UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS RURAIS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DOS ALIMENTOS

Maiara Taís Bazana

NANOEMULSÕES DO EXTRATO DO CÁLICE DE *Physalis peruviana*: DESENVOLVIMENTO, AVALIAÇÃO DA ESTABILIDADE E ESTUDOS DE TOXICIDADE

Santa Maria, RS
2019

Maiara Taís Bazana

NANOEMULSÕES DO EXTRATO DO CÁLICE DE *Physalis peruviana*: DESENVOLVIMENTO, AVALIAÇÃO DA ESTABILIDADE E ESTUDOS DE TOXICIDADE

Tese apresentada ao Curso de Pós-Graduação em Ciência e Tecnologia dos Alimentos, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Doutora em Ciência e Tecnologia dos Alimentos.**

Orientador: Prof. Dr. Cristiano Ragagnin de Menezes
Coorientadora: Profª. Drª. Cristiane de Bona da Silva

Santa Maria, RS
2019

Bazana, Maiara Taís
Nanoemulsões do extrato do cálice de Physalis peruviana: desenvolvimento, avaliação da estabilidade e estudos de toxicidade / Maiara Taís Bazana.- 2019.
137 p.; 30 cm

Orientador: Cristiano Ragagnin de Menezes
Coorientadora: Cristiane de Bona da Silva
Tese (doutorado) - Universidade Federal de Santa Maria, Centro de Ciências Rurais, Programa de Pós Graduação em Ciência e Tecnologia dos Alimentos, RS, 2019

1. Cape gooseberry 2. Citotoxicidade 3. C. elegans 4. Nanotecnologia 5. Resíduo I. Menezes, Cristiano Ragagnin de II. Silva, Cristiane de Bona da III. Título.

Sistema de geração automática de ficha catalográfica da UFSM. Dados fornecidos pelo autor(a). Sob supervisão da Direção da Divisão de Processos Técnicos da Biblioteca Central. Bibliotecária responsável Paula Schoenfeldt Patta CRB 10/1728.

E-mail: maiarabazana@gmail.com

Maiara Taís Bazana

NANOEMULSÕES DO EXTRATO DO CÁLICE DE *Physalis peruviana*: DESENVOLVIMENTO, AVALIAÇÃO DA ESTABILIDADE E ESTUDOS DE TOXICIDADE

Tese apresentada ao Curso de Pós-Graduação em Ciência e Tecnologia dos Alimentos, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Doutora em Ciência e Tecnologia dos Alimentos.**

Aprovado em 18 de outubro de 2019:

Cristiano Ragagnin de Menezes, Dr. (UFSM)
(Presidente/Orientador)

Cristiane de Bona da Silva, Dra. (UFSM)
(Coorientadora)

Aline Ferreira Ourique, Dra. (UFN)

Cristiane Franco Codevilla, Dra. (PMSS)

Cristiano Augusto Ballus, Dr. (UFSM)

Sabrina Somacal, Dra. (UFSM)

Santa Maria, RS
2019

Dedico aos meus
pais e ao Jeferson com
todo meu amor e carinho

AGRADECIMENTOS

Agradeço à Universidade Federal de Santa Maria, pelo ensino público, gratuito, de qualidade e excelência, onde tive a oportunidade de realizar minha graduação, mestrado e doutorado.

Ao Programa de Pós-Graduação em Ciência e Tecnologia dos Alimentos, pela realização desse trabalho e incentivo à pesquisa científica. Assim como, aos professores do PPGCTA pelos conhecimentos transmitidos.

Ao meu orientador, Prof. Cristiano pela orientação, ensinamentos, apoio, amizade, e pela confiança em mim depositada, sendo um exemplo de profissional.

À minha coorientadora, Profª. Cris pelos anos de orientação (parte da iniciação científica e mestrado), compreensão na minha decisão pela área de alimentos e por aceitar me coorientar, dando todo respaldo sobre a nanotecnologia.

À CAPES pelo apoio financeiro através da bolsa de estudos.

Aos membros da banca pela disponibilidade em avaliar o trabalho e por contribuírem para a qualidade desta tese.

Ao meu companheiro, Jeferson, pelo amor, a dedicação, o carinho, a paciência, a compreensão e pela força diária que necessitei em todos os momentos nessa caminhada. E por estar dividindo esse momento sublime em nossas vidas, a espera de nosso filho amado, notícia esta de muita felicidade e que me deu mais força para conseguir concluir esta etapa.

Aos meus pais, Isabel e Orlando pela educação, por acreditarem e me apoiarem em todos os meus sonhos, incentivando o meu crescimento, sempre com muito amor e carinho.

Ao Lab 106 onde fui muito bem acolhida, local onde me senti em casa durante esse período. Em especial à equipe nano, Suelen e Cris Codevilla pela amizade, conhecimento compartilhado, auxílio nas decisões de experimentos e pelos desafios com a *Physalis*. Agradeço aos colegas atuais Thai, Cassandra, Simara, Naiara, Gabi, Greice, Vandré, Vítor, Débora e Camila por todo auxílio nas análises, amizade, momentos de descontração, rodas de mate, pelo convívio diário, enfim vocês foram muito importantes. E às colegas mais antigas, Graci, Mari e Maria Fernanda que fizeram parte da minha trajetória e hoje já concluíram o doutorado, mas a amizade permanece mesmo a distância. Obrigada a todos!

Aos demais amigos e familiares que sempre estiveram torcendo e me apoiando, especialmente aos meus sogros Eneida e Saul pela compreensão com meus compromissos e prazos, pois sempre foram muito atenciosos e solícitos, sendo minha segunda família em Santa Maria.

Ao Laboratório de Desenvolvimento Farmacotécnico, pelo espaço e equipamentos cedidos para a realização das nanoemulsões. Assim como, à Nadine e Aline pelas análises no Zetasizer e Mastersizer.

Ao Prof. Juliano S. Barin pela utilização do moinho de facas e pelas análises de ORAC com auxílio da aluna Bruna N. Lucas.

Ao Prof. Marcio A. Mazutti pela utilização do HPLC e Spectra Max M5, e ao servidor Gustavo A. Ugalde pela responsabilidade com as análises cromatográficas.

À pós-doc Daniele R. N. Librelotto, pelo auxílio nas análises de citotoxicidade.

Ao Prof. Félix A. A. Soares pela parceria, em especial a Marina e Larissa, pelo auxílio no experimento de toxicidade em *C. elegans*.

Ao Prof. Roberto C. V. Santos pela parceria nas análises antibacterianas do extrato e ao seu aluno Marcos V. Missel pela ajuda nos experimentos.

Enfim a todos aqueles que de uma forma ou de outra contribuíram para a finalização desta etapa.

“A educação é a arma
mais poderosa
que você pode usar
para mudar o mundo.
Devemos
promover a coragem
onde há medo,
promover o acordo
onde existe conflito
e inspirar esperança
onde há desespero”

Nelson Mandela

RESUMO

NANOEMULSÕES DO EXTRATO DO CÁLICE DE *Physalis peruviana*: DESENVOLVIMENTO, AVALIAÇÃO DA ESTABILIDADE E ESTUDOS DE TOXICIDADE

AUTORA: Maiara Taís Bazana
ORIENTADOR: Cristiano Ragagnin de Menezes
COORIENTADORA: Cristiane de Bona da Silva

A *Physalis peruviana* apresenta uma variedade de compostos biologicamente ativos principalmente em seu cálice, o qual é considerado um subproduto, com potencial para ser explorado e reutilizado. Entre as tecnologias mais promissoras na indústria de alimentos, destaca-se a nanotecnologia, com a capacidade de encapsular variados compostos que possuem limitações. No entanto, ainda requer maiores estudos quanto à nanotoxicologia das formulações e sua aplicação em resíduos gerados pelas indústrias. Nesse sentido, o objetivo deste trabalho consistiu em desenvolver nanoemulsões contendo o extrato do cálice de *P. peruviana* e avaliar a estabilidade do extrato e das nanoemulsões frente a diferentes condições de armazenamento, capacidade antioxidante, e toxicidade *in vitro* e *in vivo*, visando sua aplicação em alimentos. Os extratos foram preparados em etanol 60% e caracterizados quanto à capacidade antioxidante, capacidade redutora, composto majoritário, atividade antibacteriana e capacidade antibiofilme. As nanoemulsões foram preparadas pelo método de emulsificação espontânea e caracterizadas físico-quimicamente. Após, avaliou-se a estabilidade dos extratos e das nanoemulsões frente a diferentes condições de armazenamento, tais como temperaturas de 7 e 25°C com ausência ou incidência de luz por 120 dias. Também, verificou-se: a liberação do composto majoritário a partir das nanoemulsões e do extrato em meio gastrointestinal simulado; a toxicidade *in vitro* em linhagens de células tumorais (MCF-7 câncer de mama) e não tumorais (3T3 fibroblastos) e *in vivo* (utilizando o modelo *Caenorhabditis elegans*). O extrato do cálice de *P. peruviana* foi caracterizado quanto a sua capacidade redutora (610 mg Eq. de ácido gálico/100 g de cálice), capacidade antioxidante (138 μmol de Trolox/g de cálice) e teor de rutina (11,3 μg/mL). A concentração inibitória mínima variou de 3,15 a 30 mg/mL de extrato, demonstrando atividade bacteriostática frente a oito patógenos e atividade bactericida na concentração de 30 mg/mL frente a seis cepas. Ainda, o extrato inibiu a formação de biofilme produzido pela *Pseudomonas aeruginosa* PA01, porém não houve destruição. As nanoemulsões apresentaram tamanho de gotícula nanométrico (160-180 nm), índice de polidispersão abaixo de 0,15, potencial zeta (-8 a -11 mV), pH levemente ácido (5,4 a 6,3), distribuição do tamanho de gotículas (pico unimodal na faixa nanométrica), morfologia (formato esférico e superfície lisa), teor de rutina (11 μg/mL) e eficiência de encapsulamento de 85%. Entre as amostras testadas, a maior estabilidade foi observada para as nanoemulsões contendo o extrato do cálice de *P. peruviana* quando armazenadas à temperatura ambiente e com ausência de luz. O extrato e as nanoemulsões do extrato não mostraram toxicidade na linhagem celular não tumoral. No entanto, à medida que a concentração das nanoemulsões aumentou, elas demonstraram citotoxicidade contra a linhagem celular tumoral. No modelo *in vivo*, todas as amostras analisadas não causaram toxicidade para *C. elegans* no teste de sobrevivência. Em relação à resistência ao estresse oxidativo, as formulações exerceram seus efeitos antioxidantes em baixas concentrações *in vivo*. Além disso, a rutina presente no extrato nanoemulsionado apresentou maior estabilidade frente às condições gastrointestinais simuladas em relação a presente no extrato livre, controlando a liberação e aumentando os níveis de bioativo liberado no duodeno e íleo, onde ocorre a absorção. Portanto, este estudo contribui para que futuras aplicações na área de alimentos sejam estudadas e aprofundadas.

