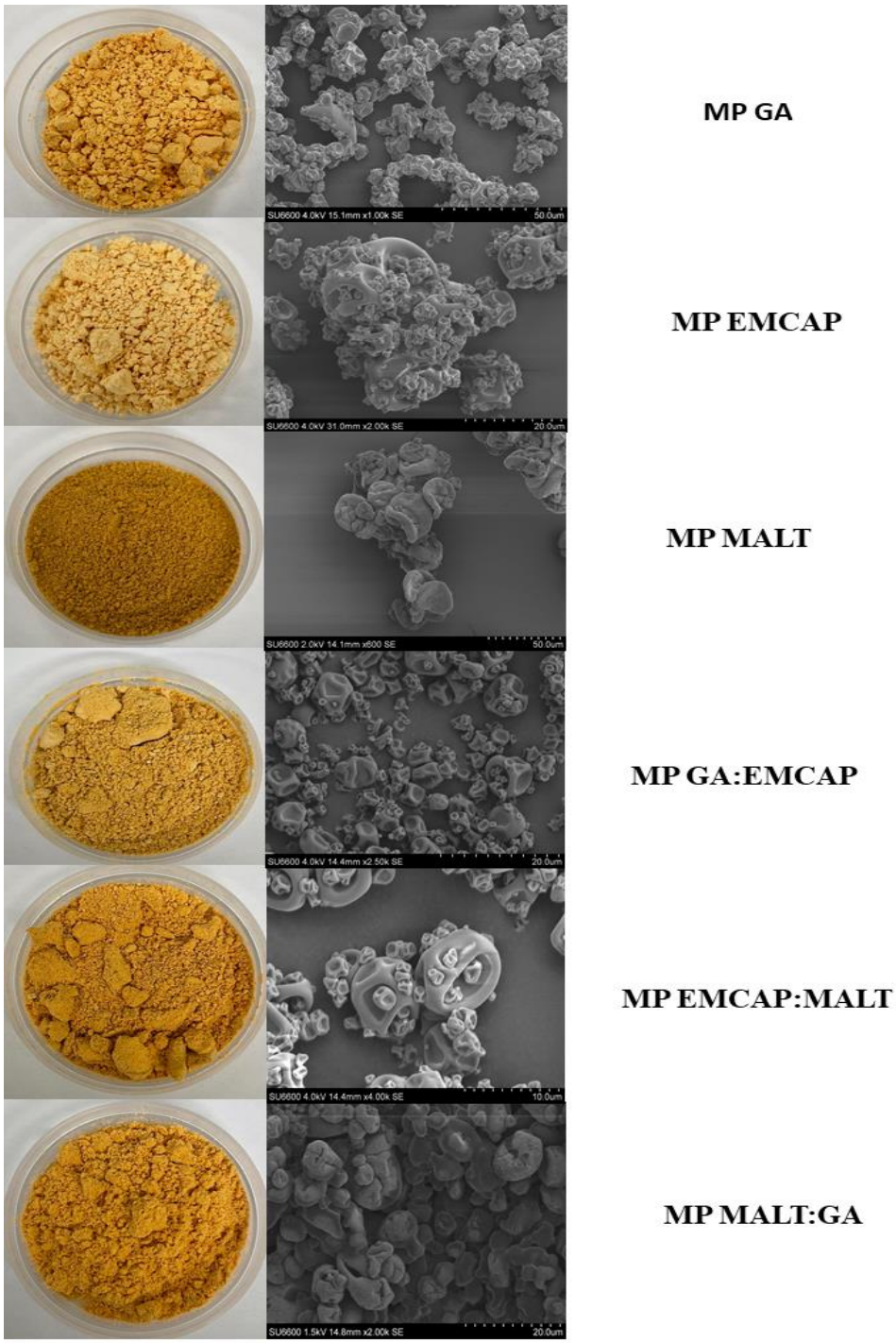
Raman spectroscopy is a non-destructive method able to provide the molecular structure of samples by means of frequency shift (or energy change) of the scattered light (Achata *et al*., 2018). Thus, the powders microparticles and *Capsicum* oleoresin were subjected to Raman analysis with the purpose of obtaining their structure properties. The most important peaks were highlighted in the graphic, as is shown in Figure 2.

Figure 2 (A-G) shows the main peaks of *Capsicum* oleoresin microparticles and *Capsicum* oleoresin acquired in the spectra ranging from 250 to 3000 cm^{-1}. According to Figure 2 (A, B, C, D, F and G) a similar trend among samples was observed, i.e., the same bands were detected in 1006 cm^{-1}($C\text{-}CH_3$ bending), 1159 cm^{-1} (C-C stretching) and 1500 or 1510 cm^{-1} (C=C stretching), characteristic of carotenoids compounds, that also were observed in food products rich in carotenoids (Portarena *et al.* 2018; Carvalho *et al.*, 2019). Microparticles composed by EMCAP: MALT (Figure E) did not present peaks in 1006 cm^{-1} and 1500 cm^{-1}. This spectrum showed an atypical behaviour in relation to other samples pointing a possible interaction between carotenoids and wall materials or oxidation of carotenoids after encapsulation that caused absence of these peaks. In addition, Raman bands at 810 cm^{-1} and 1260 cm^{-1} were found in all samples, which are related to capsaicin's presence. Similar bands in 807 cm^{-1} (C_{39}-C_{37}-C_{43} stretching) and 1264 cm^{-1} (ring stretching) were seen in capsaicin samples by Tian *et al.* (2017). Therefore, Raman technique proved that *Capsicum* oleoresin was encapsulated by different carbohydrates, since the capsaicin was detected in all microparticles spectra.

The visual aspects and micrographs of the microparticles acquired by scanning electron microscopy (SEM) are shown in Figure 3. All the formulations presented agglomerates formation, characteristics behaviour of spray-dried powders. The colour of microparticles' was affected by type of material used. As confirmed by photograph images, MP MALT, EMCAP: MALT and MALT:GA showed a strong orange colour, characteristic of malt color.

Morphological properties evidenced differences among formulations due to the use of distinct wall materials in the formulations. SEM images of microparticles composed by commercial encapsulant agents as MP GA, MP EMCAP, MP GA: EMCAP, showed a smooth surface, with hollows, depressions and without pores, characteristics typical of carbohydrate composed particles obtained by spray drying (Anthero *et al.,* 2020; Luna-Guevara *et al.,* 2017; Gómez-Aldapa *et al.,* 2019; Guadarrama-Lezama *et al.,* 2014). Otherwise, images of MP MALT, MP EMCAP: MALT and MP GA:MALT showed particles with a shape similar to blood cells with wrinkled surfaces and without concavities. These characteristics correspond to modified malt' presence. Moreover, using SEM micrographs, it was possible to estimate the average size of the *Capsicum* oleoresin microparticles to be less than ≈50μm.

Figure 3 Visual aspect and scanning electron microscopy of *Capsicum* oleoresin microparticles.



MP:Microparticles. Gum Arabic (GA), OSA-corn modified starch (EMCAP) and modified malt (MALT).

3.1 Cytotoxicity assessment

The toxicity of natural compounds depends on its concentration and passage through the body (Guldiken *et al.*, 2018). Thus, the toxicity of the bioactive compound must be evaluated to understand its behaviour, especially when the bioactive compound is encapsulated, i.e., covered by distinct wall materials.

The MTS assay evaluated the cytotoxicity of *Capsicum* oleoresin microparticles, non-encapsulated *Capsicum* oleoresin, and isolated wall materials in Caco-2 and HepG2 cells. The use of the two different cell lines can be justified by the function of each one. The Caco-2 is a model of the intestinal barrier in which 4 h of experiment represents the time to substance absorption in the intestine. On the other side, HepG2 is a hepatic cell model in which 72h of substance exposition mimics the time of compound is metabolized in the liver.

Figures 4A-D show the cytotoxicity results for all materials in five different concentrations, ranging from 5 -100 µg/ml against Caco-2 lines. Non-encapsulated *Capsicum* oleoresin, microparticles and isolated wall materials (Figure 4A-D) did not show any significant cytotoxicity effect compared to the negative control. A study of Han, Tian, and Lim (2006) also observed no toxicity effect of capsaicin (100mM) against Caco-2 cells after 4 h exposure.

In relation to cytotoxicity assay with HepG2 cell lines, all treatments were evaluated in concentrations of 5-100 µg/ml (see Figure 5A-D). No cytotoxicity was observed for all treatments after 72 hours of exposure, indicating a negligible general cytotoxicity of the samples in this studied concentration range.

Figure 4 Cytotoxicity of *Capsicum* oleoresin microparticles, non-encapsulated oleoresin, and wall materials against Caco-2 cell lines under 4h of exposure.

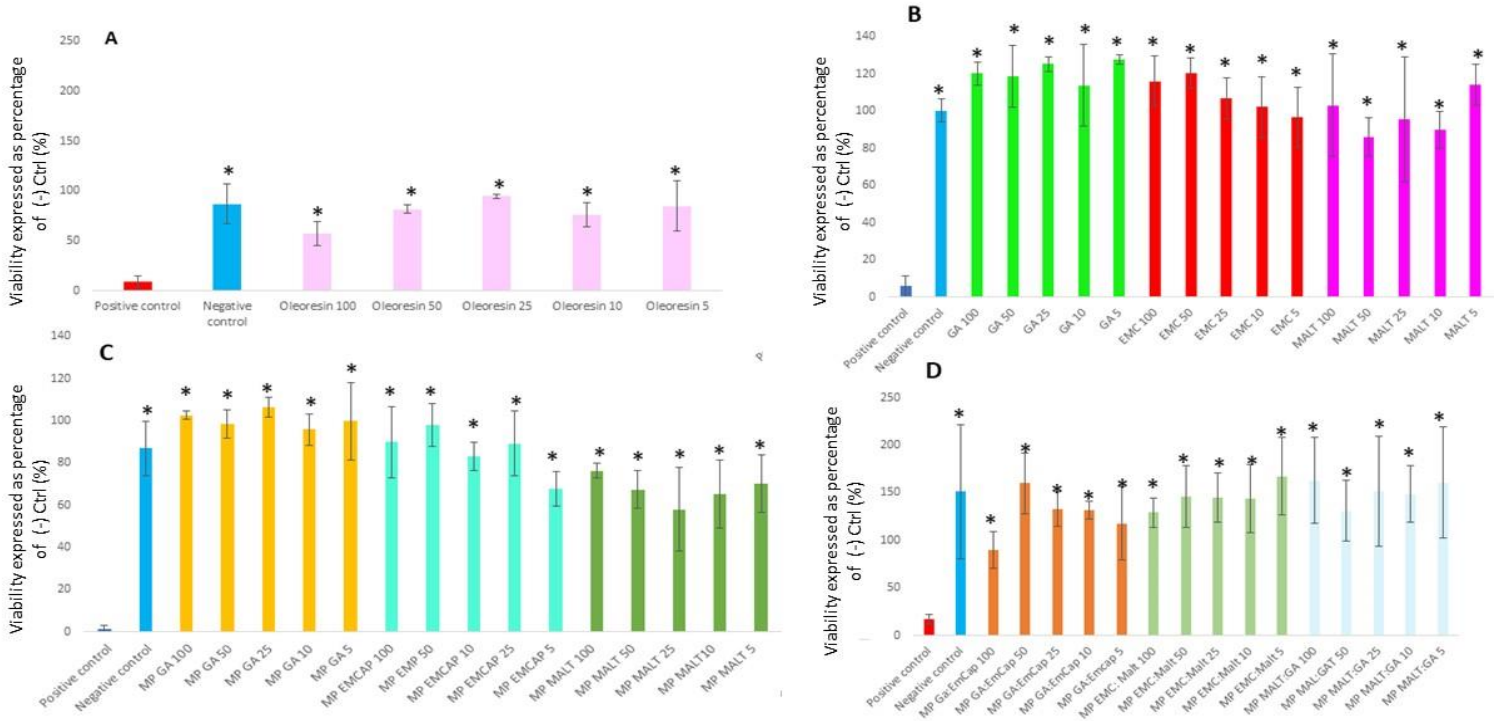


Figure **A** represents Caco-2 toxicity assay for *Capsicum* oleoresin non-encapsulated; **B** represents HepG2 toxicity assay for WM (wall materials) toxicity: Gum Arabic (GA), OSA-corn modified starch (EMCAP) and modified malt (MALT); **C** represents Caco-2 toxicity assay for microparticles of *Capsicum* oleoresin formulated by GA, EMCAP and MALT isolated; **D** represents Caco-2 toxicity assay for microparticles of *Capsicum* oleoresin formulated by combinations of GA:EMCAP; EMCAP:MALT; MALT:GA. All treatments were tested on concentration ranging from 100 to 5 µg/ml. Percentage (%) of MTS converted was compared to untreated control. One-Way ANOVA with Dunnetts's multiple comparison test *P<0.05, *P< 0.01. Each value represents the mean ±SD. n = 3 independent experiments for each concentration and time point with replicates of three. Positive control was Triton X-100™ (0.05%).

Figure 5 Cytotoxicity of *Capsicum* oleoresin microparticles, non-encapsulated oleoresin, and wall materials against HepG2 cell lines under 72h of exposure

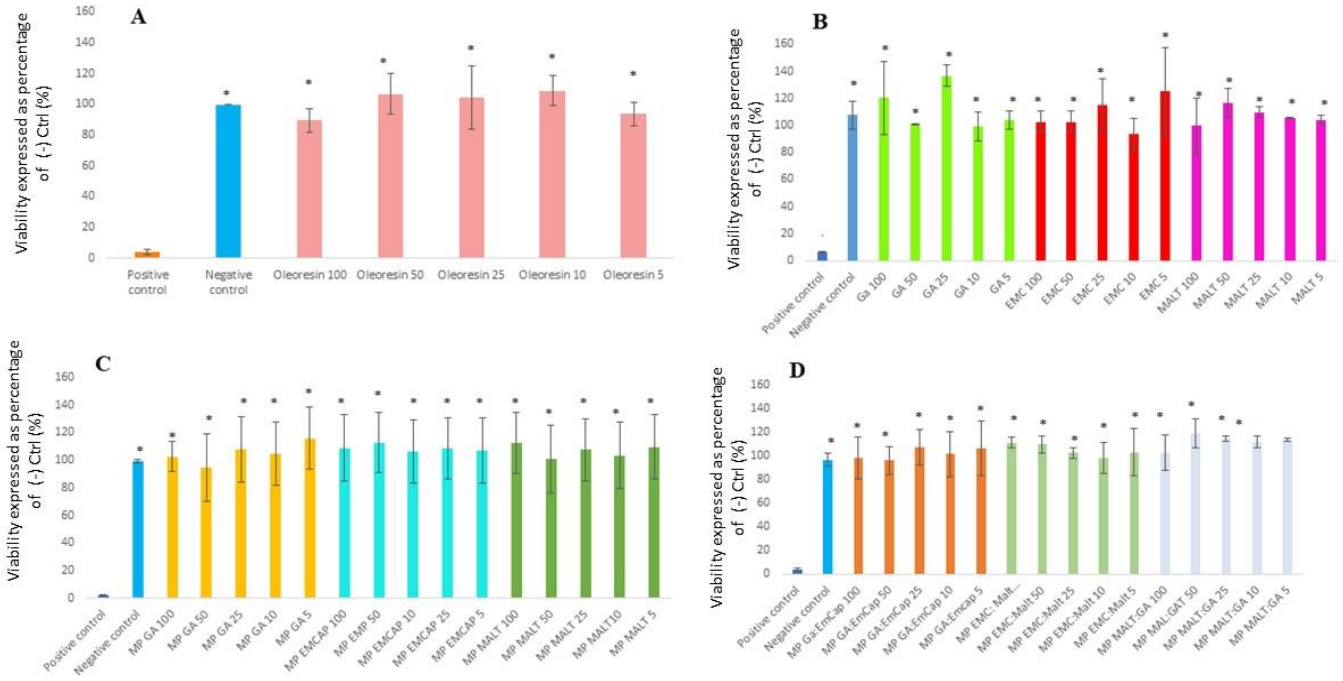


Figure **A** represents HepG2 toxicity assay for *Capsicum* oleoresin non-encapsulated; **B** represents HepG2 toxicity assay for WM (wall materials) toxicity: Gum Arabic (GA), OSA-corn modified starch (EMCAP) and modified malt (MALT); **C** represents HepG2 toxicity assay for microparticles of *Capsicum* oleoresin formulated by GA, EMCAP and MALT isolated; **D** represents HepG2 toxicity assay for microparticles of *Capsicum* oleoresin formulated by combinations of GA: EMCAP; EMCAP:MALT; MALT:GA. All treatments were tested on concentration ranging from 100 to 5 µg/ml. Percentage (%) of MTS converted was compared to untreated control. One-Way ANOVA with Dunnetts' multiple comparison test *P<0.05, *P < 0.01. Each value represents the mean ±SD. n = 3 independent experiments for each concentration and time point with replicates of three. Positive control was Triton X-100™ (0.05%).