Palavras-chave: *Cape gooseberry*. Citotoxicidade. *C. elegans*. Nanotecnologia. Resíduo.

ABSTRACT

NANOEMULSIONS OF THE *Physalis peruviana* CALYX EXTRACT: DEVELOPMENT, STABILITY ASSESSMENT, AND TOXICITY STUDIES

AUTHOR: Maiara Taís Bazana
ADVISOR: Cristiano Ragagnin de Menezes
CO-ADVISOR: Cristiane de Bona da Silva

Physalis peruviana presents a variety of biologically active compounds, especially in its calyx, which is considered a byproduct with potential to be explored and reused. Among the most promising technologies in the food industry, nanotechnology stands out with its ability to encapsulate various compounds, which have limitations. However, this technology still requires further studies on the nanotoxicity of formulations and its use in waste generated by industries. Therefore, the objective of this study was to develop nanoemulsions containing the *P. peruviana* calyx extract and evaluate the stability of the extract and nanoemulsions against different storage conditions, antioxidant capacity, and *in vitro* and *in vivo* toxicity, aiming its application in food. The extracts were prepared in 60% ethanol and characterized by antioxidant capacity, reducing capacity, HPLC-quantified major compound, antibacterial activity, and antibiofilm capacity. Nanoemulsions were prepared by the spontaneous emulsification method and physically and chemically characterized. Afterwards, the stability of the extracts and nanoemulsions were evaluated under different storage conditions, such as temperatures of 7 and 25 °C with the absence or incidence of light for 120 days. Additionally, the release of the major compound from the nanoemulsions and extract in a simulated gastrointestinal environment, the *in vitro* toxicity in tumor cell lines (MCF-7 breast cancer) and non-tumor cell lines (3T3 fibroblasts), and *in vivo* (using the *Caenorhabditis elegans* model) were evaluated. The *P. peruviana* calyx extract was characterized by its reducing capacity (610 mg Eq. of gallic acid/100 g of calyx), antioxidant capacity (138 μmol Trolox/g of calyx), and rutin content (11.3 μg/mL). The minimum inhibitory concentration ranged from 3.15 to 30 mg/mL extract, showing bacteriostatic activity against eight pathogens and bactericidal activity at 30 mg/mL concentration against six strains. Nevertheless, the extract inhibited the formation of biofilm produced by *Pseudomonas aeruginosa* PA01, although there was no destruction. The nanoemulsions had nanometric droplet size (160-180 nm), polydispersity index below 0.15, zeta potential (-8 to -11 mV), slightly acidic pH (5.4 to 6.3), droplets (unimodal peak in the nanometer range), morphology (spherical shape and smooth surface), rutin content (11 μg/mL), and encapsulation efficiency of 85%. Among the tested samples, the highest stability was observed in nanoemulsions containing the *P. peruviana* calyx extract when stored at room temperature and in the absence of light. The extract and nanoemulsions of the extract showed no toxicity in the non-tumor cell line. However, as the concentration of nanoemulsions increased, they demonstrated cytotoxicity against the tumor cell line. In the *in vivo* model, all samples analyzed did not cause toxicity to *C. elegans* in the survival test. Regarding resistance to oxidative stress, the formulations exerted their antioxidant effects at low concentrations *in vivo*. In addition, the rutin present in the nanoemulsion extract showed greater stability against the degradation of simulated gastrointestinal conditions compared to the free extract, controlling the release and increasing the levels of bioactive compounds released in the duodenum and ileum, which is where absorption occurs. Therefore, this study contributes to future applications in the food area to be studied and deepened.

Keywords: Cape gooseberry. Cytotoxicity. *C. elegans*. Nanotechnology. Residue.

LISTA DE FIGURAS

LISTA DE TABELAS

ARTIGO 1

ARTIGO 2

MANUSCRITO 1

LISTA DE ABREVIATURAS E SIGLAS

ABTS	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
A/O	Água em óleo
C. elegans	*Caenorhabditis elegans*
CBM/MBC	Concentração bactericida mínima / "*Minimum bactericidal concentration"*
CIM/MIC	Concentração inibitória mínima / "*Minimum inhibitory concentration"*
CLAE/HPLC	Cromatografia líquida de alta eficiência / "*High performance liquid chromatography"*
DPPH	2,2-difenil-1- picril-hidrazil
MEV/SEM	Microscopia eletrônica de varredura / "*Scanning Electron Microscopy"*
mL	Mililitro
nm	Nanometro
O/A	Óleo em água
ORAC	Capacidade de desativação do radical peroxila / "*Oxigen radical absorbance capacity"*
P. peruviana	*Physalis peruviana*
PdI	Índice de polidispersão / *"Polydispersity index"*
pH	Potencial hidrogeniônico
TCM/MCT	Triglicerídeos de cadeia média / *"Medium chain triglycerides"*
µg	Micrograma
µm	Micrômetro

SUMÁRIO

APRESENTAÇÃO

A tese segue as normas estabelecidas na Estrutura e Apresentação do Manual de Dissertações e Teses da UFSM – MDT 2015. Os dados do estudo estão apresentados no formato de artigos científicos integrados, sendo composta a tese por dois artigos publicados (um de revisão bibliográfica publicado na Revista *Current Opinion in Food Science,* Qualis CAPES A1 e o outro com resultados da pesquisa publicado na Revista *Food Research International*, Qualis CAPES A1) e dois manuscritos com resultados da pesquisa, sendo que o manuscrito 1 foi submetido a Revista Ciência Rural, Qualis CAPES A4 e o manuscrito 2 será submetido a Revista *Journal of Functional Foods*, Qualis CAPES A1. Ao final, encontram-se os itens **DISCUSSÃO GERAL** e **CONCLUSÃO**, apresentando uma compilação de interpretações e comentários a respeito dos resultados demonstrados nos artigos e manuscritos. As **REFERÊNCIAS** referem-se às citações apontadas nos itens **INTRODUÇÃO, REFERENCIAL TEÓRICO** e **DISCUSSÃO** dessa tese.

1 INTRODUÇÃO

Os resíduos gerados no processamento de alimentos, geralmente são descartados ou empregados na alimentação animal, sendo, atualmente, o reaproveitamento um desafio tanto para as indústrias agroalimentares quanto para os pesquisadores. Por outro lado, na indústria alimentícia a busca por diferentes fontes de compostos bioativos que possam ter um efeito benéfico em nossa saúde, tem sido tendência emergente na ciência de alimentos. Esses subprodutos tem se mostrado como uma alternativa valiosa para extrair compostos naturais com bioatividade demonstrada (BALLESTEROS-VIVAS et al., 2019b).

Physalis peruviana L. também conhecida como *goldenberry* ou *cape gooseberry*, pertence à família Solanaceae, que possui cerca de 120 espécies, distribuídas em regiões tropicais e subtropicais da África, Ásia e América (KUSPKA; JÉLEN, 2016). O processamento industrial de *goldenberries* gera dois subprodutos, a baga (incluindo sementes e pele) e o cálice (RAMADAN, 2011). O cálice é um invólucro que sustenta os frutos comestíveis, protegendo-os naturalmente ao longo do seu desenvolvimento e amadurecimento, contra insetos, aves, doenças e situações climáticas adversas. Tal estrutura consiste em uma fonte essencial de carboidratos durante os primeiros 20 dias de crescimento do fruto (TAPIA; FRIES, 2007; PUENTE et al., 2011).

O cálice de *P. peruviana* é uma fonte potencial de compostos bioativos, tais como, fitoquímicos (terpenoides, fitoesteróis e derivados do fitol), flavonoides (destacando-se a rutina como um dos compostos majoritários), ácidos fenólicos e vitanolídeos (GIRONÉS-VILAPLANA et al., 2014; BALLESTEROS-VIVAS et al., 2019a; BALLESTEROS-VIVAS et al., 2019b; MEDINA et al., 2019). Além disso, possui potente atividade antioxidante (GIRONÉS-VILAPLANA et al., 2014; TORO et al., 2014) e anti-inflamatória comprovada (FRANCO et al., 2007; FRANCO et al., 2014; CASTRO; OCAMPO; FRANCO, 2015), dentre outros usos na medicina popular como anticancerígeno, antipirético, diurético, antimicrobiano e imunomodulador (FRANCO et al, 2007).

Essas substâncias com excelentes propriedades funcionais (compostos bioativos) possuem algumas limitações que dificultam sua aplicação em alimentos, incluindo baixa solubilidade em água, baixa absorção e biodisponibilidade (SHIN; KIM; PARK, 2015). Entre os compostos bioativos que apresentam essas desvantagens, está a rutina, um dos flavonoides presentes em maior quantidade no cálice de *P. peruviana* (GULLÓN et al., 2017). Além disso, esses compostos podem se degradar pelas condições de processamento e armazenamento de alimentos como aquecimento, acidificação, luz e oxigênio. Defeitos de

qualidade, como gosto adstringente também podem ser observados quando adicionados em produtos alimentares. Assim, alguns extratos não são utilizados devido a tais obstáculos (CODEVILLA et al., 2015).

Um dos setores que tem se destacado em pesquisas com alimentos e sua aplicação é a nanotecnologia (HAMAD et al., 2018), impulsionados pela necessidade de sistemas comestíveis capazes de encapsular, proteger e liberar compostos funcionais (ARANCIBIA et al., 2017). As nanoemulsões são utilizadas na indústria de alimentos na produção de edulcorantes, óleos aromatizados, molhos para saladas, bem como em outros alimentos processados (HAMAD et al., 2018). Assim, as nanoformulações são uma alternativa para impedir a degradação de compostos bioativos, melhorando sua estabilidade e biodisponibilidade (REZAEI; FATHI; JAFARI, 2019).