3.4 Effect of microencapsulation of Capsicum oleoresin on its color and capsaicin content stability

Oleoresin of *Capsicum* contain various carotenoids such as lutein, beta-carotene, beta-cryptoxanthin, zeaxanthin, violaxanthin, capsanthin and capsorubin which are responsible for its color (Aguiar *et al,* 2022). As these components are prone to oxidation, the stability of compounds present into non-encapsulated and encapsulated *Capsicum* oleoresin was evaluated during the 60 days of stored at 25 and 45°C.

Figure 6 shows color stability of *Capsicum* oleoresin microencapsulated and non-encapsulated. It was observed some differences among formulation regarding luminosity (L*), chromaticity (C*) and variation in Hue angle (h*). During the storage, all microparticles showed a slight increase in luminosity indicanting lighter color. These characteristics could be linked to oxidation of carotenoids (Liu *et al.,* 2019).

Regarding the curve of the microparticles stored at 25 and 45 °C, it was observed that temperature contributed to the acceleration of carotenoids oxidation since the luminosity for all formulations was higher at 45 °C. *Capsicum* oleoresin presented a higher decrease of luminosity when exposed to high temperature. This trend could be associated with the oxidation of the oil, probably causing a browning in the oleoresin.

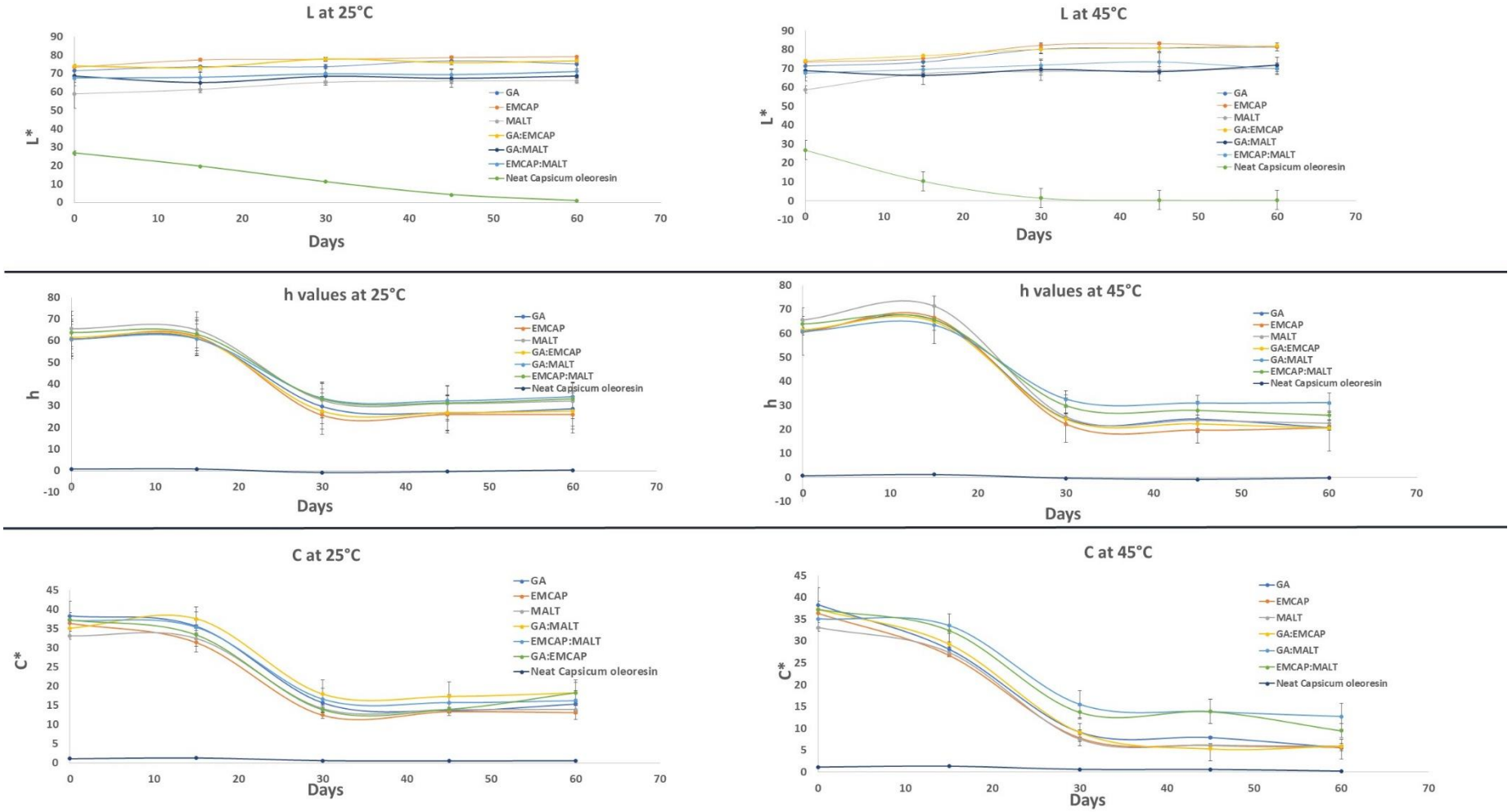
Angle hue (h) and Chroma parameter values indicated a slight decrease for all formulation notably for spray-dried particles stored at 45 °C. The H parameter represents particles with orange color loss and C values shows a reduction of saturation during the stored, i.e., all particles formulation presented orange dark color at 0 day and after 60 days a discoloration was observed.

Additionally, the effect of the wall material on color protection was observed. Particles encapsulated with EMCAP (OSA-modified starch) and EMCAP combined with gum arabic had a higher color loss after 60 days when compared to other formulation. Combinations with modified malt evidenced a better protection for carotenoids compounds. Paprika oleoresin microencapsulated with EMCAP and GA: EMCAP also showed an intense loss of color during 45 days of stored at 35 °C (Anthero *et al.,* 2021).

Non-encapsulated *Capsicum* oleoresin did not show significant loss of color. This was not expected since encapsulation should improve the carotenoids protection. This trend could be explained through difference of materials: *Capsicum* oleoresin microencapsulated and non-encapsulated. Firstly, *Capsicum* oleoresin was not submitted to thermal processing as spray-dried

microparticles. Secondly, spray-dried microparticles usually have pores and surface cracks, and some mass of the encapsulated compound may be deposited into the surface of those particles, where they are exposited to the oxidative action of air (Carneiro *et al.*, 2013). Additionally, microparticles produced by spray drying have a much larger contact surface, which increases susceptibility to oxidation. Similar results were observed by Santos *et al.* (2021) who compared non-encapsulated and encapsulated Tucumã oil during 125 days of storage. The authors also reported that carotenoids in microparticles had a greater loss than carotenoids present into unencapsulated oil.

Figure 6 Color stability of *Capsicum* oleoresin spray-dried particles and neat *Capsicum* oleoresin



Gum Arabic (GA), OSA-corn modified starch (EMCAP) and modified malt (MALT).

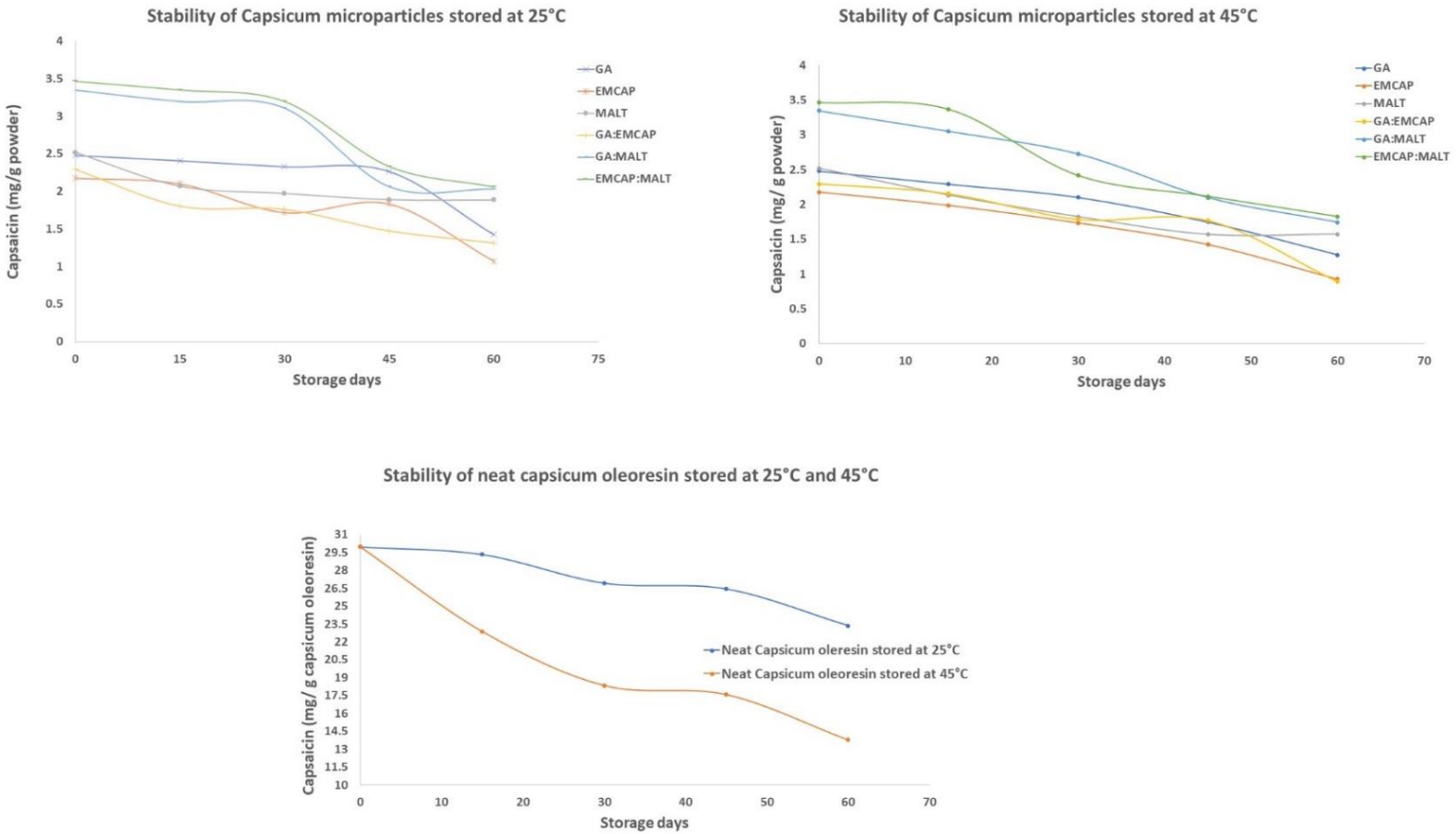
Figure 7 illustrates the kinetic curves for capsaicin degradation during storage. Neat *Capsicum* Oleoresin is more stable than microparticles stored at 25°C, as the data showed a lower capsaicin loss compared to treatments. However, *Capsicum* oleoresin stored at 45°C suffered a decrease of around 54% in capsaicin indicating high temperature sensitivity.

Among all microparticles containing modified malt proved the best choice as encapsulating material. Even presenting rougness in their particle structure, modified malt protected capsaicin compound better than other materials, and then, when this material was combined to industrial materials such as gum arabic and OSA-corn modified starch had an improved in its capsaicin stability. After 60 days of storage, MALT reached a loss of capsaicin over 25% when its storage at 25°C and 37% at 45 °C. Meanwhile, the highest values to capsaicin degradation (61%) were achieved to formulation composed by GA: EMCAP. A possible explanation is that the microparticles containing modified malt possibly are less porous which caused a lower rate of capsaicin oxidation.

Zhang *et al.* (2022) observed a similar trend for capsaicin spray-dried particles stored for 15 days. Capsaicin microencapsulated with whey protein (WP) and starch modified with OSA (OS) showed lower retention values when stored at high temperature (50°C), indicating its susceptibility to heat. They also reported the influence of wall material ratio on retention of capsaicin showing better result for composition particles of WP: OS (7:3). Overall, authors stated that lower retention rate of capsaicin is due to location of capsaicin on surface of particle which resulted in its oxidation.

Once microparticles formulated with modified malt presented better color stability and high capsaicin retention after storage, they can be considered a potential formulation to food applications.

Figure 7 Stability of *Capsicum* oleoresin non-encapsulated and microencapsulated stored at different temperatures



Gum Arabic (GA), OSA-corn modified starch (EMCAP) and modified malt (MALT).

5 Release profile of capsaicin during digestibility assay

Compounds encapsulated by one or more techniques in combination can affect their release by the type and ratio of encapsulating material used pH, enzymatic activity, and salt concentration. Moreover, the microstructure and size of food particles can change in the digestion system. Therefore, this disintegration is relevant for modulating nutrient bio-accessibility and controlling gastric conditions (Chen et al., 2011).

In-vitro digestibility of the spray-dried *Capsicum* oleoresin microparticles was assessed to evaluate the efficacy of carbohydrates solids to protect capsaicin from oral (SSF) and gastric conditions (SGF) and its intestinal phase (SIF). The capsaicin release in the three phases is provided in Figure 8.

The percentage of capsaicin released in the oral phase (SSF) varied from 7.42 to 59.08% all treatments presented a high capsaicin release after 2 min of digestion, except for one formulation, MALT: GA. The low resistance of several of the formulations to the oral phase is likely due to their composition and the high solubility of spray-dried particles. Furthermore, as α-amylase is secreted in the saliva it will begin to act on the α (1,4) bonds in the starch in the initial stages of the digestion, further accelerating degradation (Aguiar & Cazarin Year). Coelho et al. (2022) observed an initially fast released of β-carotene during *in vitro* digestibility justified by presence of many pores on the surface where compounds are adsorbed which facilitating their release (Carneiro et al., 2013). In addition, during this stage can occurs the loss of microparticle wall integrity attributed to swelling mechanisms, that water diffuses from the external solution into the microcapsule matrix causing erosion/rupture of the structure and capsaicin is released by diffusion (Coelho *et al.*, 2022).

Different behavior was observed for MALT, GA, and MALT: GA. Spray-dried microparticles have high water solubility, therefore, a greater release of the compound from the powder structure in the SSF is expected. However, the combination of modified malt and gum Arabic contributed to entrapment of capsaicin into matrix. Modified malt (MALT) presents characteristics of resistant starch, which means a decreased accessibility for the digestive enzymes (Anthero et al., 2021; Aguiar & Cazarin, 2021). As a result, MALT combined with GA reached a release only of 7.42 % of capsaicin in the SSF due to its low soluble and resistance to enzyme

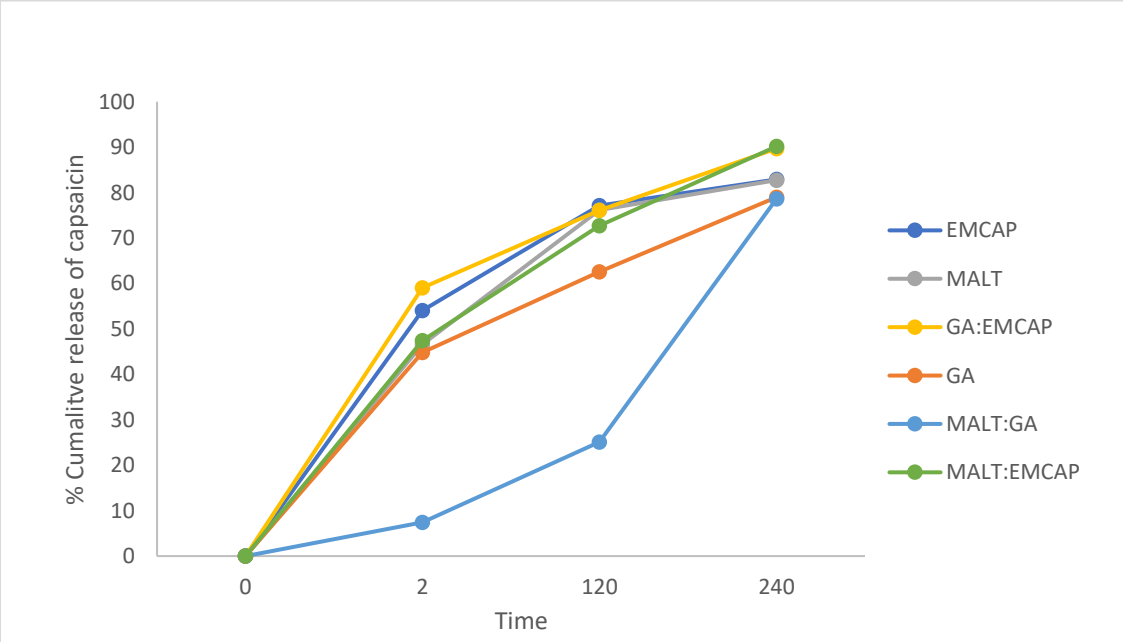
activity, justifying by material characteristics and a possible barrier to enzyme access formed by the interaction of these two-wall material.