Por outro lado, os possíveis riscos para a saúde humana e ao meio ambiente das nanopartículas, como os impactos toxicológicos não acompanharam o rápido aumento nas pesquisas e utilização dos nanomateriais. Ainda são necessários estudos para ajudar a concluir os mecanismos de toxicidade das nanopartículas, bem como os mecanismos envolvidos na sua distribuição, acúmulo em células e tecidos e sua eliminação pelo organismo (MISSAOUI; ARNOLD; CUMMINGS, 2018).

Considerando o potencial do cálice de *P. peruviana* como uma fonte promissora de fitoquímicos bioativos, este trabalho visou obter um extrato desse derivado natural e caracterizá-lo. Assim como, desenvolver nanoemulsões contendo o extrato do cálice de *P. peruviana* e avaliar se, tanto as nanoemulsões como o extrato, são estáveis quanto às características físico-químicas, capacidade antioxidante e redutora, teor de rutina frente à temperatura ambiente (25°C) e refrigerada (7°C), submetidos à incidência ou ausência de luz. Além de, estudar a toxicidade *in vitro* e *in vivo* das formulações e verificar a liberação no trato gastrointestinal simulado.

2 OBJETIVOS

2.1 OBJETIVO GERAL

Desenvolver nanoemulsões contendo extrato do cálice de *Physalis peruviana* e avaliar sua estabilidade frente a diferentes condições de armazenamento e toxicidade *in vitro* e *in vivo*.

2.2 OBJETIVOS ESPECÍFICOS

- Preparar e caracterizar o extrato hidroalcoólico do cálice de *P. peruviana* frente à capacidade antioxidante e redutora, e o teor de rutina;
- Avaliar a atividade antibacteriana e antibiofilme do extrato;
- Desenvolver nanoemulsões contendo o extrato do cálice de *P. peruviana* e nanoemulsões brancas (sem o extrato associado às nanoestruturas);
- Avaliar as características físico-químicas das nanoemulsões quanto ao diâmetro médio de gotícula, índice de polidispersão, potencial zeta, distribuição do tamanho de gotícula, pH, teor de rutina, eficiência de encapsulamento e morfologia;
- Analisar a estabilidade dos extratos do cálice de *P. peruviana* e das nanoemulsões em diferentes condições de luminosidade e temperatura, por um período de 120 dias, através da capacidade redutora, capacidade antioxidante e teor de rutina;
- Avaliar a liberação *in vitro* da rutina a partir das nanoemulsões e do extrato livre em condições gastrointestinais simuladas;
- Verificar a citotoxicidade do extrato do cálice de *P. peruviana* associado ou não às nanoemulsões em linhagens de células tumorais (MCF-7 câncer de mama) e não tumorais (3T3 fibroblastos);
- Submeter às formulações e os extratos a testes de toxicidade e atividade antioxidante *in vivo*, utilizando o modelo de *Caenorhabditis elegans*.

3 REFERENCIAL TEÓRICO

3.1 *Physalis peruviana*

Produtores e comerciantes têm se interessado pelo cultivo de pequenas frutas no Brasil. Ainda são bastante incipientes e inovadoras, as atividades econômicas com a utilização de pequenas frutas. De modo geral, caracterizam-se, pelo baixo custo de implantação e de produção, acessível aos pequenos produtores, bom retorno econômico em curto prazo, boa adaptação às condições socioeconômicas e ao ambiente local, grande exigência de mão de obra, possibilidade de cultivo no sistema orgânico e demanda maior do que a oferta (MUNIZ et al., 2011).

A *P. peruviana* Linnaeus (Figura 1) é uma planta herbácea, semi-arbustiva, vertical e perene, podendo atingir 0,6 a 0,9 metros e em alguns casos pode alcançar até 1,8 m. As flores podem ser facilmente polinizadas por insetos, pelo vento e também por autopolinização. Os frutos possuem coloração laranja-amarelada e uma baga suculenta com formato ovoide. O diâmetro varia entre 1,25 e 2,50 cm e peso entre 4 e 10 g, contendo interiormente cerca de 100 a 200 pequenas sementes amareladas. Esse fruto é protegido pelo cálice que cobre completamente os frutos ao longo do seu desenvolvimento e amadurecimento (TAPIA; FRIES, 2007; PUENTE et al., 2011). O fruto de *P. peruviana* é considerado climatérico, ou seja, são frutos que podem amadurecer na planta ou fora dela se colhidos imaturos (RODRIGUES et al., 2012).

Figura 1 – *Physalis peruviana*

Fonte: <https://www.sitiodamata.com.br/especies-de-plantas/plantas-ornamentais/physalis> e <https://commons.wikimedia.org/wiki/File:Physalis_Nahaufnahme.JPG>

Vários são os nomes atribuídos ao fruto de *P. peruviana* ao redor do mundo, sendo conhecido como *uchuva* na Colômbia, *uvilla* no Equador, *cereza del Perú* ou *aguaymanto* no Peru, *topotopo* na Venezuela e *goldenberry* ou *cape gooseberry* em países de língua inglesa (PUENTE et al., 2011). No Brasil, é encontrado em algumas regiões como no Norte e Nordeste sendo comum nos quintais, nomeados como: camapu, camambu, camaru, joá-de-capote, joá-poca, balão-rajado, mulaca, saco-de-bode, bucho-de-rã e mata-fome (LICODIEDOFF, 2012). A planta *P. peruviana* é originária de países Andinos, sendo cultivada em países sul-americanos, especialmente Colômbia, Peru e Equador; e atualmente disponível em mercados internacionais (OLIVARES-TENORIO, 2016).

Na Colômbia é cultivada para exportação e o país lidera como o maior produtor seguido pela África do Sul (MAZORRA, 2006). A Colômbia produz 11.500 toneladas/ ano de frutos de *P. peruviana*, sendo que o excedente, não destinado à exportação, atingiu 50% do total da produção, pois esta fruta não é muito exportável devido o seu pequeno tamanho, por isso vem sendo usada para novos produtos desidratados (CASTRO; RODRIGUEZ; VARGAS, 2008). As pesquisas sobre esta planta, no Brasil, iniciaram em São Paulo, no ano de 1999, pela Estação Experimental Santa Luzia que foi a pioneira no cultivo. Para o comércio na capital paulista, a estação produz de 2 a 3 toneladas anuais (RUFATO et al., 2013). No município de Lages, SC, verificaram-se valores de produção de *P. peruviana* entre 2 a 8,67 $ton.ha^{-1}$ em áreas experimentais (BRIGHENTI et al., 2008).

A fruta de *P. peruviana* possui prestígio em alguns mercados internacionais. Os europeus, por exemplo, muitas vezes pagam preços “premium” para mergulhar as bagas no chocolate ou decorar bolos e tortas. Além de ser consumida *in natura*, a fruta pode ser apreciada de diferentes maneiras, sendo um ingrediente interessante em saladas, pratos cozidos, sobremesas e geleias (RAMADAN; MOERSEL, 2007).

A *P. peruviana* é uma boa fonte de provitamina A (vitamina A equivalente de atividade de retinol - RAE 163 µg/100g e vitamina A equivalente de retinol - RE 326 µg/100g), de minerais (cálcio, cobre, ferro, magnésio, manganês, zinco e em maior quantidade o potássio 382 mg/100g), vitamina C (17,72 mg/ 100g) e de vitaminas do complexo B (vitamina B6 0,321 mg/100g e riboflavina 0,023 mg/ 100g). A fruta contém 15% de sólidos solúveis (principalmente açúcares) e alta concentração de frutose. Assim como o nível de fósforo (33 mg/100g) também é elevado para uma fruta. Além disso, seu alto teor de fibra dietética é importantíssimo, pois a pectina das frutas atua como um regulador intestinal. Os valores de fibra alimentar total são 6,5 g/100g, fibra insolúvel 5,2 g/100g e fibra solúvel 0,9 g/100g (HASSANIEN, 2011; ROCKETT et al., 2018).

Outras partes da planta têm sido objeto de pesquisa, como a obtenção de 4β-hidroxivitanolídeo, a partir do extrato das partes aéreas da planta (hastes e folhas). Esse composto é responsável por inibir o crescimento de células cancerígenas do pulmão, podendo ser um potencial quimioterápico (YEN et al., 2010). No cálice de *P. peruviana* foi, inicialmente, isolado um vitanolídeo (28-hidroxivitanolídeo E) (DINAN; SARKER; SIK, 1997). Ballesteros-Vivas e colaboradores (2019a) identificaram 56 fitoquímicos, incluindo principalmente compostos fenólicos, 17 tipos de vitanolídeos e ésteres de sacarose anti-inflamatórios, indicando a ampla variedade de compostos bioativos presentes nos cálices. De acordo com Severo et al. (2010) os teores de carotenoides totais e de fenólicos totais dos frutos foram avaliados e verificou-se que até o estágio de amadurecimento 4 (coloração amarelo-amarronzado) os teores foram crescentes. A atividade antioxidante se mostrou superior em frutos nos estágios iniciais de amadurecimento. O estudo verificou que os frutos de *P. peruviana* consistem em fontes relevantes de carotenoides e compostos fenólicos, contudo apresentam baixa atividade antioxidante, esta foi avaliada com o radical ABTS (SEVERO et al., 2010). Porém, Wu e colaboradores (2005) observaram que os extratos etanólicos de *P. peruviana* possuem boa atividade antioxidante, avaliada por três métodos diferentes: teste do ácido tiobarbitúrico, citocromo c e inibição da xantina oxidase, diferindo do estudo anterior, e além disso, um avaliou o fruto e o outro o extrato etanólico do fruto.