Regarding gastric conditions, we highlighted the protection of microparticles caused by these carbohydrates under pH 2 and by the action of pepsin. The gastric condition can protect such phenolic compounds microencapsulated from chemical or enzymatic activity as observed by Silva-Spinoza, García-Martínez & Martínez-Navarrete (2021) and Meena *et al.* (2021). As expected, low capsaicin release from *Capsicum* oleoresin microparticles obtained by different wall materials was observed.

Finally, the intestinal phase was comprised of the action of pH 7, bile salts, and pancreatic enzymes. In particular, the powder structure formed by MALT: GA was unstable in the SIF caused by activity of bile salt and pancreatin that provoke the degradation of wall material solids (Meena et al., 2021) resulting in a high release of capsaicin (53.53%).

Even though all formulations presented a cumative release of capsaicin over 78% after all digestion stages, only MALT: GA was considered a potential formulation to carry capsaicin or other compounds to be absorbed by the intestine.

Figure 8 Cumative release of capsaicin profile under in vitro gastrointestinal conditions



Gum Arabic (GA), OSA-corn modified starch (EMCAP) and modified malt (MALT).

4. Conclusion

In this research, *Capsicum* oleoresin microparticles rich in capsaicin were obtained. All pure wall materials, *Capsicum* oleoresin microparticles and free *Capsicum* oleoresin did not present any toxic affect against intestinal, or liver cells studied. Orange color was degraded after 60 days of storage especially for EMCAP and GA: EMCAP formulation. Capsaicin stability was affected by type of wall material and temperature. Microparticles composed by malt presented lower percentage of capsaicin loss (37%) stored at 45°C when compared to other formulation. Concerning *in vitro* gastrointestinal release, the formulation MALT: GA presented a high capsaicin release in the intestine phase, being an interesting formulation to carry the capsaicin and other compounds until intestine, since these carbohydrates combined are poorly soluble and resistant to amylase hydrolysis.

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CAPÍTULO 7

CAPSICUM OLEORESIN MICROPARTICLES AS POTENTIAL INGREDIENTS FOR HUNGER CONTROL AND OBESITY TREATMENT

Manuscripted prepared to be submitted in Food Research International

CAPSICUM OLEORESIN MICROPARTICLES AS POTENTIAL INGREDIENTS FOR HUNGER CONTROL AND OBESITY TREATMENT

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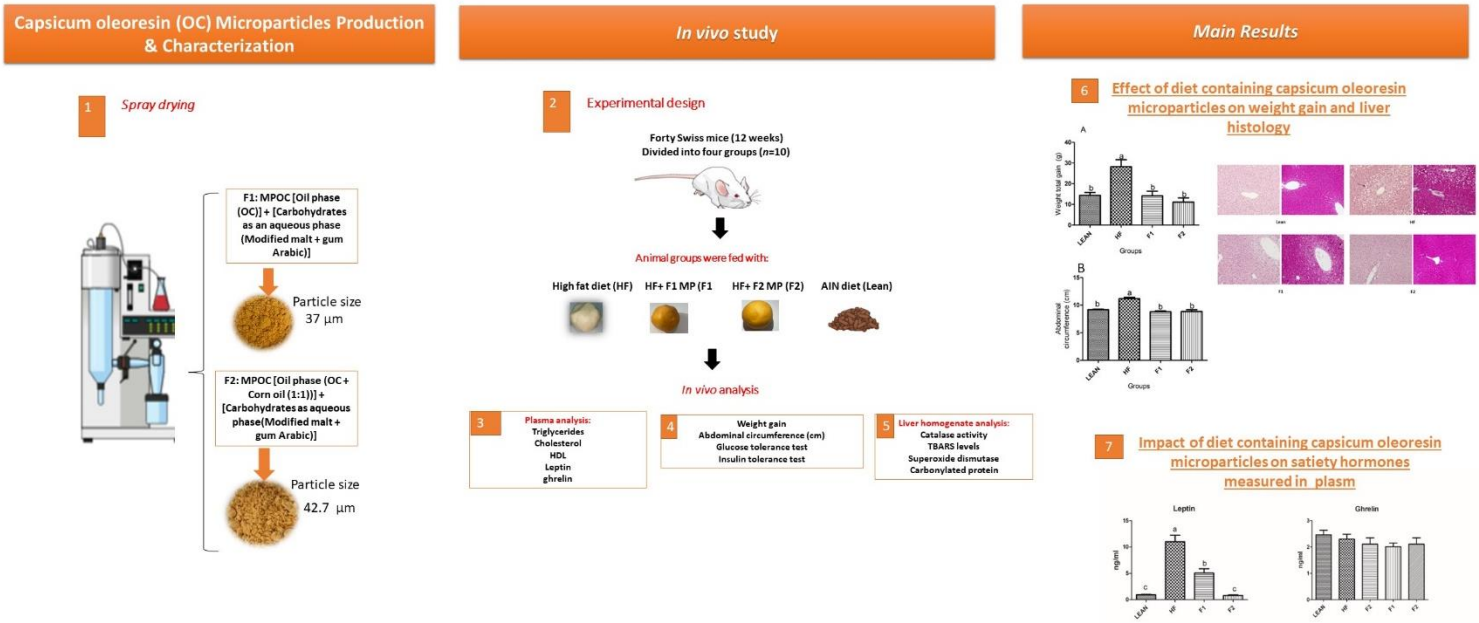
Graphic abstract



Abstract

Capsicum oleoresin is a food flavoring agent and source of capsaicin, a compound responsible for health benefits. This work investigated the effect of *Capsicum* oleoresin (CO) microparticles on glucose tolerance, lipid metabolism and leptin levels in diet-induced obese mice. Gum arabic and modified malt were used as emulsifying agents. Two formulas containing 15% solids were prepared with 5% CO and 95% emulsifier (F1), and the second formula (F2) with 2.5% corn oil plus 2.5% of CO and 95% emulsifier (F2). Rotor-stator homogenized these formulas (5000 rpm/10 min), and they were atomized in the spray dryer. Ultra-Performance Liquid Chromatography determined the capsaicin content for both formulations. Mice were divided into two groups: lean control (normocaloric AIN diet, n=10) and high-fat (HF diet: hypercaloric, n=30), which were subdivided into three subgroups, as follows: HF control diet (n=10), diet F1: HF + 20% CO oleoresin microparticles (n=10) and diet F2: HF + 20% CO microparticles with corn oil (n=10). The experimental groups received a high-fat diet containing 0.0044% (F1) and 0.0028% (F2) of capsaicin daily. The animals treated with the microparticles showed lower glucose levels compared to the HF control. Mice fed with HF-containing CO microparticles showed cholesterol blood levels like the lean group and lower (<100 mg/dl) than the group HF control (150 mg/dl). Leptin levels for mice fed with HF diet plus CO microparticles showed an average of 2-5 ng/ml whereas the fat control group developed leptin resistance. *Capsicum* microparticles evidenced a protective effect against dyslipidemia compared to the fat control group, which suggests its use as a potential ingredient to inducer satiety and obesity control.

Keywords: *Capsicum* oleoresin, spray-drying, leptin, satiety, obesity.

1. Introduction

It is well known that some processed food categories are high in salt, fat, and sugar. Therefore, depending on the frequency and amount ingested, they can increase the incidence of obesity, a high-risk factor for severe diseases such as type 2 diabetes mellitus, hypertension, and nonalcoholic fatty liver disease. In this way, food science researchers are looking for ways to overcome these drawbacks by developing food and supplements as potential agents to prevent obesity development (Song et al., 2017; Liu et al., 2019; Hanse et al., 2021).

One way to reduce the risk of obesity development is through hunger control. Satiety is characterized by the combination of sensory, gastric, and physiological signals, which stimulate the central nervous system to inhibit food intake. In this way, the modulation of the hormones leptin and ghrelin could be an alternative to control body weight gain. These hormones are released in the plasma when digestion begins and act directly on the hypothalamic center, controlling food ingestion (Mela; Boland, 2014; Hetherington, 2016; Zanchi et al., 2017). However, there is evidence in the literature that the ingestion of some substances, like capsaicin, can modulate the release of these hormones, thus preventing weight gain in experimental models (Janssens, Margriet, Westerterp-Plantenga, 2014; Kim et al., 2014; Baboota et al., 2018; Anthero et al., 2022). Also, capsaicinoids were associated with two mechanisms that could explain hypoglycemia in experimental models: a decrease in blood glucose and increased insulin levels and a decrease in glucose absorption in the ileum (Zhang et al., 2018). Furthermore, it is described in the literature other controlling mechanisms associated with *Capsicum* and its derivatives, such as capsaicin and capsaicinoids, that comprise cell cycle arrest, thermogenesis activation, and inhibition of lipogenesis (Sarkar & Thirumurugan, 2019). However, these compounds are known for high pungency and low solubility in water, making their intake difficult, as their bioaccessibility and application in food (Berke & Shieh, 2012; Wu et al., 2022).

In this sense, microencapsulation techniques are widely used to make the application and ingestion of these compounds more accessible. Once these techniques can mask the taste, increase solubility, improve the delivery of compounds to the intestine and increase the bioaccessibility of bioactive compounds (Kim et al., 2014; Aguiar et al., 2022; Wu et al., 2022). For instance, encapsulated *Capsicum* oleoresin is a source of capsaicin and a functional additive for savory foods (Akbas, Soyler & Oztop, 2019; Zhou et al., 2020; Aguiar et al., 2021; Anthero et al., 2022).

A previous work verified that *Capsicum* oleoresin spray-dried particles present potential action against hepatic steatosis and promote weight loss in mice (Anthero et al., 2022); however, however, the mechanisms underlying these effects were not fully investigated. For this reason, the present study focused at evaluating several parameters related to obesity and hepatic steatosis in mice that received a high fat diet supplemented with CO microparticles obtained by spray drying. This investigation included the assessment of ghrelin and leptin levels, glucose homeostasis, blood, and liver lipid profile, as well as liver histology and oxidative stress parameters.

2. Material and method

2.1 Material

The gum arabic was purchased from Nexira (São Paulo, Brazil). Modified malt (MALT) with ultrasound and stearic acid was obtained in previous work by Anthero et al. (2021). *Capsicum* oleoresin was bought from Synthite Industries Ltd. (Kerala, India). The capsaicin standard was acquired from Cayman Chemical (Ann Arbor, USA, Purity > 95%), and corn oil (Liza, Cargill, Campinas, Brazil) was purchased from a local supermarket. All other reagents, like chemicals and solvents, were of analytical grade.

2.2 Production of Capsicum oleoresin microparticles

Capsicum oleoresin emulsions were formulated with gum arabic and modified malt as emulsifying agents. Two formulas were prepared to contain 15% solids, one consisting of 5% *Capsicum* oleoresin and 95% emulsifier (F1), whereas F2 contained 2.5% corn oil plus 2.5% of *Capsicum* oleoresin and 95% emulsifier (F2). Dispersions composed of aqueous (95%) and oil phase (5%) were homogenized by rotor-stator (L5M-A Laboratory Mixer, Chesham, Buckinghamshire, UK) at 5000 rpm for 10 min. Emulsions (500 g) were atomized in the Mini Spray Dryer B-290 atomizer (Switzerland 290, Büchi, Flawil), using a 0.7 mm double liquid nozzle. The drying air flow was 35 m³/h with an inlet temperature of 160 ºC. The compressor air pressure was maintained at 0.06 MPa with an emulsion feed flow of 15 mL/min and an outlet temperature varied from 75 °C to 78 °C.

2.3 Microparticles characterization

Moisture and water activity: Capsicum oleoresin powders were subjected to an infrared balance to analyze moisture content (Shimadzu, model Moc63u, Kyoto, Japan). The water activity (a_w) was determined by Aqualab model 3TE digital hygrometer (Decagon, Pullman, USA) at 25 °C.

Particle size: Capsicum oleoresin microparticle size was determined by the light scattering technique in the Mastersizer 2000 instrument (Malvern Instruments Ltda, Malvern, UK) using SIROCCO optical unit with ethanol solvent dispersion. The mean diameter of the microparticles was expressed as the volumetric mean diameter $D_{[4,3]}$ and polydispersity index (Span).

Capsaicin quantification: The capsaicin content in microparticle formulations F1 and F2 was determined by High-Performance Liquid Chromatography (HPLC) following the same methodology described in our previous work (Anthero et al., 2022).

2.4 Experimental procedure

The experimental protocol of the present study was submitted and approved by the Ethics Committee in the Use of Animals of the State University of Maringá (CEUA/UEM nº 3305020621). This experiment followed institutional ethics and the Brazilian National Council for Animal Experimentation Control (CONCEA). Forty days old male Swiss mice remained during the three months referring to the experimental period in an acclimatized room (controlled temperature 22-25ºC), with free access to diet and water, with a light/dark cycle of 12 h. The animals were maintained for an adaptation period of 1 week on a commercial diet. After this period, the animals were divided into two groups: lean control (Lean) (norm caloric AIN diet, n=10) and high-fat (HF diet: hypercaloric, n=30), which were subdivided into three subgroups, as follows: HF control diet (n=10), diet F1: HF + 20% oleoresin microparticles (n=10) and diet F2: HF + 20% oleoresin microparticles with corn oil (n=10). The high-fat subgroups received a high-fat diet of AIN 93G-modified/added lard (35% lipids, 31% lard + 4% soybean oil). In contrast, the lean group consumed the AIN 93G diet recommended by the American Institute of Nutrition, as described in the article by Reeves (1997). The diets and their compositions are presented in **Table 1**. During the 12 weeks of the experiment, body weight gain was monitored once a week, and food consumption three times a week, obtaining a monthly intake mean.

Table 1. Diet formulation and intake of capsaicin by animal groups.

Groups	*Microparticles composition	Diet composition	Intake of capsaicin per day by animal (mg/day)
Lean		AIN 93G (Reeves, 1997).	-
High-fat (HF)		High-fat: AIN 93G-modified to contain 35% lipids (31% lard and 4% soybean oil)	-
F1	100 g of emulsion containing: 15% total solid (95% is emulsifier and 5% is *Capsicum* oleoresin)	High-fat diet containing 20% of *Capsicum* oleoresin microparticles composed by MALT:GA	2.65
F2	100 g of emulsion containing: 15% total solid (95% is emulsifier: GA:MALT, 2.5% *Capsicum* oleoresin, 2.5% corn oil)	High-fat diet containing 20% of *Capsicum* oleoresin microparticles composed by MALT:GA with corn oil	1.22

*GA:MALT: emulsifier materials in a ration of 1:1; GA (gum arabic), MALT (modified malt

2.5 Liver oxidative stress parameters

Two grams of freezed-clamped liver tissue were homogenized in a Dounce homogenizer with a ratio of 1:10 of sample: phosphate buffer (0.1 M, pH 7.4) at 4°C to assess oxidative stress parameters. The protein concentrations in the homogenate were determined using bovine serum albumin as a standard (Lowry, 1951).

a) Liver Lipid Peroxidation (TBARS) and Protein Carbonylation

The lipoperoxide levels in liver homogenate were measured using the thiobarbituric acid reactive substance (TBARS) (Buege & Aust, 1978). Then, the lipoperoxide level in the total homogenate was calculated using a standard curve constructed with 1,1',3,3'-tetra ethoxy-propane, and the results were expressed in nmol MDA equivalents. (mg. protein) $^{-1}$.