Portanto, *P. peruviana* tem um grande potencial para o processamento de novos alimentos funcionais, em decorrência de suas propriedades nutricionais, bem como os seus componentes biologicamente ativos como compostos fenólicos, vitanolídeos, carotenoides, compostos aromáticos e voláteis (HASSANIEN, 2011). O fruto apresenta uma abundância de constituintes, dentre os quais se destacam os flavonoides (ROP et al., 2012; SATHYADEVI; SUBRAMANIAN, 2015); vitamina E (RAMADAN; MORSEL, 2003), β-caroteno (RAMADAN; MORSEL, 2003; BRIONES-LABARCA et al., 2013; ETZBACH et al, 2018) e ácido ascórbico (ROP et al., 2012; BRIONES-LABARCA et al., 2013). De acordo com Teixeira e colaboradores (2016) o fruto apresenta compostos fenólicos, que são variáveis de acordo com o local de plantio, já que os frutos cultivados na região de Huánuco no Peru apresentaram 149,3 ± 1,62 mg/Eq de ácido gálico/100 g de fruto e nas demais regiões (Junín, Ancash e Cajamarca) se observou valores menores de compostos fenólicos.

Para a preservação das propriedades nutracêuticas é sugerida a aplicação de tecnologias inovadoras para a estabilização da polpa de *goldenberry*. Quanto às tecnologias, a aplicação de alta pressão hidrostática (APH) tem o potencial de produzir alimentos de alta qualidade que exibem características de produtos frescos, microbiologicamente seguros e têm

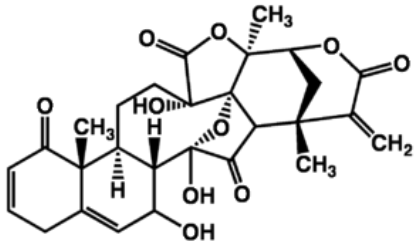
uma vida útil prolongada (FERRARI, MARESCA; CICCARONE, 2010; HUANG et al., 2014b). O emprego da encapsulação, também, é considerada uma alternativa muito promissora, como o estudo realizado em nosso grupo de pesquisa por Silva (2018) que desenvolveram nanoemulsões contendo o extrato da fruta, as quais apresentaram pH de 3,84, diâmetro médio de gotícula de 268 ± 7 nm, índice de polidispersão de 0,113 e potencial zeta negativo de 13,94 mV. A formulação se manteve estável pelo período de 60 dias sob refrigeração ao contrário do extrato livre que a partir do 30º dia perdeu 56% de compostos fenólicos. Além disso, as análises de toxicidade *in vitro* e *in vivo* demonstraram que tanto o extrato livre quanto associado às nanoemulsões não apresentaram toxicidade nas concentrações de 50, 200 e 400 µg/mL no teste de citotoxicidade frente a linhagem não tumoral (3T3 fibroblastos) e nas concentrações de 0,5; 1,0 e 2,0 mg/mL no teste de sobrevivência dos *Caenorhabditis elegans* (SILVA, 2018). Assim como, a microencapsulação do suco de *P. peruviana*, utilizando-se maltodextrina misturada com goma arábica, alginato e pectina para aumentar a estabilidade em fluídos de digestão simulados. As microcápsulas mostraram reter mais de 75% dos compostos fenólicos para todos os tipos de goma. *In vitro* foi possível verificar que a liberação de compostos fenólicos das microcápsulas foi maior no fluido intestinal simulado do que no meio gástrico (DAG; KILERCIOGLU; OZTOP, 2017).

3.2 CÁLICE DE *P. peruviana*

O nome *Physalis* provém do grego, em que "*Physa*" significa bolha ou bexiga, referindo-se ao cálice que sustenta seus frutos comestíveis (LICODIEDOFF, 2012). O cálice, formado por cinco sépalas, também conhecido como cesta de frutas ou lanterna chinesa é um invólucro que protege naturalmente o fruto ao longo do seu desenvolvimento e amadurecimento, protegendo-o contra pragas, doenças e clima. A estrutura, ainda, é considerada uma excelente fonte carboidratos durante os primeiros 20 dias de crescimento e desenvolvimento do fruto (TAPIA; FRIES, 2007; PUENTE et al., 2011). Outra característica que merece destaque relaciona-se à sua coloração que indica o ponto de colheita. Rodrigues e colaboradores (2012) enfatizam que o fruto de *P. peruviana* deve ser colhido quando o cálice apresentar coloração amarelo-esverdeado até amarelo-amarronzado. Nessas fases os frutos apresentam maiores massas, diâmetros e acúmulo de sólidos solúveis totais. Ademais, o cálice aumenta a conservação do fruto, pois a vida de prateleira aumenta, alcançando um mês; enquanto que sem o cálice é em torno de 4 a 5 dias (CEDEÑO; MONTENEGRO, 2004).

O cálice é importante, ainda, por ser o principal depósito das fisalinas que foram identificadas na espécie *P. alkekengi* (JI et al., 2012; XU et al., 2012; HUANG et al., 2014a), as quais são moléculas exclusivas do gênero *Physalis*. As fisalinas são moléculas de estruturas bastante complexas, apresentam lactona e γ lactona fundida ao anel D, sendo derivados esteroidais do tipo 13,14-sec 16,24 ciclo ergostano, carbonilados em C-15 (TOMASSINI et al., 2000). As concentrações desses compostos na planta são variáveis, sendo a fisalina A (Figura 2) a mais abundante e, dependendo das condições de conservação, mais estável em relação às fisalinas O e P (XU et al., 2012). Já foram descobertas várias fisalinas, sendo a mais recente a fisalina X (HUANG et al., 2014a) e também já foram identificadas as isofisalinas (JI et al., 2012). Além disso, estudos identificaram os vitanolídeos como moléculas da *Physalis*, extraídos por etanol, cuja atividade antitumoral, imunossupressora e hepatoprotetora vêm sendo estudada (GAUTAM; DWIVEDI; KUMAR, 2015). O local de colheita e a parte da planta de onde se obtém o extrato, consistem em outros fatores importantes, visto que já foi verificado que tanto o fruto como o cálice são os locais de maior acúmulo de compostos fenólicos, sendo mais proeminente no cálice, de acordo com o observado por Medina-Medrano e colaboradores (2015) que compararam as diferentes partes da planta (fruto, folha e cálice) de espécies de *Physalis* (*P.angulata, P. hederifolia var. hederifolia, P. solanacea*, *P. patula* e *P. subulata).*

Figura 2 – Estrutura química da fisalina A



Muitos utilizam o cálice em decoração (de doces finos ou outros produtos alimentícios), contudo na indústria de alimentos, muitas vezes, essa parte da planta é considerada subproduto (LICODIEDOFF, 2012). No entanto, alguns estudos relacionados ao cálice encontram-se descritos, destacando seus benefícios. Avaliou-se com o uso de um modelo de fagocitose *in vitro* (infecção por *Leishmania panamensis* em macrófagos murinos), o efeito anti-inflamatório dos extratos etanólicos ou etéreos dos cálices de *P. peruviana* no processo fagocítico. A atividade anti-inflamatória descrita neste modelo relaciona-se a um efeito imunomodulador exercido sobre macrófagos infectados, que "bloqueiam" direta ou

indiretamente sua capacidade de secretar mediadores pró-inflamatórios solúveis (MARTÍNEZ et al., 2010).

Castro e colaboradores (2015) avaliaram a atividade anti-inflamatória do extrato etéreo total de cálices de *P. peruviana* em protocolos preventivos e terapêuticos em um modelo de colite em ratos, induzido por ácido TNBS. O tratamento com o extrato produziu melhora significativa no tecido do cólon, tanto a nível macroscópico como histológico. Deste modo, o extrato apresentou atividade anti-inflamatória intestinal, demonstrando que o cálice desta espécie apresentou-se como uma fonte promissora de metabólitos que poderiam ser utilizados no tratamento da doença inflamatória intestinal.

Para Franco e colaboradores (2007), 38 frações secundárias foram logradas por cromatografia em coluna a partir da fração hidroalcoólica primária de extratos do cálice, das quais seis foram avaliadas no modelo de inflamação aguda em ratos. Identificou-se as principais frações responsáveis pela atividade anti-inflamatória, promissoras no desenvolvimento de fitoterápicos, porém, faz-se necessária a realização de estudos adicionais buscando evidenciar como isolar e identificar os compostos responsáveis pela atividade e para o mecanismo envolvido no efeito anti-inflamatório.

Os mesmos pesquisadores avaliaram a melhor fração obtida pela extração dos cálices de *P. peruviana*, a qual foi purificada por vários métodos cromatográficos para obter uma mistura inseparável de dois novos ésteres de sacarose denominados: peruviose A (1) e peruviose B (2). Elucidou-se as estruturas dos novos compostos utilizando métodos espectroscópicos e transformações químicas. A atividade anti-inflamatória da mistura de peruvioses foi avaliada pelo método de edema de pata em ratos. Como resultado, as peruvioses não produzem efeitos colaterais no fígado e rins. Além disso, atenuaram significativamente a inflamação induzida por λ-carragenina em uma dose-dependente. Este trabalho foi o primeiro a relatar a presença de ésteres de sacarose em cálices de *P. peruviana* demonstrando um potente efeito anti-inflamatório. Estes resultados sugerem o potencial dos ésteres de sacarose do gênero *Physalis* como uma alternativa natural para tratar doenças inflamatórias (FRANCO et al., 2014).

Conforme Toro e colaboradores (2014) a fração butanólica do cálice foi considerada promissora devido às suas atividades anti-inflamatórias e antioxidantes. Portanto, foi utilizada uma abordagem guiada por bioensaio para isolar e identificar a rutina e a nicotoflorina a partir dos dados de espectroscopia por ressonância magnética nuclear (RMN) e espectrometria de massas (MS). A identificação da rutina em cálices de *P. peruviana* apoia a possível utilização deste material de resíduos para preparações fitoterapêuticas, nutracêuticas e cosméticas.