The levels of protein carbonyl groups were quantified using the spectrophotometer at 370 nm using the 2,4-dinitrophenylhydrazine. Results were calculated using the molar extinction coefficient (ε) of 2.20×10^4 M^{-1} cm^{-1} and expressed in nmol (mg. protein) $^{-1}$ (Levine et al., 1990).

b) Reactive oxygen species (ROS)

The ROS levels were determined in the supernatant of liver homogenate through the reaction with the 2'-7'-dichlorofluorescein-diacetate (DCFH-DA) (Siqueira et al., 2005). The formation of oxidized dichlorofluorescein (DCF) was measured in a spectrofluorimeter (excitation at 504 and emission at 529 nm). A standard curve was constructed with oxidized dichlorofluorescein, expressing the results in µmol. $min^{-1}mg^{-1}$).

c) Catalase and superoxide dismutase activity

Antioxidant enzyme activities were measured in the supernatant of liver homogenates. Catalase (CAT) activity was evaluated by measuring the transformation of hydrogen peroxide (H_2O_2) at 240 nm and expressed as µmol. min^{-1}. (mg. protein) $^{-1}$. Superoxide dismutase (SOD) activity was determined by its ability to inhibit pyrogallol autooxidation in an alkaline medium. This phenomenon was monitored spectrophotometrically at 420 nm (Marklund & Marklund, 1974). One superoxide dismutase unit (U) comprises the amount of enzyme responsible for 50% inhibition of pyrogallol autoxidation. Thus, SOD was expressed in $U.mg^{-1}$.

2.5 Determination of liver and plasma lipid content

The total lipid content of the liver was determined by gravimetry, and the lipid contents were expressed in percentage terms (g/100 g wet liver weight) following the methodology of Folch et al. (1957). Hepatic total cholesterol and triglycerides (TAG) were determined after Triton (2%) fat suspension, followed by vortexing and heating to 55°C for 24h. Liver lipid content was measured using standard assay kits (Laborlab®, Guarulhos, SP).

Blood samples were collected from the cava vein and then centrifuged (1000g at 4º C) for plasma separation. Plasma total cholesterol, high-density lipoprotein (HDL-cholesterol), and TAG were analyzed by standard methods using Laborlab® assay kits.

2.6 Assessment of Glucose and insulin tolerance test

The oral glucose tolerance test (OGTT) was assessed after 6 hours of fasting. Blood glucose was measured at 0, 5, 15, 30, 45, and 60 min after oral administration of glucose solution (1.5 mg/g body weight). The insulin tolerant test (ITT) was assessed after 8 hours of fasting, and blood glucose was measured after administration of human insulin (1 U/kg of body weight, human insulin, *Novolin®* 100 U/ml) via intraperitoneal at 0, 5, 10, and 15 min. The blood glucose of the mice in both tests was monitored by a blood sample collected through the tail vein using a Free Lite G-Tech® glucometer (Infopia Co., Ltd; South Korea). The tests were performed one week apart.

2.7 Satiety signals by quantification of ghrelin and leptin

The levels of leptin (Catalog No. E-EL-M0551) and ghrelin (Catalog No. E-EL-M0551) in the plasma samples were determined using ELISA kits (Elabscience®, Texas, United States) and following the manufacturer's instructions.

2.8 Liver histological assay

Histology assays were performed on liver samples to evaluate hepatic steatosis and morphological changes. In turn, the samples were fixed for 24 hours at room temperature (24ºC) in 10% buffered formalin and then immersed in ethanol (70%). The slices were cut into 5 μm-thick sections and stained with hematoxylin and eosin to visualize the morphology of the

hepatocytes, as previously described by Flister et al. (2018). The NAFLD activity score (NAS) was applied by a semiquantitative analysis of the three defined NASH criteria described by Kleiner et al. (2005): steatosis (0–3), ballooning (0–3) and lobular inflammation (0–2) using a Leica Microscope DMI 4000 B (Switzerland).

2.9 Statistical analysis

All results were performed at least in sextuplicated and subjected to analysis of variance (ANOVA) using the GraphPrism version 5 software. Tukey's test determined significant differences (p-value <0.05) among animal groups.

3. Results and discussion

3.1 Characterization of Capsicum oleoresin microparticles

Capsicum oleoresin (CO) microparticles were analyzed regarding their physicochemical properties. Results for moisture, water activity, particle size, and capsaicin content are shown in **Table 2**. Microparticles with only CO (F1) or CO plus corn oil (F2) did not show any difference for moisture and water activity (a_w). For moisture and water activity, F1 and F2 reached maximum values of 5.83 ± 0.51 g water per 100 g powder and a_w of 0.128 ± 0.013, respectively, properties typical of particles obtained by spray drying. All formulations showed $D_{[4,3]}$ values higher than 37 μm and a high span mean. Previous work also observed these characteristics using the same wall materials (Anthero et al., 2022). In this case, particle size was influenced by biopolymers combination such as gum arabic and modified malt, which modified malt was probably caused by the agglomeration of particles (Guo et al., 2021). The capsaicin content ranged from 3.54 ± 0.467 to 1.63 ± 0.378 (mg capsaicin/g powder), showing that CO F2 microparticles have about 54% less capsaicin than F1, an expected result since F2 has half as much CO assembly as F1.

Table 2. Physicochemical properties of *Capsicum* oleoresin microparticles

Microparticles	Moisture (g water/ 100 g powder)	Water activity (a_w)	Particle size		Capsaicin content (mg capsaicin/ g powder)
			D $_{[4,3]}$ (µm)	Span	
F1	5.83 ± 0.51	0.128 ± 0.013	37.0 ± 5.7	7.31 ± 0.778	3.54 ± 0.467
F2	5.08 ± 0.32	0.105 ± 0.016	42.7 ± 8.5	4.84 ± 0.557	1.63 ± 0.378

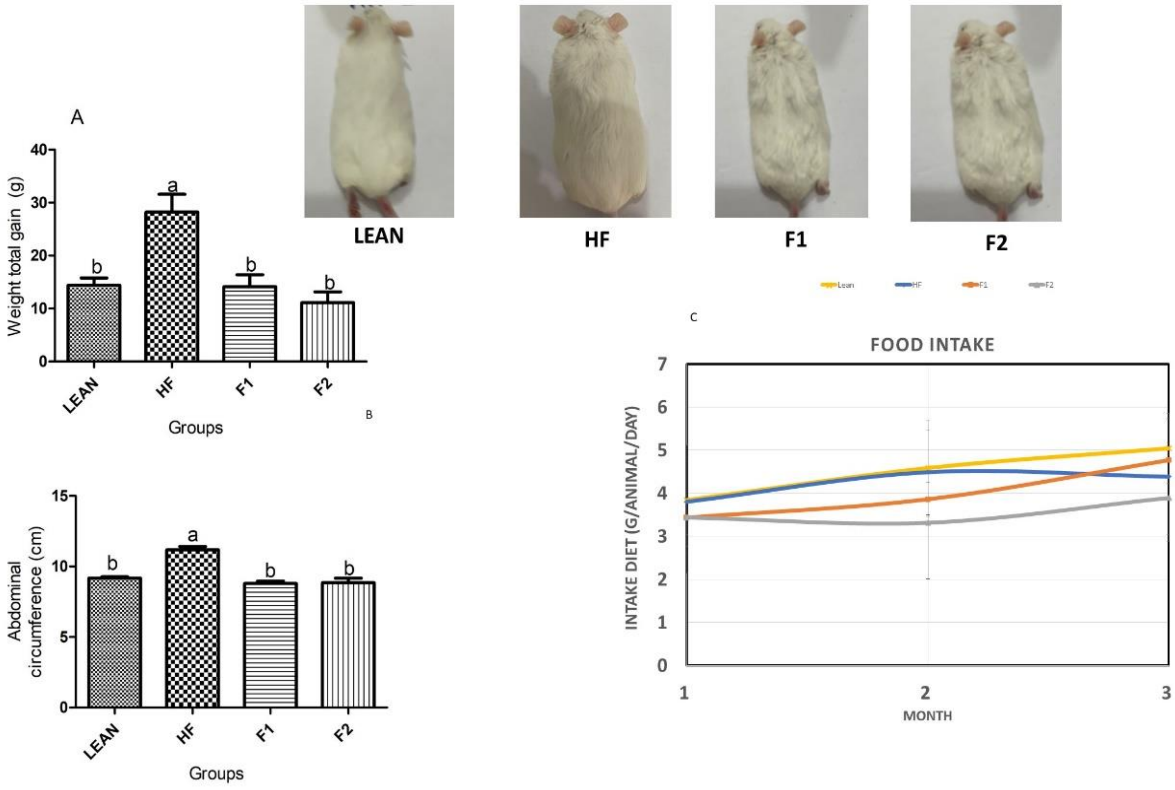
Results are expressed in mean ± standard deviation (SD). One-way ANOVA followed by Tukey (comparison between microparticles formulation); different letters indicate statistical difference ($p < 0.05$). Abbreviations: F1 (*Capsicum* oleoresin microparticle containing GA:MALT (gum arabic and modified malt) and 5% *Capsicum* oleoresin); F2 *Capsicum* oleoresin microparticle containing GA:MALT and 2.5% *Capsicum* oleoresin, 2.5% corn oil.

3.2 Effect of Capsicum oleoresin microparticles added in high-fat diet to prevent obesity

Figure 1 shows the appearance of the animal after intake for three months high-fat diet. Mice fed with only high-fat showed to be bigger than animals from groups fed with lean or high-fat diets containing *Capsicum* oleoresin microparticles (F1 and F2). Obesity damage was visualized in the liver from the HF group.

So, mice fed with a high-fat diet successfully achieved obesity. Animals fed with only a high-fat diet gained around 26 g after three months of the experiment. Diets supplemented with *Capsicum* oleoresin (CO) microparticles (F1 and F2) promoted a lower weight gain for the animal. Its results were like the Lean group, even ingesting around 500 kJ/100 g more than them. Also, all HF groups showed similar calorie intake; however, the animals fed with F1 and F2 diets showed a lower abdominal circumference measure. These results suggest a protective effect of oleoresin *Capsicum* intake against weight gain.

Figure 1 Weight total gain, abdominal circumference, and consumption diet



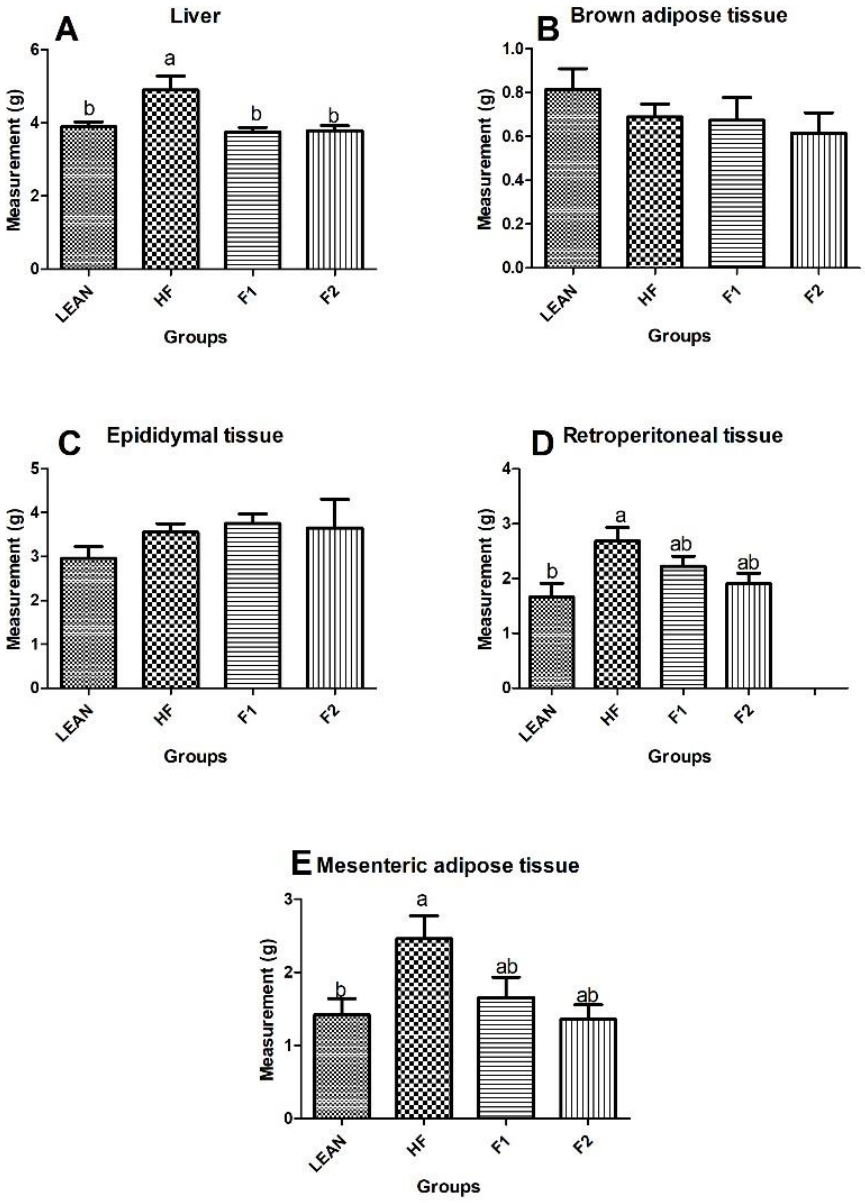
All column bars are expressed in mean ± standard deviation (SD). One-way ANOVA followed by Tukey (comparison between animal groups); different letters indicate statistical difference ($p < 0.05$). Abbreviations: LEAN: AIN 93G diet (Reeves, 1993); HF: High-fat diet; F1: High-fat diet containing *Capsicum* oleoresin microparticle composed by GA: MALT (gum arabic and modified malt) and 5% *Capsicum* oleoresin; F2: High-fat diet containing *Capsicum* oleoresin microparticle composed by GA: MALT and 2.5% *Capsicum* oleoresin, 2.5% corn oil.

3.3 Tissue and deposits of fat of mice fed with high-fat diet supplemented with Capsicum oleoresin microencapsulated

Brown tissue contributes to the increased thermogenic action of fat, whereas white tissue is responsible for storing lipids and producing pro-inflammatory cytokines (Chen et al., 2022; Wang et al., 2022). Thus, an increase in white tissue indicates inflammation influenced by obesity. According to **Figure 2B** the Lean group had more brown tissue than the other groups fed a high-fat diet; however, there is not a significant difference among them. **Figure 2 C, D, and E** represent values for white tissue accumulated in the animals. In this case, we can see that the high-fat group (HF) presented significantly more accumulation of retroperitoneal and mesenteric adipose tissue than the lean group ($p<0.05$) caused by the effect of high lipid content in the diet. Also, although

F1 and F2 presented differences in capsaicin content, results did not show any difference between these groups regarding white adipose tissue measurements. Moreover, excluding the epididymal adipose tissue, we can observe that the consumption of diets F1 and F2 showed a tendency to decrease the accumulation of retroperitoneal and mesenteric fat similar to that observed in lean animals.

Figure 2 Measurements of relative tissue weight: (A) liver, (B) Brown adipose tissue, (C) Epididymal tissue, (D) Retroperitoneal tissue and (D) Mesenteric adipose tissue.