Relevante, também, é o alto teor de compostos fenólicos e capacidade antioxidante no cálice. Estudo analisou separadamente os frutos e os cálices de *P. peruviana* obtidos da Colômbia. Verificou-se maior quantidade de compostos fenólicos e um teor muito maior no cálice (195,44 ± 3,88 mg.100 g^{-1} de peso seco) do que na fruta (2,18 ± 0,71 mg.100 g^{-1} de peso seco). A capacidade antioxidante foi avaliada por ORAC (*Oxigen Radical Absorbance Capacity*), sendo que o cálice de *P. peruviana* apresentou 24,29 ± 3,11mmol Trolox.100 g^{-1}, enquanto o fruto resultou em uma menor capacidade antioxidante (3,29 ± 0,58 mmol Trolox.100 g^{-1}). Ainda, foi possível identificar no cálice dois diferentes glicosídeos de quercetina (sendo a rutina o composto fenólico majoritário) e de kaempferol, mas apenas foram encontrados vestígios de rutina (quercetina 3-O-rutinosídeo) e kaempferol 3-O-rutinosídeo na fruta. Os ácidos 3-O-cafeoilquínico e 5-O-cafeoilquínico também foram identificados no cálice, contudo, na fruta não foram observados. Menciona-se, ainda, que o cálice apresentou as maiores concentrações de flavonóis e derivados de ácido hidroxicinâmico comparados aos frutos latino-americanos analisados, salientando a importância da utilização do cálice em novos produtos (GIRONÉS-VILAPLANA et al., 2014).

3.3 A IMPORTÂNCIA DOS BIOATIVOS EM RESÍDUOS DE FRUTAS E ANÁLISE DOS COMPOSTOS FENÓLICOS

No que tange à produção de frutas no mundo, o Brasil ocupa a terceira posição e com isso o processamento dos sucos de frutas nas indústrias resulta em um grande volume de resíduos, os quais podem ser explorados para a produção de substâncias altamente valorizadas (FORSTER-CARNEIRO et al., 2013). Um problema ocasionado por isso consiste no descarte desses resíduos, uma vez que é agravado por restrições ambientais atuais, tais como as que constam na Política Nacional de Resíduos Sólidos e na Resolução do CONAMA nº 313 de 29 de setembro de 2002, que dispõe sobre o Inventário Nacional de Resíduos Industriais. Assim, ressalta-se a importância da utilização destes resíduos, pois muitas vezes, representam uma importante fonte de açúcares, minerais, ácidos orgânicos, fibra dietética e compostos fenólicos. Além disso, esses compostos possuem várias ações benéficas para a saúde que incluem atividade antitumoral, antiviral, antibacteriana, cardioprotetora e antimutagênica (SONJA; JASNA; GORDANA, 2009). Nas frutas o processamento origina dois principais subprodutos, a casca e as sementes, sendo que o extrato desses resíduos contém uma quantidade considerável de compostos bioativos (GOOT et al., 2016). Para exemplificar, vários estudos relataram a exploração de resíduos de algumas frutas, como uma nova fonte de

ingredientes alimentares, tais como sementes de *Zizyphus spina-christi* (jujuba de espinhos de Cristo) (EMBABY; MOKHTAR, 2011), sementes de manga (TORRES-LEÓN et al., 2016), bagaço de maçã (SUDHA; BASKARAN; LEELAVATHI, 2007) e subproduto da polpa de *goldenberry* ou do processamento de suco (resíduo em pó) (MOKHTAR; SWAILAM; EMBABY, 2018). O processamento industrial de *P. peruviana* gera dois tipos de resíduos, a baga (incluindo sementes e pele) que representa 27 % do peso das frutas, e o cálice que representa 5% da fruta crua. Cerca de 33 toneladas de resíduos de cálice são gerados por hectare cultivado de *P. peruviana* (RAMADAN, 2011).

Entende-se como relevante o destaque à importância do conhecimento detalhado sobre esses resíduos e sua caracterização química, contribuindo para sua utilização como fonte de compostos bioativos (ABDENNACER et al., 2015). Esses, são encontrados em pequenas quantidades nos alimentos e são considerados como ingredientes não nutricionais, porém vitais para a manutenção da saúde humana (PATIL et al., 2009). Em meados de 1980, surgiu no Japão a terminologia de alimentos funcionais. O conceito se refere a alimentos usados como parte de uma dieta normal, fisiologicamente benéficos, reduzindo o risco de doenças crônicas, além de suas funções básicas nutricionais. Esses alimentos, designados para “uso específico de saúde” (FOSHU, do inglês *foods for specified health use*), apresentam um selo de aprovação do Ministério da Saúde e Bem-Estar japonês. Deste modo o conceito foi rapidamente adotado no mundo (COSTA; ROSA, 2016).

Entre os compostos bioativos, os fenólicos são compostos poli-hidroxilados, constituindo um dos grupos mais extensos de produtos químicos presentes no reino vegetal. Estes mostram diversidade estrutural variando de fenólicos simples a complexos, bem como compostos altamente polimerizados. Os compostos fenólicos de alto peso molecular com estrutura complexa são frequentemente referidos como polifenóis (BALASUNDRAM et al., 2006; SINGH et al., 2017). Além disso, eles são considerados os principais constituintes das frutas e dos seus resíduos (BATAGLION et al., 2015). Os polifenóis são compostos que possuem mais de 9000 substâncias identificadas e podem ser divididos em grupos de acordo com à sua estrutura química: flavonoides (isoflavonoides, antocianidinas, flavanois, flavonois, flavanonas e flavonas) e não flavonoides (ácidos hidroxicinâmicos e hidroxibenzoicos, estilbenos, lignoides e cumarinas) (TSAO, 2010). Em geral, a presença de compostos fenólicos em produtos alimentares é de extrema importância, pois eles possuem muitos benefícios para a saúde, podendo atuar como anticancerígenos, antitrombóticos, antiulcerativos, antiarteriogênicos, antialérgicos, anti-inflamatórios, antioxidantes,

imunomoduladores, antimicrobianos, agentes cardioprotetores, vasodilatadores e analgésicos (BALASUNDRAM et al., 2006; SINGH et al., 2017).

Para a extração de compostos fenólicos existem muitos métodos disponíveis, porém nenhum pode ser categorizado como um método padrão. Tal extração é principalmente afetada por vários parâmetros (como o tamanho de partícula das amostras, o tipo de solvente utilizado, a relação soluto/solvente, taxa de agitação, eficiência de transferência de massa e temperatura utilizada). A etapa importante é selecionar as condições ótimas necessárias para a extração. Além disso, o passo crucial na quantificação desses compostos é o método de preparação das amostras e a influência da precisão e da repetibilidade durante a análise (ZHAO et al., 2011). As amostras em pó fino melhoram consideravelmente a extração de compostos fenólicos devido ao aumento da área superficial e ruptura das paredes celulares presentes no material vegetal utilizado. Geralmente solventes orgânicos puros, tais como metanol, etanol, acetona e acetato de etila, bem como as suas combinações com água (dependendo da seletividade, preço e recuperação) são utilizados para extrair esses componentes (KHODDAMI; WILKES; ROBERTS, 2013; TALUKDAR, 2013).

As técnicas espectrofotométricas (como as que utilizam o reagente Folin-Ciocalteu) são muito empregadas para quantificação dos compostos fenólicos totais (KHODDAMI; WILKES; ROBERTS, 2013; TALUKDAR, 2013). Apesar de serem econômicas, simples e rápidas, não são seletivas e não quantificam compostos fenólicos individuais (KHODDAMI; WILKES; ROBERTS, 2013). Por outro lado, a cromatografia líquida de alta eficiência (CLAE) é comumente usada para elucidar os perfis de compostos fenólicos presentes em muitas espécies vegetais (KHODDAMI; WILKES; ROBERTS, 2013; TALUKDAR, 2013).

Portanto, o cálice de *P. peruviana* é um resíduo, que ainda pode ser mais explorado, o qual apresenta variados compostos bioativos, destacando-se o flavonoide (rutina), como um dos compostos majoritários, e pode ser analisado utilizando essas técnicas de análise (BALLESTEROS-VIVAS et al., 2019a; GIRONÉS-VILAPLANA et al., 2014; MEDINA et al., 2019).

3.4 CAPACIDADE ANTIOXIDANTE E RUTINA

As espécies reativas de oxigênio (EROs) são um subproduto do metabolismo celular normal em plantas. Porém, quando estas são submetidas a condições de estresse ocorrem alterações na produção de EROs afetando a homeostase. Estas espécies reativas formadas começam a inativar enzimas rapidamente, degradar macromoléculas importantes na

manutenção orgânica da planta, danificar organelas celulares vitais e desestabilizar as membranas celulares, principalmente, através da indução da degradação de pigmentos, proteínas, lipídios e ácidos nucléicos, acarretando o processo de morte celular (KARUPPANAPANDIAN et al., 2011).

Assim como, no corpo humano a molécula de oxigênio pode produzir uma reação gerando EROs desencadeados por alguns fatores exógenos e/ou processos metabólicos endógenos. As EROs incluem uma série de moléculas quimicamente reativas, como o peróxido de hidrogênio (H_2O_2), superóxido ($O_2^{\bullet-}$) e radical hidroxila (•OH). Quando a geração de EROs é induzida por vários estímulos no organismo e excede a capacidade antioxidante do mesmo, resultará em uma variedade de processos fisiopatológicos (YANG; GUO; YUAN, 2008).

Nos últimos anos, inúmeros sequestradores naturais e sintéticos das EROs e antioxidantes vêm sendo pesquisados e desenvolvidos para proteger biomoléculas contra o ataque de EROs e/ou para suprimir o dano resultante (YANG; GUO; YUAN, 2008). Como por exemplo, extratos aquosos de 30 plantas foram investigados por suas propriedades antioxidantes usando o teste de capacidade de eliminação de radicais (DPPH e ABTS), teste da capacidade de desativação do radical peroxila (ORAC), ensaio de superóxido dismutase (SOD) e teste de potencial antioxidante redutor férrico (FRAP). O teor fenólico total também foi determinado pelo método de Folin-Ciocalteu. Sendo que, as propriedades antioxidantes e o conteúdo fenólico total diferiram significativamente entre as plantas selecionadas. Verificou-se que os extratos aquosos de carvalho (*Quercus robur*), pinho (*Pinus maritima*) e canela (*Cinnamomum zeylanicum*) possuíam as maiores capacidades antioxidantes na maioria dos métodos utilizados e, portanto, poderiam ser fontes ricas de antioxidantes naturais. Os extratos aquosos de erva mate (*Ilex paraguariensis*) e cravo (*Eugenia caryophyllus clovis*) também apresentaram fortes propriedades antioxidantes e um elevado teor fenólico. Foi possível observar uma relação significativa entre a capacidade antioxidante e o conteúdo fenólico total, indicando que os compostos fenólicos são os principais contribuintes para as propriedades antioxidantes dessas plantas (DUDONNÉ et al., 2009).