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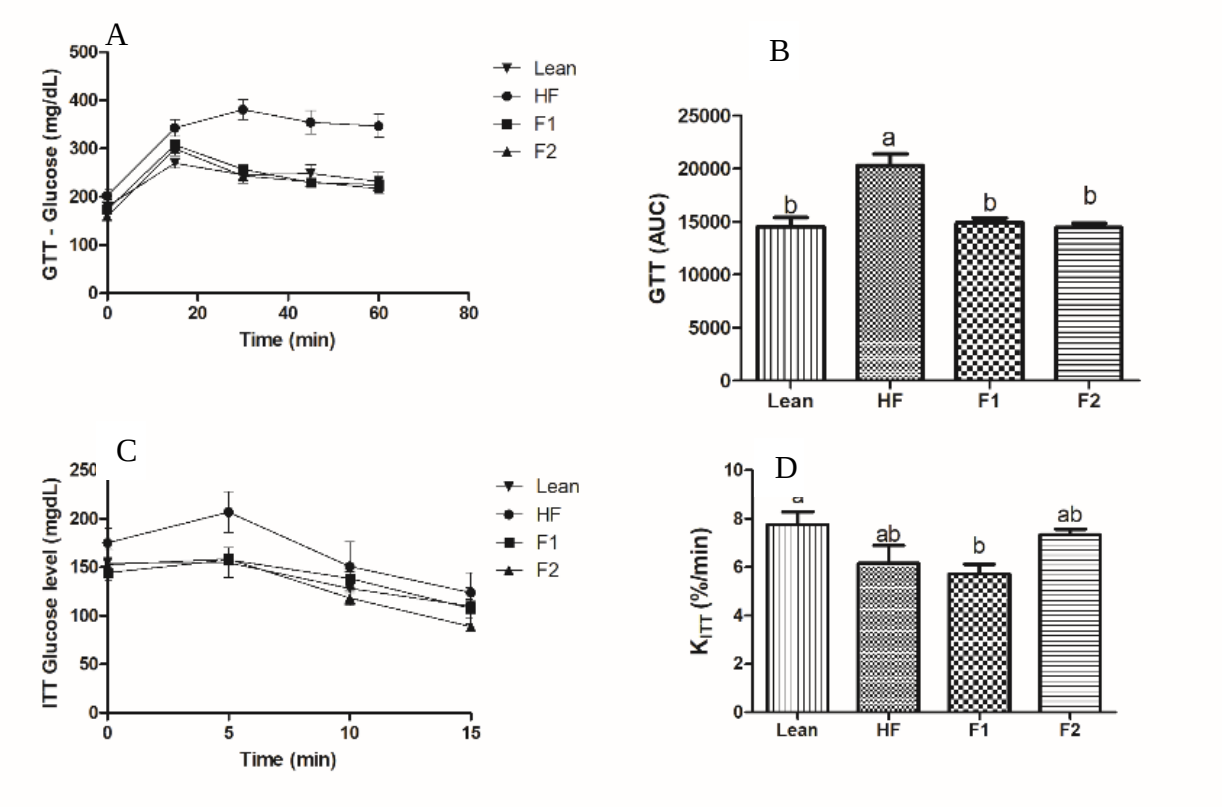
3.4 Influence of Capsicum oleoresin microparticles on oral glucose and insulin tolerance, and leptin and ghrelin hormones

The oral glucose tolerance (OGTT) and insulin tolerance (ITT) tests were performed to evaluate the effect of *Capsicum* oleoresin microparticles added to the high-fat diet on glucose homeostasis and protection against the development of insulin resistance. **Figure 3A** evidence that there was no significant difference in basal blood glucose (time 0 min) between treatments ($p < 0.05$), and in all experimental groups, the blood glucose peak occurred 30 minutes after the oral administration intraperitoneal injection of glucose solution. However, after 60 min, it was possible to see that the group of animals that received the HF diet had more resistance to normalizing the glycemia; on the other hand, animals fed with F1 and F2 diets showed the same profile as the Lean group. Furthermore, the area under the curve (**Figure 3B**) displays a bigger area for the HF group evidencing the development of glucose intolerance in the model. However, there was no difference between the experimental groups in the insulin tolerance test (ITT); we can see that the HF group compared to the Lean group, shows a tendency to decrease its responsivity to the insulin, suggesting a beginning of an insulin intolerance development. Nonetheless, the F2 diet intake seems to protect the animals from developing insulin resistance since the response of this group was like the Lean group. This finding was consistent with a study by Kang et al. (2010), which also reported that mice fed a high-fat diet for ten weeks supplemented with 0.0015% capsaicin had a reduction in obesity-induced glucose intolerance as well as reduced body weight.

Some mechanisms involved in this protective effect have been described in the literature. Firstly, capsaicin in microparticles can inhibit the α-amylase and α-glucosidase activity, which reduces sugar absorption (Sarkar & Thirumuruga, 2019). Secondly, capsaicin can stimulate the secretion of GLP-1 by increasing the concentration of short-chain fatty acids in the intestine, stabilizing the blood glucose in obese mice (Song et al., 2017).

Figure 3 Glycemic curve for 60 min after intraperitoneal injection of glucose solution (A), area under the curve during GTT (B) and glucose response (C) and glucose decay rate (D) in the insulin tolerance test. KITT calculated from the blood glucose collected in the insulin tolerance test.



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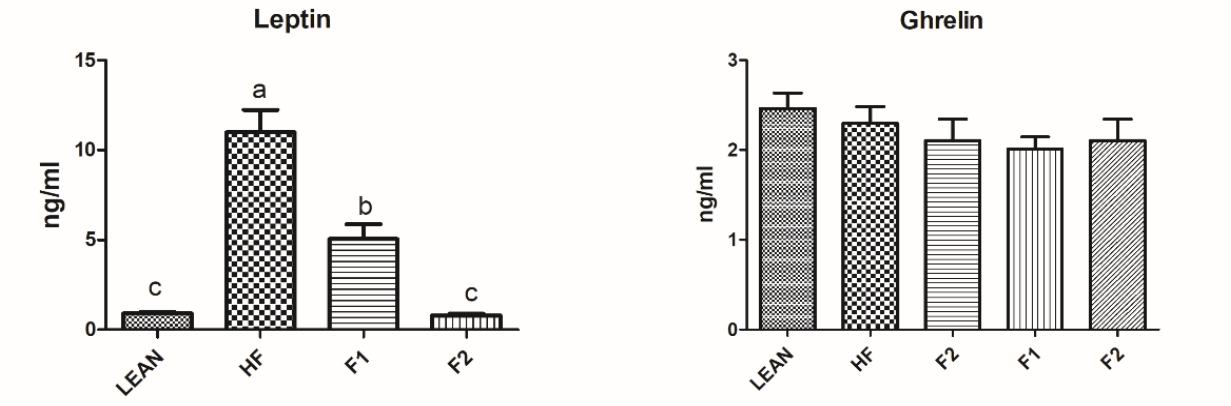
Regarding hormones involved in appetite regulation, *Leptin* and *ghrelin* were determined. *Ghrelin* is a peptide produced in stomach cells and is related to short-term energy balance. *Leptin* is a type of adipokine that plays a key role in regulating energy intake and expenditure, being responsible for appetite suppression, and obese individuals tend to be resistant to the action of this hormone (Mela et al., 2014; Mukherjee et al., 2015).

As shown in **Figure 4** the ghrelin levels did not change significantly among groups receiving different diets. The hyperlipidemic diet did not affect the levels of this hormone, and the *Capsicum* oleoresin microparticles did not promote any change in it.

On the other hand, plasma leptin levels were different ($p<0.05$) among the groups. The high-fat group showed a high leptin content, wherea treated groups showed low values approaching the lean control. This finding clarifies the action of these diets regarding leptin

resistance and the function of controlling appetite, thermogenic and anti-obesity action (Feng et al., 2014; Mukherjee et al., 2015). Similarly, a clinical study showed that the ingestion of 0.9 g red pepper (0.25% capsaicin) promoted satiety and reduced energy and fat intake, being considered an anti-obesity agent (Westerterp-Plantenga et al., 2005).

Figure 4 Leptin and ghrelin levels in the plasma of mice



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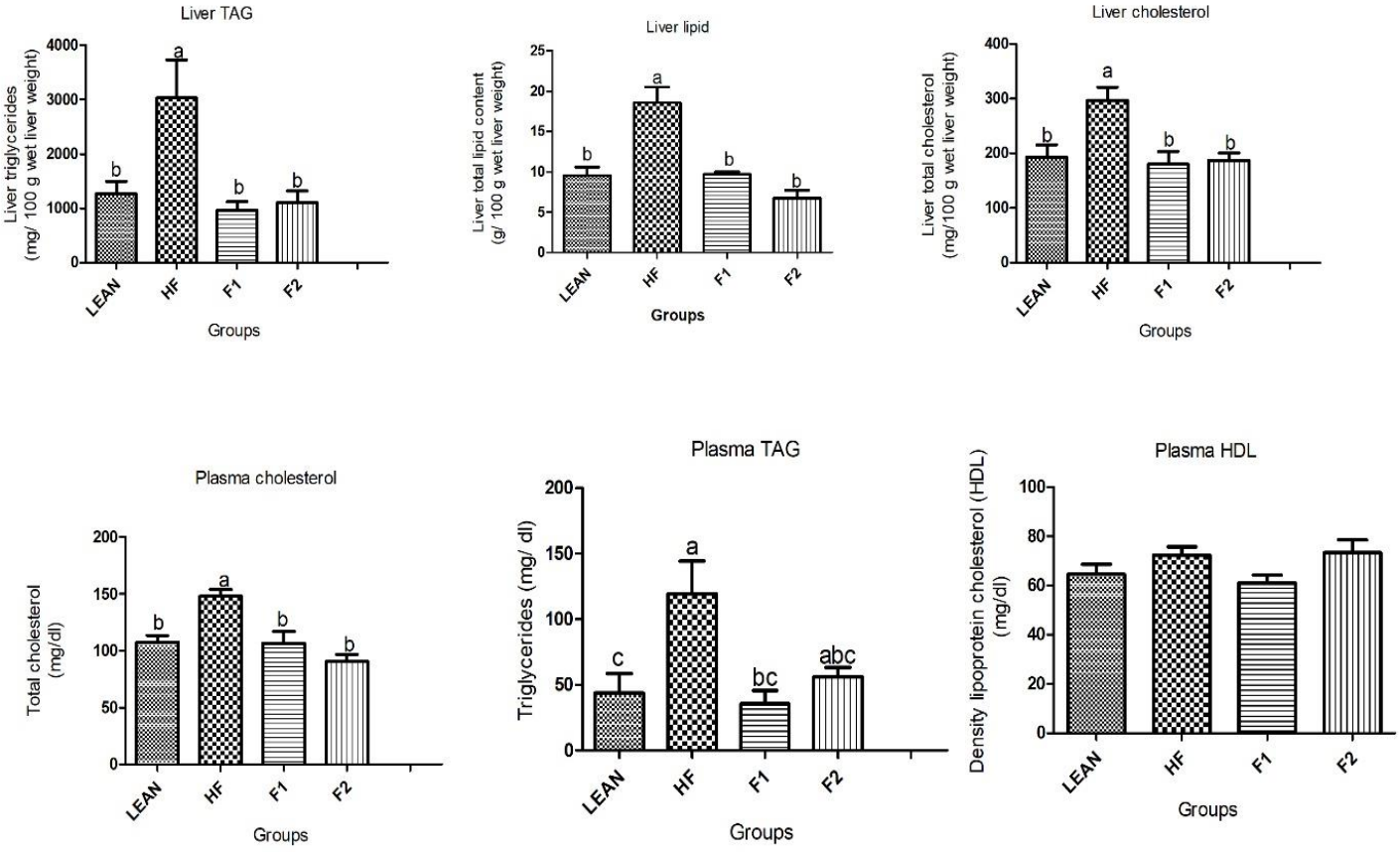
3.5 Lipids concentration on liver and plasm

The HF group showed the highest liver weight compared to the other groups, which can be explained by the accumulation of total lipids, cholesterol, and triglycerides in the liver as are shown in **Figure 5.** The development of hepatic steatosis is a common outcome associated with a high-fat diet intake, which increases the risk of liver failure and cirrhosis.

The consumption of the diets with oleoresin microparticles (F1 and F2), on the other hand, showed a lower lipid content, triacylglycerols, and cholesterol in the liver compared to the HF group. In the same way, the treated groups showed a similar serum lipid profile to the Lean group. Interestingly, a study with Mongolian squirrels observed that *Capsicum* oleoresin reduced blood triglyceride and cholesterol levels by 66% and 70%, respectively. The hypothesis raised by the

authors was related to a decrease in intestinal absorption of exogenous cholesterol, impacting enterohepatic circulation after feeding the animals with a diet rich in cholesterol (1%) and pepper (1%) for 12 weeks (Gupta; Dixit; Dobhal, 2002). The higher fecal excretion of bile acids leads the liver to use endogenous cholesterol to synthesize new bile acids, decreasing cholesterol in circulation.

Figure 5 Lipid profile in plasma and liver of mice



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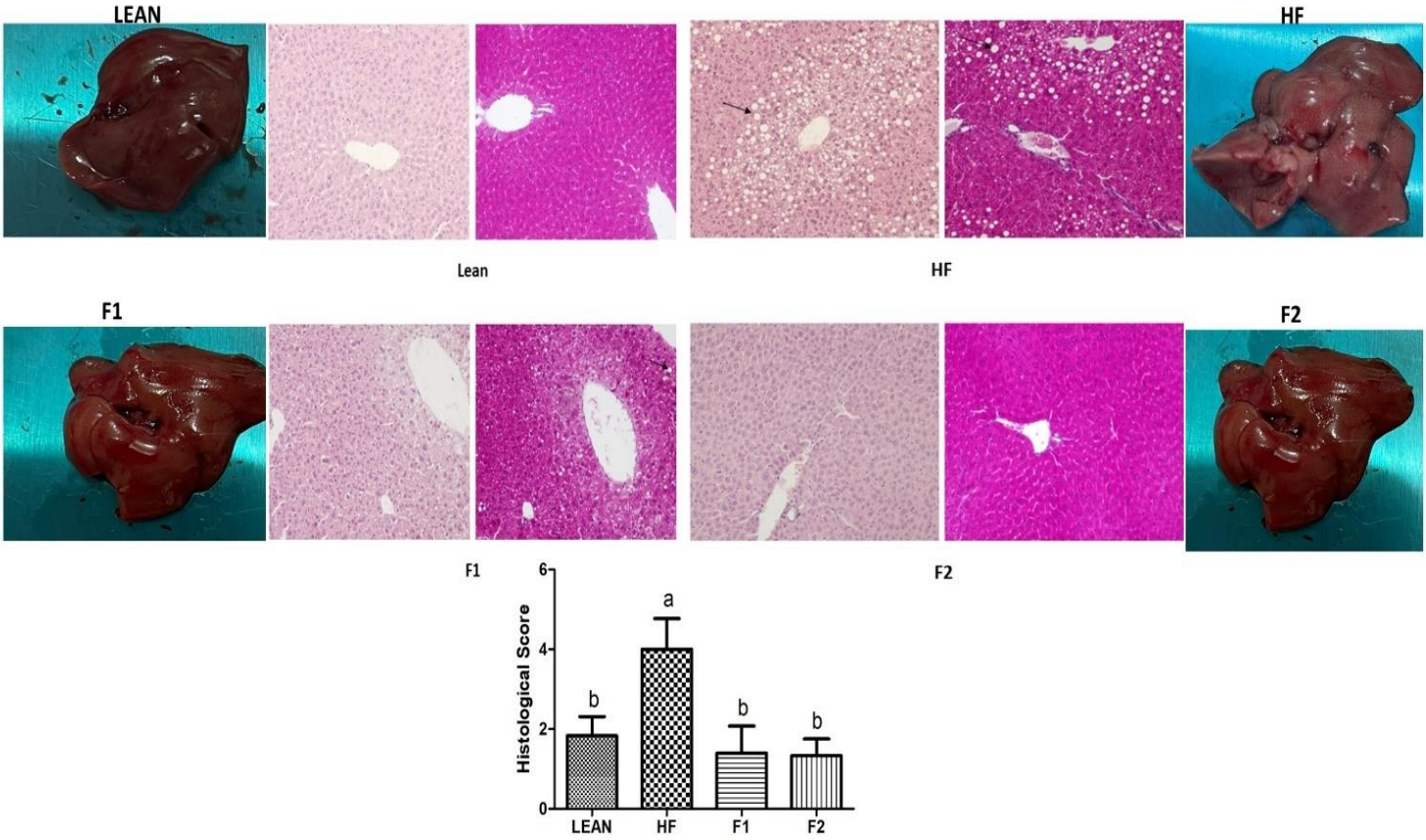
3.6 Histology analysis

The results in **Figure 5** for lipid profile in the liver agree with histologic analysis (**Figure 6A**) of the liver since the control of the high-fat group shows severe hepatic steatosis. Also, it is possible to observe in **Figure 6** that *Capsicum* oleoresin added to the animals' diet promoted protection and a lower fat accumulation in the hepatocytes, agreeing with previous studies (Shin, Yang & Han, 2020; Anthero et al., 2022; Lee et al., 2022). The histological score shows that the HF group presented higher values for steatosis, ballooning, and lobular inflammation ($p< 0.05$) corroborating with damages observed in images of HF liver. This outcome shows a promising protective effect caused by *Capsicum* oleoresin microparticles against fat accumulation, especially because steatosis can progress to cirrhosis, causing loss of liver function. The mechanisms by which the consumption of F1 and F2 diets promoted less fat accumulation in the liver of animals need to be better investigated and elucidated; however, there are indications in the literature that capsaicin and other capsaicinoids present in *Capsicum* can modulate the inflammatory responses contributing to fatty acid oxidation in the liver due to thermogenic action (Li, Yang & Lu, 2019, Shin, Yang & Han, 2020).