Flavonoides são conhecidos por suas significantes propriedades de eliminação de radicais de oxigênio *in vivo* e *in vitro*. Além desses efeitos importantes, eles possuem propriedades estabilizadoras da membrana e também afetam alguns processos de metabolismo intermediário (BOMBARDELLI; MORAZZONI, 1993). Além disso, muitos estudos demonstram a importância da ação contra radicais livres dos flavonoides. Dentre eles, extensas pesquisas com o objetivo de beneficiar seres humanos foram descritos, ressaltando

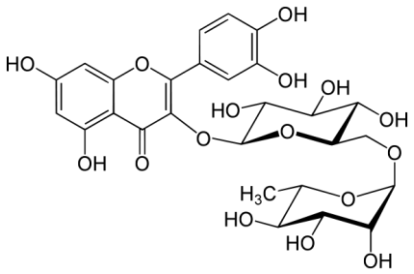
que os flavonoides possuem numerosas atividades biológicas, como antioxidantes, anti-inflamatórias, estrogênica, antitumoral, citotóxica, antiviral e outras (HARBORNE; WILLIAMS, 2000).

De acordo com Zhang e colaboradores (2010), antioxidantes sintéticos têm seu uso generalizado como aditivos alimentares em muitos países. No entanto, antioxidantes naturais são preferidos aos sintéticos, pois possíveis problemas de saúde podem estar relacionados aos sintéticos (HOU, 2003; PRIOR, 2004). Atualmente, compostos naturais com capacidade antioxidante, como tocoferóis e flavonoides vêm atraindo um interesse crescente (ERKAN; AYRANCI; AYRANCI, 2008).

A capacidade dos flavonoides de eliminar os radicais livres e quelar íons metálicos levaram a análise de suas atividades como antioxidantes na peroxidação lipídica. A atividade antioxidante está relacionada à sua estrutura química, coeficientes de partição e taxa de reação com os radicais de interesse (BOYLE et al., 2000).

Nesse sentido, a rutina (quercetina-3-rutinosídeo) (Figura 3) é um derivado natural da flavona que foi descoberto pela primeira vez no século XIX em trigo mourisco ou também chamado de trigo sarraceno. É um composto de baixo peso molecular amplamente distribuído em vegetais, frutas, bebidas derivadas de plantas, como o chá e vinho. Pouca ou nenhuma rutina dietética é absorvida intacta uma vez que a microflora intestinal metaboliza a rutina a uma variedade de compostos que podem ser absorvidos (YANG; GUO; YUAN, 2008). O banco de dados de suplementos alimentares lista mais de 860 produtos que contêm rutina atualmente comercializados nos EUA (GULLÓN et al., 2017).

Figura 3 – Estrutura química da rutina



Mais de 70 espécies de plantas contêm rutina. Trigo sarraceno (*Fagopyrum esculentum* Moench) da família Polygonaceae é uma importante fonte de rutina (KIM et al., 2005). O trigo sarraceno é de origem chinesa e tem um cultivo histórico devido ao seu interesse na medicina tradicional. Há relatos em vários livros agrícolas antigos e obras

medicinais (GULLÓN et al., 2017). No Japão, o trigo sarraceno é cultivado, não apenas para ser usado em alimentos tradicionais, mas também como ingrediente de alimentos saudáveis (SUZUKI et al., 2015). Outras fontes comerciais de rutina incluem *Ruta graveolens* L. (Rutaceae), *Sophora japonica* L. (Fabaceae) e *Eucalyptus* spp. (Myrtaceae) (CHUA, 2013).

A atividade antioxidante da rutina *in vitro* é comparável à do ácido ascórbico (YANG; GUO; YUAN, 2008) e superior à do alfa-tocoferol (FRANKEL; MEYER, 2000). A rutina tem sido amplamente utilizada no tratamento de doenças devido suas diversas atividades farmacológicas, incluindo atividade antialérgica (OLIVEIRA; FERNANDES; VIEIRA, 2006), anti-inflamatória e vasoativa, antitumoral, antibacteriana, propriedades antivirais e antiprotozoárias (CALABRÒ et al., 2005). Além disso, também foram relatadas atividades hipolipidêmica e citoprotetora (CASA et al., 2000), antiespasmódica e anticancerígena (WEBSTER; GAWDE; BHATTACHARYA, 1996) e prevenção da ulceração da mucosa gástrica em modelos animais (LIU et al., 2013). Outros estudos demonstraram um efeito dose-resposta da rutina na inibição da peroxidação das lipoproteínas de baixa densidade (LDL) (JIANG et al., 2007) e atividade antioxidante da rutina na reação de Fenton (CAILLET et al., 2007). Em um estudo *in vivo*, foi avaliada a inibição da peroxidação lipídica e a melhora de sua ação antioxidante no fígado, rim e cérebro de ratos diabéticos. Eles descobriram que a administração via oral de rutina (100 mg/kg) por um período de 45 dias teve um efeito antioxidante significativo na diabetes induzida experimentalmente por estreptozotocina (KAMALAKKANNAN; PRINCE, 2006).

Por outro lado, a principal desvantagem da rutina é a sua baixa biodisponibilidade, causada principalmente por sua baixa solubilidade em meio aquoso, baixa estabilidade e permeabilidade à membrana limitada (GULLÓN et al., 2017).

3.5 NANOTECNOLOGIA E A INDÚSTRIA DE ALIMENTOS

A demanda atual dos consumidores por produtos alimentícios saudáveis e mais naturais levou a um interesse crescente da indústria de alimentos na substituição de ingredientes sintéticos por outros mais naturais (WALKER, DECKER; MCCLEMENTS, 2015; MCCLEMENTS; BAI; CHUNG, 2017). Somado a isso, a indústria e os pesquisadores estão dando ênfase à nanotecnologia para abordar questões relevantes para a alimentação e nutrição, impulsionados pela necessidade de sistemas comestíveis capazes de encapsular, proteger e liberar compostos funcionais (SILVA, CERQUEIRA; VICENTE, 2012; ARANCIBIA et al., 2017).

Entre os conceitos de nanotecnologia existentes, destaca-se a ISO/TC 229, que a define como: 1) compreensão e controle da matéria e processos em nanoescala, em geral, mas não exclusivamente, abaixo de 100 nanometros em uma ou mais dimensões, em que o aparecimento de fenômenos ligados ao tamanho geralmente possibilita novas aplicações; e 2) utilização de propriedades dos materiais em escala nanométrica que diferem das propriedades dos átomos, das moléculas e da matéria a granel, para o desenvolvimento de materiais, dispositivos e sistemas aprimorados que exploram essas novas propriedades. Ou seja, para um dispositivo ser considerado nanotecnológico, não basta ter dimensões nanométricas; ele deve apresentar propriedades diferenciadas associadas à nanoescala.

A nanotecnologia tem uma aplicação próspera em vários setores, e sua aplicação na ciência de alimentos é um evento recente. A segurança e a qualidade dos alimentos são uma grande preocupação, desta forma, os pesquisadores buscaram por várias tecnologias na tentativa de melhorar essas questões. Em especial, a aplicação da nanotecnologia na indústria alimentar levou à produção de alimentos com melhor estabilidade térmica, solubilidade e com maior biodisponibilidade oral. Além disso, a incorporação de elementos funcionais aos alimentos vem sendo pesquisada por um bom tempo, e a nanotecnologia preparou-se para isso, levando o desenvolvimento de nanoemulsões e nanocompósitos (HAMAD et al., 2018).

O aumento da vida de prateleira dos produtos alimentícios, o melhor rastreamento de contaminantes, o armazenamento aprimorado, a incorporação de suplementos saudáveis ou agentes antibacterianos nos alimentos, são alguns exemplos da grande contribuição da nanotecnologia na ciência dos alimentos (NEO et al., 2013). Independente do meio escolhido para a preservação é importante ressaltar que a qualidade e o sabor dos alimentos não devem ser interferidos e devem permanecer tão intactos quanto possível. A incorporação de nutracêuticos, a liberação de nutrientes, agentes de gelificação e viscosificantes, a fortificação com vitaminas e minerais, bem como a nanoencapsulação de sabor e aroma são algumas das formas de processamento de alimentos com nanomateriais (HUANG; YU; RU, 2010).

Vale salientar que o valor de mercado das embalagens de alimentos da indústria aumentou US$ 2,5 bilhões em 2012 (SCOTT; CHEN, 2013). É pertinente notar que com esta tecnologia, o problema da escassez de alimentos em algumas partes do mundo pode ser resolvido com mais facilidade. Várias formas de nanossistemas, como nanopartículas sólidas, nanofibras, nanocápsulas e nanoemulsões são alguns dos nanomateriais que encontraram seu caminho nos setores de processamento de alimentos, embalagens e preservação (DUNCAN, 2011).

No desenvolvimento de embalagens, a aplicação da nanotecnologia pode fornecer diversas funções como: incorporação de nanopartículas com propriedades antimicrobianas; nanosensores capazes de detectar produtos químicos, agentes patogênicos e toxinas em alimentos; nanopartículas bioativas capazes de manter os compostos em condições ideais, até a sua migração para o produto alimentício; e nanocompósitos que melhoram as propriedades de flexibilidade, barreira a gases, umidade e quanto à absorção de irradiação UV dos materiais aos quais são incorporados, assim como a estabilidade frente à temperatura (ALMEIDA et al., 2015).

Entre os nanossistemas, neste trabalho foi dado ênfase às nanoemulsões, as quais são uma classe de emulsões com diâmetro de gotículas (O/A e A/O) na escala nanométrica. O tamanho de gotículas pode ser bastante variável, sendo considerados diâmetros de 1-100 nm (MASON et al., 2006), 20-200 nm (SOLANS et al., 2005), 20-300 nm (YILDIRIM, OZTOP; SOYER, 2017), 50-500 nm (CHAARI et al., 2018) e 50-1000 nm (EZHILARASI et al., 2013). Essas variações encontradas na literatura em relação ao diâmetro de gotícula se devem as diferenças na composição do sistema (tensoativos/ co-tensoativos, óleo e água) e no método de preparação utilizado (SALEM; EZZAT, 2018).

As nanoemulsões e microemulsões óleo-em-água são dispersões coloidais adequadas para encapsulação e entrega de componentes lipofílicos na indústria. É possível produzir tanto microemulsões como nanoemulsões, utilizando os mesmos componentes (óleo, água e tensoativo), porém usados em diferentes proporções, sendo que as nanoemulsões geralmente apresentam-se como um líquido opaco/ leitoso e as microemulsões possuem aspecto transparente/translúcido. A principal diferença entre essas duas formulações é que as nanoemulsões são termodinamicamente instáveis, enquanto as microemulsões são termodinamicamente estáveis. Contudo, deve salientar-se que as microemulsões são apenas termodinamicamente estáveis sob uma gama particular de composições e condições ambientais, e podem degradar se estas condições forem alteradas (por exemplo, devido à diluição, adição de ingredientes ou alterações de temperatura). Além disso, as microemulsões apresentam uma alta concentração de tensoativos, sendo uma característica negativa. (RAO; MCCLEMENTS, 2012).

As nanoemulsões, do ponto de vista físico-químico, são sistemas cineticamente estáveis. A alta estabilidade cinética pode ser obtida quando o método de preparação, a composição e os componentes do sistema são adequadamente selecionados (SOLANS; SOLÉ, 2012). A preparação pode ser realizada por uma variedade de métodos, classificados como métodos de alta energia e baixa energia. A abordagem de alta energia utiliza

dispositivos mecânicos capazes de gerar forças disruptivas intensas que conduzem à formação de gotículas de óleo, enquanto rompem-se as fases óleo e água (tais como, homogeneizadores de válvulas de alta pressão, microfluidizadores e métodos de sonicação). Já as abordagens de baixa energia dependem da formação espontânea de gotículas de óleo dentro de sistemas mistos de emulsionantes/óleo/água quando a solução ou as condições ambientais são alteradas, por exemplo, métodos de inversão de fase, deslocamento de solvente e emulsificação espontânea (MCCLEMENTS, 2012; SILVA; CERQUEIRA; VICENTE, 2012).

No presente trabalho, as nanoemulsões foram preparadas usando o mecanismo de emulsificação espontânea que ocorre quando uma fase orgânica e uma fase aquosa são misturadas, e após realiza-se a evaporação/eliminação do solvente orgânico. A fase orgânica é uma solução homogênea de óleo, tensoativo lipofílico e solvente miscível em água, a fase aquosa consiste em tensoativo hidrofílico e água. Destaca-se que a composição da fase orgânica inicial é de grande importância para o processo de emulsificação espontânea e, portanto, para as propriedades físico-químicas das emulsões obtidas (BOUCHEMAL et al., 2004).

A emulsificação espontânea pode ser utilizada para encapsular compostos bioativos na indústria de alimentos, bebidas e farmacêutica. Esse método possui várias vantagens em relação aos métodos de alta energia e outros de baixa energia, incluindo proteção de compostos sensíveis frente às condições severas dos métodos de alta energia, capacidade de minimizar a quantidade de tensoativo, remoção de cotensoativos e estabilidade térmica (MEHRNIA et al., 2016).

Na indústria de alimentos, as nanoemulsões são utilizadas na produção de edulcorantes, óleos aromatizados, molhos para saladas, bem como outros alimentos processados. A operação se dá a partir da liberação de diferentes aromas e sabores com vários estimulantes, como calor, pH, ondas ultrassônicas, dentre outros (HAMAD et al., 2018). Elas retêm as características de forma eficaz e impedem as reações enzimáticas, bem como as de oxidação. As nanoemulsões possuem várias vantagens em relação às emulsões convencionais, uma vez que são termicamente estáveis e de menor tamanho. Devido à sua grande área superficial, as nanoemulsões podem interagir com uma série de componentes biológicos, tais como as enzimas no trato gastrointestinal (TGI). Por exemplo, a lipase facilmente digere as pequenas gotículas de nanoemulsões no TGI (ZARIF, 2003). Além disso, as nanoemulsões sob a forma de carboidratos ou proteínas ajudam a melhorar a textura e levam à uniformidade do sorvete. Também, podem conter agentes antimicrobianos em sua composição, como os tensoativos não iônicos e alguns óleos essenciais, sendo utilizadas para evitar o crescimento

microbiano e consequentemente reduzir a deterioração dos alimentos, assim como, para fins de descontaminação de embalagens de alimentos (PRADHAN et al., 2015).

Até o momento, não foram encontradas pesquisas com nanoemulsões contendo extrato do cálice de *P. peruviana*, comprovando o ineditismo do estudo realizado. Somente, nanopartículas de prata (AgNPs) foram sintetizadas usando o cálice de *P. peruviana* (PATRA et al., 2018). Rashid e Sabir (2014) realizaram a biossíntese de nanopartículas de prata (AgNPs) utilizando o extrato aquoso das frutas comestíveis de *P. peruviana*. E em nosso grupo de pesquisa, Silva e colaboradores (2019) desenvolveram nanoemulsões contendo o extrato da fruta de *P. peruviana*. Alguns estudos de nanopartículas com outras espécies de *Physalis* e com outros focos de aplicação também foram encontrados. Nanopartículas de óxido de zinco (ZnO-NPs) foram sintetizadas usando *Physalis alkekengi* (QU et al., 2011). Assim como, nanopartículas de prata (AgNPs) foram sintetizadas com fotoindução verde utilizando o extrato aquoso das folhas de *Physalis angulata* (KUMAR et al., 2017).

3.6 NANOTOXICOLOGIA

Em relação a nanotecnologia, ainda há uma necessidade urgente de mais estudos farmacocinéticos e toxicológicos sobre os nanomateriais, especialmente antes de serem comercializados (MISSAOUI; ARNOLD; CUMMINGS, 2018). O perfil toxicológico dos nanomateriais engenheirados nos ecossistemas e na saúde humana deve ser cuidadosamente avaliado. A nanotoxicologia é uma área de pesquisa interdisciplinar dedicada à avaliação dos perigos associados à esses nanomateriais e está se expandindo rapidamente. Muitas técnicas físico-químicas e metodologias bioquímicas foram propostas e atualmente são usadas para caracterizar nanopartículas do ponto de vista toxicológico (BETTAZZI; PALCHETTI, 2019).

Testes preliminares são realizados *in vitro* como o de citotoxicidade (HILLEGASS et al., 2010). No entanto, há relatos que os ensaios *in vivo* seriam mais adequados para avaliar a biossegurança das nanopartículas. Porém, o custo mais alto e o tempo experimental mais longo dos ensaios *in vivo*, acabam limitando o uso da maioria das análises convencionais que utilizam mamíferos (WU et al., 2019).

O modelo animal que utiliza o nematódeo *Caenorhabditis elegans* (*C. elegans*) foi postulado em 1965 por Sydney Brenner, fornecendo aos pesquisadores a possibilidade de investigar os efeitos biológicos com custo mais baixo (BRENNER, 1974). Em termos de nanotoxicidade *in vivo*, a distribuição e liberação das nanopartículas, bem como seus impactos nos tecidos ou órgãos, são geralmente avaliados. *C. elegans* possui quatro sistemas orgânicos,

iguais aos dos vertebrados, incluindo o sistema neural, digestivo, imunológico e reprodutivo, os quais permitem que as descobertas com *C. elegans* sejam confiáveis e de grande valor. Além disso, esse modelo possui algumas vantagens, tais como, um ciclo de vida curto, um corpo minúsculo e transparente, fácil manuseio e informações genéticas bem caracterizadas. Foi o primeiro organismo multicelular a ter todo o seu genoma sequenciado e, a partir de 2012, foi o único organismo a ter o seu conectoma, isto é, diagrama de ligações neuronais concluído (WU et al., 2019).

Levando em consideração a temática abordada, neste estudo foi realizado o desenvolvimento de nanoemulsões contendo o extrato do cálice de *P. peruviana*. A estabilidade frente às diferentes condições de armazenamento foi analisada, quanto às características físico-químicas das formulações, bem como, a capacidade antioxidante e teor de rutina. Além disso, os aspectos de nanotoxicidade *in vitro* e *in vivo* foram avaliados e as formulações foram submetidas as condições gastrointestinais simuladas, a fim de avaliar as possíveis toxicidades e o perfil de liberação do composto bioativo majoritário nas diferentes etapas do trato.

4 ARTIGO 1 – NANOENCAPSULATION OF BIOACTIVE COMPOUNDS: CHALLENGES AND PERSPECTIVES

Artigo publicado na **Revista Current Opinion in Food Science, v. 26, p. 47-56, 2019.**

Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Food Science

Nanoencapsulation of bioactive compounds: challenges and perspectives

Maiara Taís Bazana, Cristiane Franco Codevilla and Cristiano Ragagnin de Menezes

Nanotechnology is increasingly used in food science, and one of the lines of research is the nanoencapsulation of bioactive compounds. While these compounds promote improvements in human health, they are often improperly absorbed. Thus, nanostructured systems improve several characteristics, such as protection against degradation, solubility, stability, and bioavailability, among others. However, the development of nanostructures faces many challenges, from choosing the best method to obtain them to identifying the type of nanomaterial ideal for a bioactive compound of interest. In addition, the characterization of toxicological effects is sought by specific regulation for safety in human consumption and the environment, such as the use of green synthesis.

Address
Department of Food Science and Technology, Rural Science Center, Federal University of Santa Maria, Santa Maria, Brazil

Corresponding author:
de Menezes, Cristiano Ragagnin (cristiano.ufsm@gmail.com)

Current Opinion in Food Science 2019, **26**:47–56

This review comes from a themed issue on **Functional foods and nutrition**

Edited by **Tatiana Emanuelli**

For a complete overview see the Issue and the Editorial

Available online 21st March 2019

https://doi.org/10.1016/j.cofs.2019.03.005

Introduction

Nanotechnology is generally defined as the design, production, and application of structures, devices, and systems where the control of the size and shape of the material is on the scale of 10^{-9} m [1]. This technology has thriving applications in many sectors, and its rapid development has transformed many areas of food science, especially those involving processing, packaging, storage, transport, functionality, and other aspects of food safety [2]. In this sense, industry and academic researchers are emphasizing nanotechnology to address issues relevant to food and nutrition, driven by the need for edible systems capable of encapsulating, protecting, and releasing functional compounds [3,4•].