Figure 6 Histologic analysis of liver



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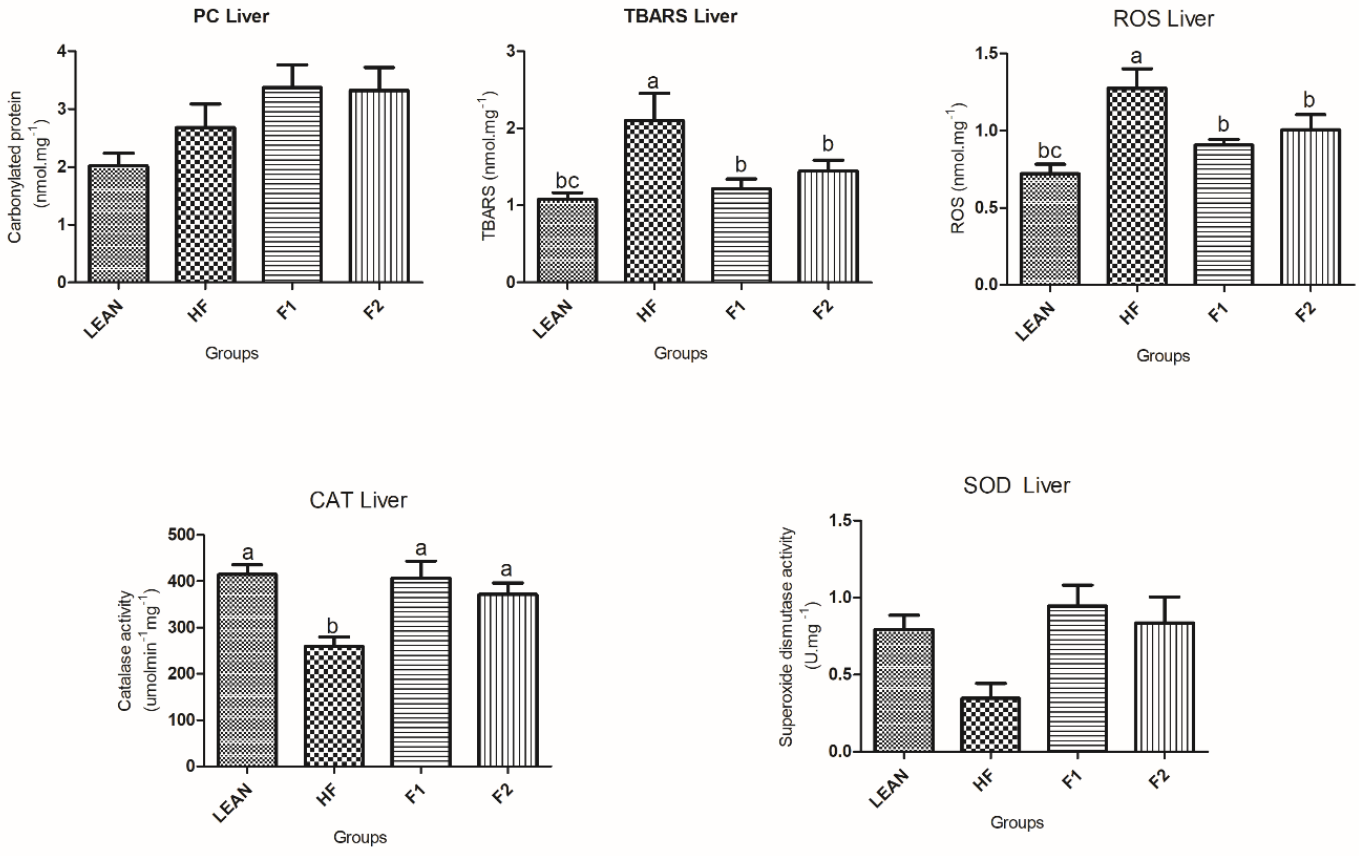
3.7 Oxidative stress

Liver analyses were performed to investigate the oxidative stress caused by the intake of a high-fat diet. First, two antioxidant enzymes' activity were measured, namely, superoxide dismutase (SOD) and catalase (CAT), as shown in **Figure 7 A-B**. The higher the antioxidant activity of the enzymes means less damage caused to the liver by the high-fat diet. It can be noted that the ingestion of an HF diet greatly decreased the activities of both enzymes, especially CAT, compared to the Lean group. The consumption of the high-fat diet supplemented with *Capsicum*

oleoresin microparticles (F1 and F2) prevented the decrease in CAT activity and tended to prevent the decrease in SOD activity, although statistical significance was lacking for the latter.

The results of liver oxidative stress markers such as protein carbonyls, reactive oxygen species (ROS) and lipid peroxidation (TBARS), are shown in **Figure 7C-E**. Administration of the HF diet increased TBARS and ROS levels, although no differences were observed for protein carbonylation. These results evidence that there was oxidative damage in the liver of the HF group, which can result in DNA inactivation or mutation, protein alterations, and oxidation of polyunsaturated fatty acids in cell membranes (Yang et al., 2019). The supplementation with CO microparticles (F1 and F2) attenuated all the modifications induced by the HF diet.

Figure 7 Oxidative stress analysis: (A) Catalase activity-CAT, (B), superoxide dismutase activity-SOD, (C) Carbonyl protein -PC, (D) Lipid peroxidation by reactive substance to the thiobarbituric acid-TBARS, (E) Oxigen-reactive species levels (ROS).



All column bars are expressed in mean ± standard deviation (SD). One-way ANOVA followed by Tukey (comparison between animal groups); different letters indicate statistical difference ($p < 0.05$). Abbreviations: LEAN: AIN 93G diet (Reeves, 1997); HF: High-fat diet; F1: High-fat diet containing *Capsicum* oleoresin microparticle composed by GA: MALT (gum arabic and modified malt) and 5% *Capsicum* oleoresin; F2: High-fat diet containing *Capsicum* oleoresin microparticle composed by GA: MALT and 2.5% *Capsicum* oleoresin, 2.5% corn oil.

4. Conclusion

In conclusion, the diets containing *Capsicum* oleoresin prevented mice from a disorder caused by obesity. Animals fed 2.65 mg (F1) and 1.22 mg (F1) of capsaicin per day had liver protection against hepatic steatosis, decreased triglycerides and cholesterol levels in the liver and plasma, also reduced oxidative stress, and prevented weighted gain. The beneficial effect of dietary *Capsicum* oleoresin microparticles on glucose tolerance was also observed. Furthermore, groups treated with a high-fat diet plus microparticles did not develop leptin resistance, as observed for HF control. Our results suggest that the intake of microparticles with only 5% *Capsicum* oleoresin is a helpful supplement for reducing obesity disorders and improving leptin sensitivity.

Conflict of interests

No conflict of interest to be declared by the authors.

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CAPÍTULO 8

DISCUSSÃO GERAL

8.1 DISCUSSÃO GERAL

O objetivo principal desta tese foi desenvolver um novo material de parede à base de malte em combinação com a goma arábica e amido de milho modificado (OSA) para encapsular a oleoresina de pimenta por *spray dring*. Além disso, o trabalho teve o propósito de avaliar as propriedades físico-químicas, morfológicas, térmicas e funcionais das micropartículas de pimenta através de estudos *in vitro* e *in vivo*. A motivação desta pesquisa foi associada ao escasso número de estudos avaliando diferentes carboidratos como agentes encapsulantes de oleoresinas e a aplicação das partículas de *spray drying* ricas em pimenta em dieta hiper-lípidica para avaliar seus benefícios na saúde.

A oleoresina de pimenta também conhecida como *Capsicum* é rica em compostos bioativos tendo como principal grupo os capsaicinóides. Dentre os capsaicinóides, a capsaicina é um componente de interesse em muitos grupos de pesquisa devido aos efeitos termogênicos, redução do tecido adiposo, controle nos hormônios relacionados à saciedade e melhora no metabolismo lípidico (ZHANG *et al.*, 2018; LI, YANG & LU, 2019; YANG *et al*., 2019).

A microencapsulação da oleoresina de pimenta foi proposta devido ao seu alto teor de capsaicina, baixa solubilidade em água, e à alta pungência o que pode causar irritação na pele ao ser manipulada sem proteção. A produção de micropartículas de pimenta rica em capsaicina foi realizada com baixa concentração de oleoresina e alta concentração de materiais de parede. Os carboidratos foram selecionados para esta finalidade por apresentarem baixo custo, não alergênicos, disponíveis e comumente aplicados na formação de partículas por *spray drying* (ZHU, ET AL. 2017).

O amido de milho esterificado com octenil succínico e a goma arábica são carboidratos amplamente utilizados na indústria de alimentos, eles não são alergênicos e apresentam boas propriedades emulsificantes, tornando-os excelentes materiais de parede na produção de micropartículas de *spray drying*. Apesar disso, poucos estudos utilizaram esses carboidratos para encapsularem oleoresina de pimenta. Contudo, o projeto teve o propósito de comparar materiais comerciais comumentes aplicados na indústria e comparar com um novo agente encapsulante ainda não explorado.

Nesse sentido, na primeira o malte de cevada foi escolhido para ser utilizado como novo material encapsulante por ser rico em amido, fibras, compostos bioativos e por apresentar

capacidade antioxidante. Alguns estudos já aplicaram o amido do malte de cevada e a fibra do malte de cevada como agentes encapsulantes de compostos bioativos e obtiveram alta eficiência de encapsulação (JEON ET AL., 2003; SALGADO, RODRÍGUES-ROJO, COCERO, 2017). No entanto, como o malte apresenta baixa solubilidade em água e baixa capacidade emulsificante até o momento nenhum estudo aplicou esse cereal como agente encapsulante.

Como a utilização do malte na forma nativa como material encapsulante é limitada a sua modificação foi proposta. Para isso, a modificação físico-quimica do malte de cevada foi realizada utilizando o ultrassom com diferentes densidades energéticas (33,8, 60, 72,5, e 107,5 MJ/m^3) em combinação com o acido esteárico (2% m/m) resultando em quatro diferentes maltes. O efeito das diferentes densidades energéticas sobre as propriedades físico-químicas e microestrutura dos materiais obtidos foi investigado. Primeiramente, foi determinado o grau de substituição dos maltes, para avaliar se houve esterificação, em seguida foi quantificado o teor de amilose, dextrose equivalente e teor de proteína. As técnicas de FTIR, DR-X e MEV provaram que a combinação dos dois métodos foi eficiente na mudança estrutural e química do malte. Esse resultado é suportado por outros estudos, que também constataram que a energia do ultrassom é eficiente na quebra das moléculas de amido e exposição das moléculas, favorecendo dessa forma, as reações de substituições no grânulo de amido (ZHU ET AL., 2014; MONROY, RIVERO, GARCÍA, 2018; SILVA ET AL., 2022).

Posteriormente a caracterização, os quatros materiais foram utilizados no preparo de emulsões óleo em água, para avaliar assim, suas propriedades funcionais como material emulsificante. Utilizando um homogeneizador de alta velocidade, dispersões aquosas contendo os quatro maltes obtidos foram dispersos em óleo de canola, que foi escolhido como óleo modelo. Teste avaliando concentração de sólidos totais (10, 15 e 20%) da emulsão foi realizado e formulações contento 15% de sólidos (75% de malte modificado e 25% de óleo) foi selecionada devido aos melhores resultados de estabilidade obtidos. Além disso, uma emulsão contendo o malte nativo foi produzida como controle, mas ela sofreu separação de fase instantaneamente após o processo de homogeneização, impossibilitando posteriores análises de caracterização. As emulsões produzidas com os maltes modificados foram caracterizadas quanto à estabilidade cinética (0,1,2,3,4,5,6 e 24 h), tamanho e distribuição de tamanho, morfologia, comportamento reológico e cor. Resultados mostraram que o aumento das densidades de energia teve diferentes efeitos nas propriedades do malte devido a um aumento no grau de substituição do grupo éster,

acréscimo no teor de amilose e aumento significativo nos valores dextrose equivalente. Em suma, o processo de ultrassom em combinação com ácido esteárico aumentou a solubilidade do malte e sua temperatura de gelatinização, causando mudanças na cristalinidade do amido e na região amorfa, que por sua vez, contribuiu na formação do malte com boas propriedades emulsificantes e estabilizantes para emulsões óleo em água.

Na sequência, como os maltes obtidos por diferentes densidades energéticas não apresentaram diferença significativa quanto suas propriedades emulsificantes, o malte modificado com apenas 25% de amplitude de ultrassom (em relação a potência nominal 700W), ou com 33,8 MJ/m^3, foi selecionado para formular emulsões contendo oleoresina de pimenta junto aos materiais comerciais, como a goma arábica e o amido de milho modificado com OSA (EMCAP). O uso dos materiais isolados e combinados foi motivado pelo interesse em investigar possíveis interações entre os biopolímeros, uma vez que quando os bipolímeros são combinados eles podem aumentar a estabilidade da emulsão e dessa forma, melhorar a eficiência de encapsulação (FATHI, MARTIN, McCLEMENTS, 2014). O sinergismo entre GA: MALT e EMCAP: MALT foi capaz de reduzir os valores de tensão interfacial (<8,7 mN/m) possibilitando a formação de emulsões cineticamente estáveis (Índice de Estabilidade Turbiscan <4,4). Demais estudos apresentaram resultados que mostram que o aumento do grau de substituição também aumenta a estabilidade da emulsão (ARSHAD, ALI e HASNAIN, 2018; GARCÍA-TEJEDA ET AL., 2018)

Todas as emulsões foram atomizadas para obtenção das micropartículas em pó, e como resultado, foram obtidas micropartículas com diferentes características físico-químicas, térmicas e morfológicas, sendo estas inerentes às propriedades de cada material de parede. Durante a caracterização das micropartículas, a eficiência de encapsulação de capsaicina foi realizada de duas maneiras, a primeira lavando cerca de 1g de micropartículas em metanol através de um filtro de papel para remover a capsaicina superficial. O permeado foi submetido à análise no HPLC (Cromotagrafia Líquida de Alta Eficiência). A quantidade total de capsaicina foi obtida através da homogeneização de 1g de micropartículas com metanol mais água (75:25). O total encapsulado foi obtido pela diferença entre o teor total capsaicina e o teor superficial. No entanto, os valores obtidos para o teor superficial foram acima de 100%, o que levou a autora apresentar apenas os dados de teor de capsaicina total nas micropartículas. Assim, os resultados de teor superficial foram superiores à 100% foi atribuído ao teor de água presente no solvente metanol que em conjunto com as concavidades presente nas micropartículas de *spray drying* causou a lixiviação

da capsaicina e solubilização parcial das micropartículas. O mesmo problema foi observado por Consoli et al. (2019) na determinação da eficiência de encapsulação do resveratrol por s*pray drying*. Os autores apresentaram valores superiores à 100% de eficiência de encapsulação.

Outro objetivo do estudo foi testar o efeito das micropartículas compostas por malte modificado sobre o efeito metabólico de camundongos, a formulação goma arábica mais malte modificado foi selecionada para ser adicionada na dieta hiper-lípidica devido ao teor de capsaicina na micropartícula e por apresentar maior rendimento de pó após a atomização.

O primeiro ensaio animal foi conduzido com o propósito de verificar se os animais consumiriam a dieta hiper-lípidica adicionada de micropartículas, e se a referida dieta causaria algum efeito adverso após 28 dias de consumo. Assim, para essa investigação, dietas hiper-lípidicas foram formuladas e adicionadas de micropartículas de oleoresina de pimenta contendo GA e MALTE e a segunda contendo 50% da oleoresina de pimenta e 50% de óleo de milho (OM) (GA:MALTE:OM). O propósito da adição do óleo de milho foi associado à sua estrutura, uma vez que este óleo apresenta uma maior concentração de ácidos graxos poliinsaturados. Esses ácidos graxos cotribui na formação de micelas resultando em uma melhor biodisponibilidade da capsaicina. As duas formulações de micropartículas foram adicionadas em concentrações diferentes na dieta (10 e 20%). Como esperado, as micropartículas tiveram efeito positivo em relação a redução do ganho de peso em comparação ao grupo controle gordo e nenhum efeito tóxico foi observado. O mais interessante desse resultado foi que as micropartículas suplementadas na dieta apresentaram efeito dose-dependente.

A digestibilidade das micropartículas e a toxicidade *in vitro* foram investigadas. Os dados apresentados mostraram que as formulações tiveram impacto na liberação da capsaicina nos fluidos digestivos simulados da fase oral, gástrica e intestinal. A dificuldade de comparar os resultados de digestibilidade com a literatura foi associada à falta de estudos que mostram o comportamento das partículas de *spray-drying* na fase oral, uma vez que estas micropartículas são facilmente solubilizadas, logo o composto é liberado da estrutura da partícula. Um outro gargalo deste estudo foi a quantificação de capsaicina presente nos fluidos digestivos, uma vez que o tempo de retenção da capsaicina no equipamento de cromatografia líquida de alta eficiência mostrou um deslocamento no pico em relação à curva de calibração, que pode ser atribuído à presença de sais nas amostras de digestão. Para contornar tal situação, foi construída uma curva de calibração da

capsaicina diluída em soluções tampão, contendo concentrações de sais utilizados no processo de digestão. Esse procedimento contribuiu para a obtenção de dados mais confiáveis.

Quanto à toxicidade, foram investigadas diferentes concentrações de microparticles 5 - 100 µg de micropartículas ou oleoresin/ ml de Dimetilsulfóxido (DMSO), as quais foram limitadas ao máximo de partículas solubilizadas no solvente DMSO. Alguns estudos mostraram que as partículas contendo capsaicina poderiam apresentar alguma toxicidade às células, mas que esta toxicidade está relacionada à sua concentração (HALME ET AL., 2016). O tempo de exposição das células intestinais e hepáticas foram determinados em relação ao tempo que a subtância permanece em cada fase após a ingestão. Como a oleoresina estava em baixa concentração nas micropartículas e protegida pela microencapsulação as formulações não apresentaram toxicidade nos ensaios *in vitro*. Os gráficos de toxicidade apresentados no artigo "*Capsicum* Oleoresin Loaded Spray-Dried Microparticles: Stability, Toxicological Study, and *in vitro* Digestibility" evidenciaram que dentro das concentrações de dose de oleoresina testada (5-100 µg/mL), apenas a concentração de oleoresina de pimenta acima de 100 µg/m podem afetar negativamente a viabilidade celular.

Em etapa posterior, foi realizado um outro ensaio animal com o intuito de avaliar o efeito da dieta suplementada com micropartículas de oleoresina de pimenta sobre o metabolismo de camundongos. Para este estudo foram selecionadas as duas dietas que tiveram melhor desempenho no controle de peso e no metabolismo hepático dos animais. O estudo inicial tinha o propósito de suplementar as dietas com alto teor de gordura (dieta rica em gordura com aspecto mais mole) e as dietas com teor normal de gordura, conhecida também como dieta magra (aspecto de ração seca) e comparar entre os grupos. No entanto, os animais não consumiram a dieta magra, não aceitando essa dieta devido à pungência mais acentuada dessa ração seca. Como no preparo dessa ração vai água, diferente da dieta rica em gordura, houve possivelmente a liberação da capsaicina deixando assim a dieta mais "picante" o que levou à rejeição pelos animais.

Cabe ressaltar que após três meses de tratamento os animais que consumiram as dietas *high-fat* adicionadas de micropartículas de pimenta não apresentaram nenhuma irritação ou anomalia no estômago e/ou intestino.

Poucos estudos na literatura incorporaram partículas de *spray drying* em dietas normal ou rica em gordura, o que dificultou realizar comparações. No entanto, o estudou mostrou que as micropartículas ricas em pimenta quando adicionadas à dieta hipercalórica podem contribuir na

melhora da sensibilidade à insulina resistência à glicose, atenuação dos efeitos da obesidade quanto ao estresse oxidativo, acúmulo de gordura no fígado e na liberação de hormônios de saciedade.

Nesse sentido, como as micropartículas de *spray* podem ser consideravelmente solúveis em água, o presente estudo sugere a aplicação dessas formulações em produtos à base lipídica, ou na produção de cápsulas para serem liberadas no intestino.

CAPÍTULO 9

CONCLUSÃO GERAL E SUGESTÕES PARA TRABALHOS FUTUROS

9.1 CONCLUSÃO GERAL

Como foi já mencionado no primeiro capítulo deste trabalho, a motivação do estudo foi associada às características específicas da oleoresina de pimenta, como sua elevada pungência e insolubilidade em água. O malte modificado por ultrassom em combinação com ácido esteárico apresentou propriedades emulsificantes na estabilização da emulsão utilizando o óleo modelo de canola e a também a oleoresina de pimenta.

A combinação do malte modificado com os biopolímeros industriais como o amido de milho esterificado com ácido succínico e a goma arábica melhorou as propriedades emulsificantes da emulsão da oleoresina de pimenta em água, resultado atribuído à redução da tensão interfacial causada pela sinergia desses materiais.

As emulsões após a atomização formaram micropartículas ricas em capsaicina e suas propriedades físico-químicas foram influenciadas pelas propriedades de cada material de parede utilizado. Assim como observado na formação de emulsão, as partículas em pó apresentaram diferentes tonalidades em relação à cor, e estabilidade de capsaicina durante o armazenamento, sendo a formulação malte modificado mais goma arábica considerada a mais estável na proteção da capsaicina armazenada nas temperaturas de 25 e 45°C.

Nenhum efeito tóxico das micropartículas de oleoresina de pimenta compostas por MALT:GA com ou sem óleo de milho foi observado no fígado dos animais, resultado que corroborou com o segundo estudo, de toxicidade *in vitro*. O referido estudou mostrou que as micropartículas, materiais de parede e oleoresina de pimenta dentro da concentração estudada (5-100 µg/mL) não reduziram de forma significativa a viabilidade das células intestinais e hepáticas.

A digestibilidade *in vitro* da oleoresina microencapsulada mostrou que todas as formulações foram rapidamente solubilizadas na fase oral, exceto a formulação composta pelo malte e goma arábica. O malte modificado quando combinado com a goma arábica apresentou uma liberação controlada e mais acentuada na fase intestinal. Assim, devido à estabilidade apresentada, a referida formulação pode ser considerada a melhor formulação para entrega da capsaicina para ser absorvida no intestino.

O efeito benéfico das micropartículas de pimenta foi claramente mostrado nos dois experimentos utilizando animais, suportando os estudos que afirmavam que a pimenta e seus

derivados apresentam efeito positivo na redução da gordura abdominal, a qual pode estar associada ao efeito de saciedade e controle glicêmico. Os nossos resultados provaram que a oleoresina de pimenta mesmo em baixa concentração na micropartícula pode proteger o fígado dos danos causados pela obesidade como a esteatose hepática. O consumo durante os três meses de tratamento evidenciou que o ganho de peso para os grupos que consomem dieta rica em gordura suplementada com fonte de capsaicina foi notavelmente inferior quando comparado ao grupo controle gordo. As dietas suplementadas com micropartícula de oleoresina de pimenta contribuíram no controle dos níveis de leptina dos animais que consumiram dieta hipercalórica e melhoraram o metabolismo dos lipídios no plasma e no fígado.

Devido a necessidade do desenvolvimento de formulações alimentícias naturais as micropartículas de oleoresina de pimenta são ingredientes promissores a serem investigados para o controle do apetite e obesidade.

9.2 SUGESTÕES DE TRABALHOS FUTUROS

Os resultados apresentados nessa tese são promissores, o que leva a sugerir mais estudos em continuação para obtenção de novos e melhores resultados.

- Estudar diferentes métodos de microencapsulação utilizado a oleoresina de pimenta como material ativo e os carboidratos como materiais encapsulantes;
- Aumentar a concentração de oleoresina de pimenta nas formulações das emulsões e avaliar o impacto nas propriedades das emulsões e das micropartículas;
- Desenvolver alimentos, como produtos cárneos, massas e molhos com adição de micropartículas de oleoresina de pimenta;
- Avaliar a estabilidade da capsaicina presente nas micropartículas quando adicionadas em alimentos, a fim de observar se há alguma interação entre o produto e o material encapsulado.

CAPÍTULO 10

REFERÊNCIAS GERAIS

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APÊNDICES

Apêndice I: Atividades Gerais e Divulgação dos Resultados

Artigos completos publicados em periódicos

1 Comunian, Talita A.; **da Silva Anthero**, Ana Gabriela, Bezerra, Eveling Oliveira; Moraes, Izabel Cristina Freitas; Hubinger, Miriam Dupas. (2020). Encapsulation of Pomegranate Seed Oil by Emulsification Followed by Spray Drying: Evaluation of Different Biopolymers and Their Effect on Particle Properties. *Food and Bioprocess Technology*, 13 (1). pp.53-66**.**

2 **Da Silva Anthero, Ana Gabriela**; Bezerra, E. O.; Comunian, T. A.; Hubinger, M.D. (2021) Barley Malt Esterification after Ultrasound and Stearic Acid Treatment: Characterization and Use as Stabilizing Agent in Oil-in-Water Emulsions. *Food and Bioprocess Technology*, v. 2, p. 310-323.

3 Comunian, Talita & Roschel, Gabriela & **Anthero, Ana** & Castro, Inar & Hubinger, Miriam. (2020). Influence of heated, unheated whey protein isolate and its combination with modified starch on improvement of encapsulated pomegranate seed oil oxidative stability. *Food Chemistry*, 326, 126995. 10.1016/j.foodchem.2020.126995**.**

4 **Da Silva Anthero, Ana Gabriela**; Bezerra, E. O.; Comunian, T. A.; Hubinger, M.D. (2020) Effect of modified starches and gum arabic on the stability of carotenoids in paprika oleoresin microparticles, *Drying Technology*, 1-14. DOI: 10.1080/07373937.2020.1844227.

5 **Da Silva Anthero, Ana Gabriela**; Comunian, Talita Aline; Bezerra, Eveling Oliveira; de Figueiredo Furtado, Guilherme; Hubinger, Miriam Dupas. Physicochemical Properties of *Capsicum* Oleoresin Emulsions Stabilized by Gum Arabic, OSA-Modified Corn Starch, and Modified Malt. *Food and Bioprocess Technology*, v. 14, p. 1-12, 2022.

6 **Da Silva Anthero, Ana Gabriela**; Maria Tomazini Munhoz Moya, Amanda; Souza Torsoni, Adriana; Baú Betim Cazarin, Cinthia; Dupas Hubinger, Miriam. Characterization of *Capsicum* oleoresin microparticles and in vivo evaluation of short-term capsaicin intake. *Food Chemistry-X*, v. 13, p. 100179, 2022.

7 De Aguiar, Ana Carolina; Viganó, Juliane; **Da Silva Anthero, Ana Gabriela**; Dias, Arthur Luiz Baião; Hubinger, Miriam Dupas; Martínez, Julian. Supercritical fluids and fluid mixtures to obtain high-value compounds from *Capsicum* peppers. *Food Chemistry-X*, v. 13, p. 100228, 2022.

8 Silva, E. K**.; Anthero, A. G. S**.; Emerick, Lucas B.; Zabot, G. L.; Hubinger, M. D.; Meireles, M. A. A. Low-frequency ultrasound-assisted esterification of Bixa orellana L. seed starch with octenyl succinic anhydride. *International Journal of Biological Macromolecules*, v. 207, p. 1-8, 2022.

Artigo em revisão

Capsicum oleoresin loaded spray-dried microparticles: stability, cytotoxicity, and in vitro digestibility studies na Food Structure journal.

Capítulo de livro publicado em periódico

1 Anthero, A.G. S., Comunian, T.A.; Kurozawa, L.E., Pollonio, M. Hubinger, M.D. (2021). Chapter 7 - Application of nano/microencapsulated ingredients in meat products, Editor(s): Seid Mahdi Jafari, In Nanoencapsulation in the Food Industry, Application of Nano/Microencapsulated Ingredients in Food Products, Academic Press, Volume 6, 2021, Pages 305-343, ISBN 9780128157268, https://doi.org/10.1016/B978-0-12-815726-8.00007-6.

Resumos simples publicados em Anais de Congressos

1 Ana Gabriela da Silva Anthero, Eveling Oliveira Bezerra, Talita Aline Comunian, Miriam Dupas Hubinger. *Effect of different stabilizing agents in paprika oleoresin-loaded emulsions on carotenoids retention and physicochemical properties* (Pôster). In: *1st International Congress on Bioactive Compounds*, realizado nos dias 22 e 23 de novembro de 2018, Campinas, São Paulo.
2 Ana Gabriela da Silva Anthero, Talita Aline Comunian, Eveling Oliveira Bezerra, Miriam Dupas Hubinger. *Modified malt by the combination ultrasound-stearic acid and it use as stabilizer into emulsion (O/W) (Pôster).* In: *8th International Symposium on "Delivery of Functionality in Complex Food Systems*, realizado em Porto, Portugal, durante os dias 7-10 de julho de 2019
3 Ana Gabriela da Silva Anthero, Eveling Oliveira Bezerra, Talita Aline Comunian, Miriam Dupas Hubinger. *Physicochemical properties of Capsicum oleoresin emulsions using modified malt as novel encapsulating agent for foodstuff application* (Oral). In: *8th International Symposium on "Delivery of Functionality in Complex Food Systems*, realizado em Porto, Portugal, durante os dias 7-10 de julho de 2019.
4 Ana Gabriela da Silva Anthero, Eveling Oliveira Bezerra, Fernanda Ramalho Procópio, Talita Aline Comunian, Miriam Dupas Hubinger. *Effect of different biopolymers on stability of carotenoids from paprika oleoresin encapsulated by spray drying* (Pôster). In: *13th Latin American Symposium of Food Science*, Campinas, São Paulo, realizado nos dias 10-12 de novembro de 2019.
5 *Capsicum* oleoresin microparticles as a source of capsaicin and its short-term ingestion effect on the in vivo model (Appendix I) was presented by Ana Gabriela da Silva Anthero in the 49th ANNUAL FOOD SCIENCE AND TECHNOLOGY CONFERENCE. The Book of Abstracts has now been published: On Arrow https://arrow.tudublin.ie/ehsicon/4 Or doi is https://doi.org/10.21427/1qxb-1s79.
6 ANTHERO, A. G. S.; Bonetti, C.I.; Bracht, L.; BAÚ BETIM CAZARIN, CINTHIA; Hubinger, M.D . Oral gavage of free and microencapsuled *Capsicum* oleoresin in mice fed with the high-fat diet. In: First Research Meeting on Biochemistry, 2021, Maringá. *First Research Meeting on Biochemistry*, 2021**.**
7 Ana Gabriela da Silva Anthero, Bridget Hogg, Sinead M. Ryan, Graham O'Neill, Miriam Dupas Hubinger and Jesús Maria Frías Celayeta. *Capsicum* oleoresin-loaded microparticles:

formulation, toxicological study and in vitro digestibility presented in EFFOST conference, Dublin, Ireland, 2022.

Estágio de Capacitação de Docente

1 Participação como bolsista do Programa de Estágio Docente (PED), categoria C, na Disciplina "TA932 – Projeto Industrial" no 2º semestre de 2017 e 1° semestre de 2018, sob orientação da Professora Ana Carla K. Sato. Participação como bolsista PED.

Participação em eventos científicos

1 Workshop on Liquid Atomization and Spray systems, a Brazilian German Initiative on Cooperative Research, realizado nos dias 2 e 3 de outubro, 2018 em São Paulo.
2 *1st International Congress on Bioactive Compounds*, realizado nos dias 22 e 23 de novembro de 2018, Campinas, São Paulo.
3 *8th International Symposium on "Delivery of Functionality in Complex Food Systems",* que ocorreu em Porto, Portugal, durante os dias 7-10 de julho de 2019.
4 *13th Latin American Symposium of Food Science*, Campinas, São Paulo, durante os dias 10 a 12 de novembro de 2019.

Elaboração de projetos para financiamento de pesquisa

1 Participação na escrita do projeto *"Vencendo barreiras na aplicação de oleoresinas: Estabilidade e digestibilidade de sistemas co-encapsulados com aplicação em produto alimentício",* para submissão como Auxílio à Pesquisa Regular pela Fapesp, com aprovação em 2018 sob o número de chamada 2018/20466-8.

Coorientação de aluno de iniciação científica

1 Participação como coorientadora do projeto de iniciação científica *"Estabilidade dos carotenoides e propriedades físico-químicas da oleoresina de páprica microencapsulada por spray drying"* realizado pela aluna Eveling Bezerra de Oliveira, com bolsa concedida pelo CNPq, durante o período de agosto de 2018 a julho de 2019.

Estágio de Pesquisa no exterior

A Doutoranda realizou estágio de pesquisa no exterior no periodo de dezembro de 2019 a marco de 2021 na Technological University Dublin (TU Dublin) and University College Dublin (UCD). Durante este periodo a aluna realizou treinamento em algumas técnicas *in vitro* como, toxicidade, ensaio de transporte de substâncias e digestibilidade.

Lab demonstrator: Durante o estágio de pesquisa na TU Dublin, a aluna atuou como demonstrador de laboratório na disciplina de química de alimentos e projetos, auxiliando alunos

em experimentos envolvendo análises físico-químicas em alimentos e desenvolvendo projetos de conclusão de curso.

Apêndice II: Certificados da Comissão de Ética no Uso de Animais

a. Certificado da Comissão de Ética no Uso de Animais do Capítulo 5

UNICAMP

CEUA/UNICAMP

CERTIFICADO

Certificamos que a proposta intitulada **Microparticulas carreadas com capsaicina obtida com diferentes materiais de parede e avaliação do efeito indutor de saciedade**, registrada com o nº **5154-1/2019**, sob a responsabilidade de **Profa. Dra. Cinthia Bau Betim Cazarin**, **Ana Gabriela da Silva Anthero** e **Amanda Maria Tomazini Munhoz Moya**, que envolve a produção, manutenção ou utilização de animais pertencentes ao filo *Chordata*, subfilo *Vertebrata* (exceto o homem) para fins de pesquisa científica (ou ensino), encontra-se de acordo com os preceitos da **LEI Nº 11.794, DE 8 DE OUTUBRO DE 2008**, que estabelece procedimentos para o uso científico de animais, do **DECRETO Nº 6.899, DE 15 DE JULHO DE 2009**, e com as normas editadas pelo **Conselho Nacional de Controle da Experimentação Animal (CONCEA)**, **tendo sido** aprovada pela **Comissão de Ética no Uso de Animais** da **Universidade Estadual de Campinas - CEUA/UNICAMP**, em **14 de março de 2019**.

Finalidade:	( ) Ensino (X) Pesquisa Científica
Vigência do projeto:	01/04/2018 - 01/06/2020
Vigência da autorização para manipulação animal:	01/04/2019 - 01/06/2020
Espécie / linhagem/ raça:	Camundongo isogênico / C57BL/6J
No. de animais:	30
Idade/Peso:	28 dias / 20 g
Sexo:	Machos
Origem:	CEMIB/UNICAMP
Biotério onde serão mantidos os animais:	Biotério do Laboratório de Ensaios Biológicos, FEA/UNICAMP

A aprovação pela CEUA/UNICAMP não dispensa autorização junto ao **IBAMA**, **SISBIO** ou **CIBio** e é **restrita** a protocolos desenvolvidos em biotérios e laboratórios da Universidade Estadual de Campinas.

Campinas, 01 de abril de 2019.

Prof. Dr. Wagner José Favaro
Presidente

Rosangela dos Santos
Secretária Executiva

IMPORTANTE: Podence atenção ao prazo para envio do relatório final de atividades referente a este protocolo: até 30 dias após o encerramento de sua vigência. O formulário encontra-se disponível na página da CEUA/UNICAMP, área do pesquisador responsável. A não apresentação do relatório no prazo estabelecido impedirá que novos protocolos sejam submetidos.

b. Certificado da Comissão de Ética no Uso de Animais do Capítulo 7

UEM

Comissão de Ética no Uso de Animais da Universidade Estadual de Maringá

CERTIFICADO

Certificamos que a proposta intitulada "Microparticulas De Oleoresina De Capsicum Obtidas Com Diferentes Materiais De Parede E Avaliação Do Efeito Indutor de Saciedade", protocolada sob o CEUA nº 3305020621 (ID 3030631), sob a responsabilidade de **Lívia Bracht** *e equipe; Ana Gabriela Anthero; Miriam Dupas Hubinger* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade Estadual de Maringá (CEUA/UEM) na reunião de 10/06/2021.

We certify that the proposal "Capsicum Oleoresin microparticles obtained with different wall materials and evaluation of satiety-inducing effect", utilizing 60 Heterogenics mice (60 males), protocol number CEUA 3305020621 (ID 3030631), under the responsibility of **Lívia Bracht** *and team; Ana Gabriela Anthero; Miriam Dupas Hubinger* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the State University of Maringá (CEUA/UEM) in the meeting of 06/10/2021.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 08/2021 a 08/2022 Área: Dbq-Bioquímica

Origem:	Biotério Central da UEM				
Espécie:	Camundongos heterogênicos	sexo: Machos	Idade:	40 a 45 dias	N: 60
Linhagem:	Swiss		Peso:	30 a 40 g	

Local do experimento: Laboratório de Metabolismo Hepático - Departamento de Bioquímica - UEM

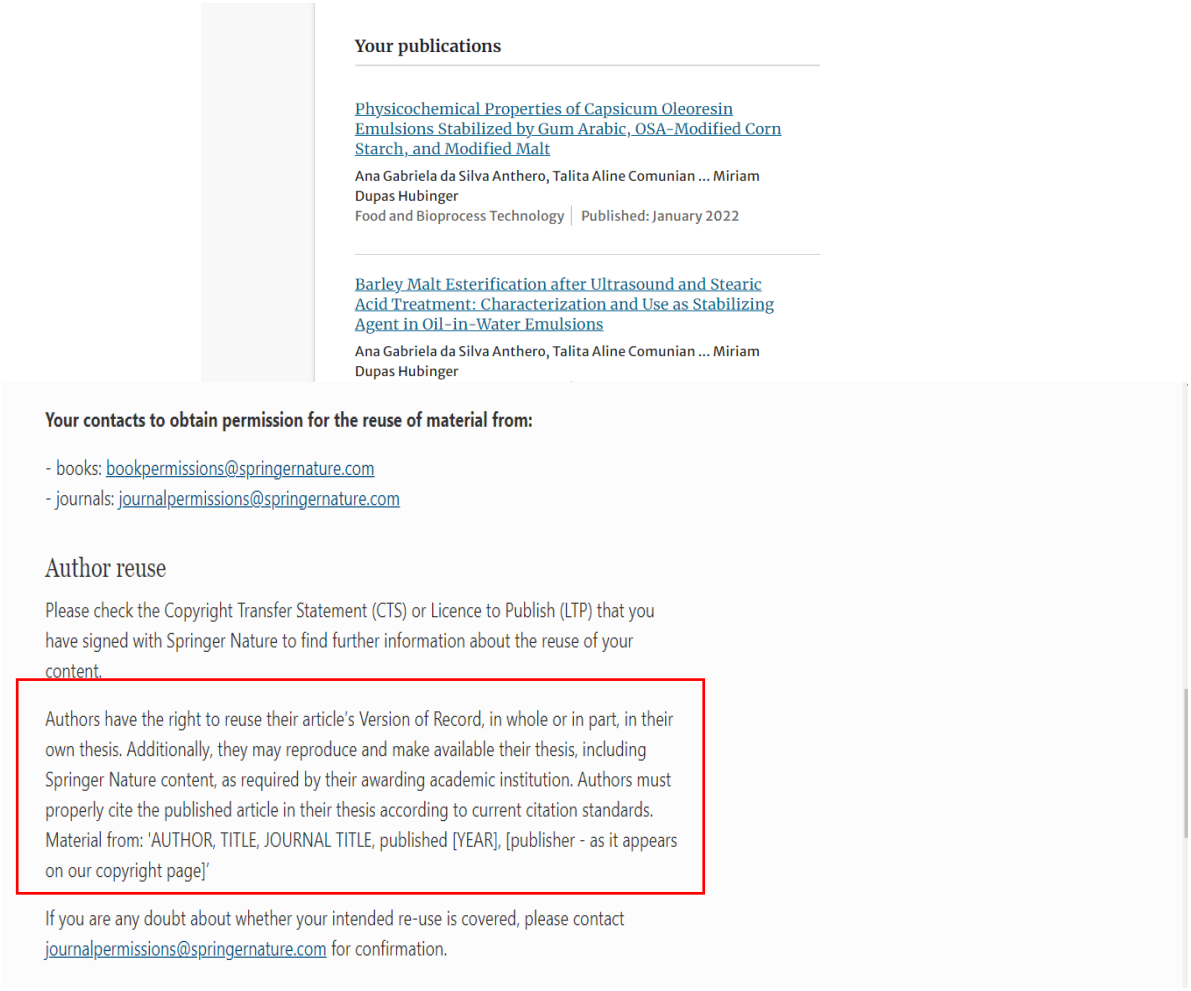
Maringá, 28 de junho de 2021

Profa. Dra. Tatiana Carlesso dos Santos
Coordenadora da CEUA/UEM
Universidade Estadual de Maringá

Profa. Dra. Erika Seki Kioshima Cótica
Coordenadora Adjunta da CEUA/UEM
Universidade Estadual de Maringá

ANEXOS

Anexo I: Permissão para uso integral do artigo correspondente ao Capítulo 3 e 4



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Physicochemical Properties of Capsicum Oleoresin Emulsions Stabilized by Gum Arabic, OSA-Modified Corn Starch, and Modified Malt

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Food and Bioprocess Technology | Published: January 2022

Barley Malt Esterification after Ultrasound and Stearic Acid Treatment: Characterization and Use as Stabilizing Agent in Oil-in-Water Emulsions

Ana Gabriela da Silva Anthero, Talita Aline Comunian ... Miriam Dupas Hubinger

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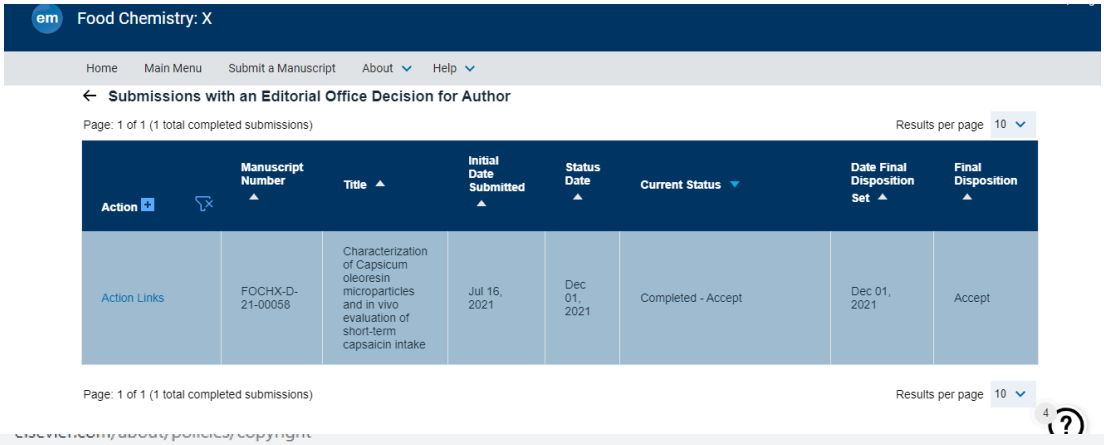
Anexo II: Permissão para uso integral do artigo correspondente ao Capítulo 5



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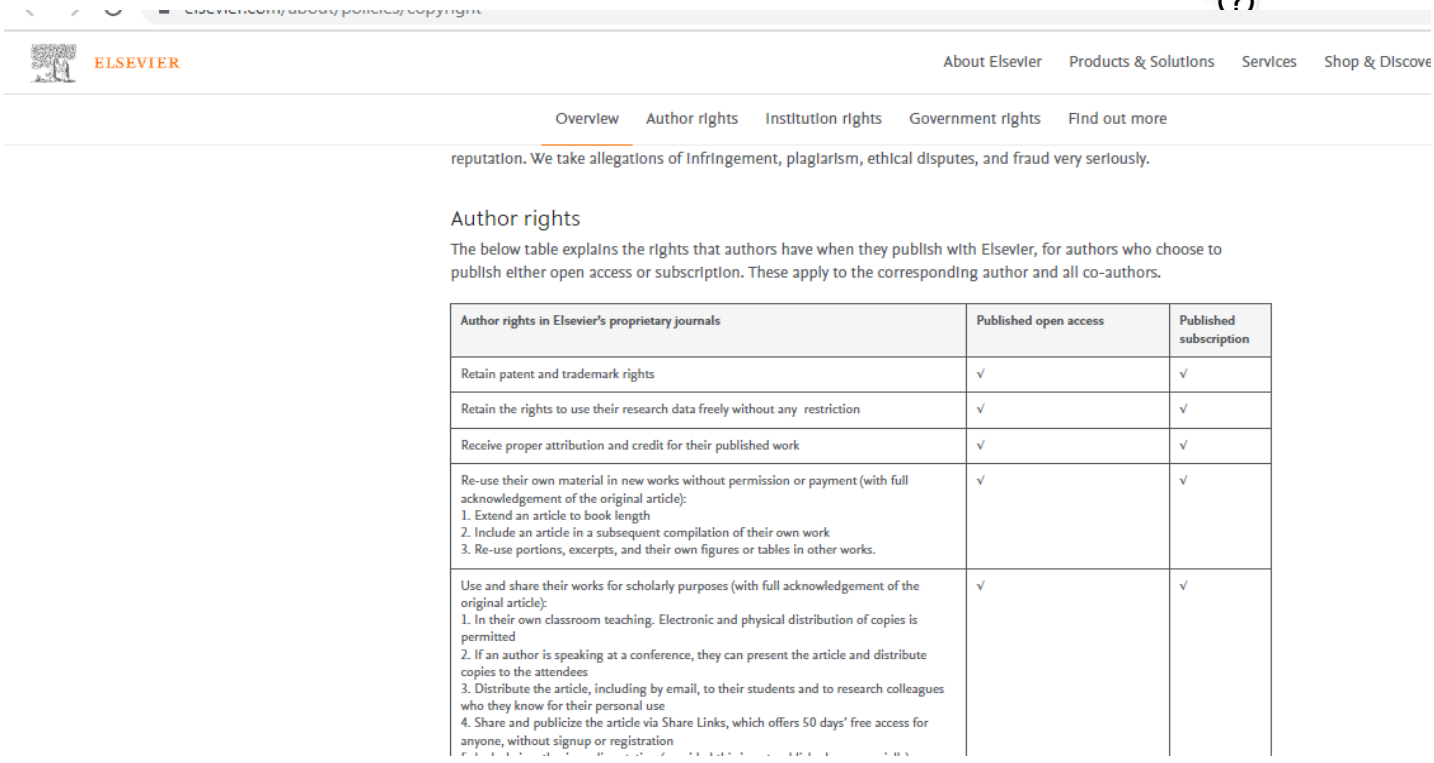
Action	Manuscript Number	Title	Initial Date Submitted	Status Date	Current Status	Date Final Disposition Set	Final Disposition
Action Links	FOCHX-D-21-00058	Characterization of Capsicum oleoresin microparticles and in vivo evaluation of short-term capsaicin intake	Jul 16, 2021	Dec 01, 2021	Completed - Accept	Dec 01, 2021	Accept

Page: 1 of 1 (1 total completed submissions) Results per page 10



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