The current consumer demand for healthier and more natural food products has led to growing interest in the food industry in replacing synthetic with natural ingredients, such as bioactive compounds [5,6]. Thus, since many biologically active compounds are lipophilic and poorly soluble, nanoencapsulation facilitates the delivery of these poorly bioavailable compounds into functional food ingredients [7] by increasing their absorption into cellular structures through favorable particle properties of form, size, and surface. These properties allow nanoparticles to increase potential solubilization, alter absorption pathways by modifying the rate and site of release, influence gastrointestinal dispersion, and prevent premature metabolic degradation of a bioactive compound [8].

With gathering research in food nanotechnology, public concerns about the safety of these products for human consumption and use also arise [9]. Therefore, a comprehensive assessment of toxicity and potential health risks is essential before they are made commercially available. However, no universal guidelines have been specifically developed for the safety assessment of nanomaterials in food. In light of this, further studies and regulations on the impacts of these nanomaterials on human and environmental health must be conducted and established to ensure food safety [10•].

On the basis of what has been learned so far, this review will address the key benefits of, challenges for, regulatory aspects of, and future prospects for the nanoencapsulation of bioactive compounds, as well as examining different aspects of the toxicity and safety of nanocarriers.

Benefits of nanoencapsulation for food science and technology

Bioactive compounds, considered essential for human health, are mostly hydrophobic and poorly soluble. Examples include phenolic compounds, carotenoids, essential oils, essential fatty acids, and insoluble vitamins. The main challenges for the application of these compounds in the pharmaceutical and food industries include low bioavailability and stability [7].

The incorporation of functional elements in foods has long been researched. Nanotechnology is a useful tool for this purpose, with nanoemulsions and nanocomposites both being developed [11]. Nanoencapsulation is a favorable alternative to preserve and protect bioactive

compounds against inappropriate environmental circumstances and increase their bioavailability and stability, thus favoring their application in food and pharmaceutical products [7]. In addition, encapsulation decreases volatility and increases chemical and thermal stabilities; protects against oxygen, light, pH, moisture, and gastric digestion; masks unpleasant taste and aroma; promotes controlled release; improves the solubility of lipophilic compounds in aqueous media, and enables the prolonged absorption of nutrients; and may confer and facilitate the development of packaging materials with antimicrobial and antioxidant potential. All of these advantages are made possible by reducing the size of nanoparticles, thus increasing the area per unit volume [12] (Figure 1).

Increasing the shelf life of food products, better tracking contaminants, improving storage, and incorporating healthy supplements or antibacterial agents into food are just a few examples of nanotechnology's major contributions to food science [13]. Incorporation of nutraceuticals; release of nutrients, gelling agents, and viscosifiers; vitamin and mineral fortification; and flavor and aroma nanoencapsulation are some forms of food processing with nanomaterials [14].

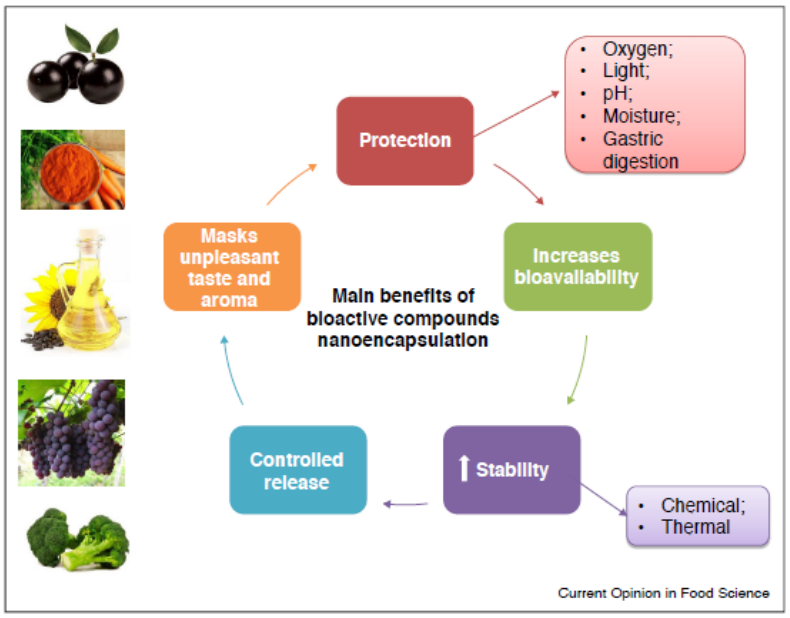
Nanotechnological challenges

Methods of nanoencapsulation and green nanotechnology

Nanoencapsulation involves the encapsulation of substances in nanocarriers by absorption, incorporation, chemical interaction, or dispersion [15,16]. Several nanoencapsulation techniques for bioactive compounds and nutraceuticals have been studied recently, including the production of different nanocarriers such as nanoemulsions; nanostructured lipid transporters; nanosuspensions; solid lipid nanoparticles (SLNs); liposomes and nanometer phytosomes; biopolymer SLNs; and micelles made of proteins, polysaccharides, or their complexes or conjugates [17]. In addition, the literature discusses other nanotransporters, such as inclusion complexes through cyclodextrins, amylose, and yeast cells, nanogels, nanofibers, nanosponges, or nanoparticles made with lipids and biopolymers [7] (Table 1). In light of this range of nanosystems, different technologies can clearly be used to nanoencapsulate food ingredients. The process requires some general equipment, including homogenizers, mills, and mixing devices, but implementing some techniques of nanoencapsulation requires specialized equipment, such as electrospinners, electrospray, nanospray dryers, and microfluidic devices [17].

Several natural and synthetic polymers are employed for nanoencapsulation. Natural encapsulating materials include polymers such as chitosan, alginates, cyclodextrins, and phospholipids, among others [30]. Synthetic polymers include biodegradable esters, such as poly(lactic-co-glycolic acid) (PLGA), polymers of poly-ε-caprolactone, and methacrylate [15]. Few studies have directly compared various methods or materials for a specific bioactive compound or ingredient, making it difficult

Figure 1



Benefits of bioactive compounds nanoencapsulation.

Table 1

Studies using different nanocarriers for the nanoencapsulation of bioactive compounds

Type of nanocarrier	Method of preparation	Bioactive compound	Major findings	References
Nanocapsules	Spray drying and electrospraying	Folic acid	High encapsulation efficiency (83.9%). Improved folic acid stability under different storage conditions over 60 days.	[18]
Nanotubes	Nanotube synthesis	Caffeine	α-Lactalbumin nanotubes synthesized in the presence of Mn^{2+} were highly stable during freeze-drying. α-LA nanotubes were very efficient at caffeine encapsulation (around 100%). Low temperature and high pH prevented caffeine release from α-LA nanotubes.	[19]
Poly(ethylene oxide) (PEO) nanofibers	Electrospinning	Tea tree oil	The nanofibers were plasma-treated to modify their surface; after plasma treatment, the release efficiency of nanofibers and the antibacterial activity of tea tree oil improved. The nanofibers increased the shelf life of beef.	[20]
Zein nanofibers	Electrospinaning	Fish oil and ferulic acid	Zein nanofibers exhibited nanoscale size of ~440 nm and showed high encapsulation efficiency (94%) and loading capacity of 20%. Loading ferulic acid into the nanofibers enhanced the oxidative stability of encapsulated fish oil. Fish oil loaded nanofibers showed an excellent release profile under gastrointestinal and enzymatic conditions.	[21]
Nanoemulsions	Low-energy emulsification method	Vitamin E	Revealed mean particle size around 86 nm; high encapsulation efficiency of near 100%; improved antioxidant and antimicrobial activity.	[22]
Cross-linked, pectin-coated solid lipid nano-particles (SLNs) and nanostructured lipid carriers (NLC)s	Solvent-diffusion and hot emulsification	Curcumin	SLNs and NLCs both exhibited high loading capacity. SLNs revealed excellent stability under storage conditions and in simulated gastrointestinal conditions.	[23]
Niosomes	Film-hydration method	α-Tocopherol	Niosomes were found on the nanometer scale (106.8–190 nm). The optimum condition for the encapsulation of α-tocopherol enhanced stability, encapsulation efficiency, and prolonged release.	[24]
Phytosome nanoparticles	Thin-film hydration method	Quercetin	The phytosome nanocapsules exhibited excellent encapsulation efficiency (98.4%). Phytosome nanocapsules enhanced the therapeutic benefits of quercetin in ovariectomized rats.	[25]
Nanogels	Self-assembly method	Rosemary essential oils	Particle size of less than 100 nm was achieved. The antibacterial activity of nanogel-encapsulated essential oils against *S. aureus* increased. Encapsulated essential oil in nanogels increased the transparency and tensile strength of starch-carboxy methyl cellulose films.	[26]
Biopolymer nanoparticles (nanospheres)	Thermal processing and electrostatic complexation of whey protein– pectin mixtures	Anthocyanins	Mean particle diameter was less than 200 nm. Exhibited high loading capacity before heating (55%). Enhanced thermal stability and lower antioxidant activity.	[27]
Nanoliposomes	Supercritical-assisted formation process	Olive pomace extract	The encapsulation efficiency of the supercritical-assisted liposome formation process was six times larger than the conventional thin-layer hydration method.	[28]
Cyclodextrin nanosponges (CDNS)	Solvent evaporation	Curcumin (CUR)	The photostability of CUR was enhanced 1.7 fold in pyromellitic dianhydride (PMDA)-crosslinked nanosponges. *In vitro* cytotoxicity study revealed increased toxicity of the drug nanosponge complex for MCF-7 cells at a lower concentration. PMDA-CDNS was found to be a potential nanocarrier for curcumin compared to di-phenyl carbonate (DPC)-CDNS.	[29]

to establish which method or material is superior for a specific bioactive compound [16]. Many encapsulation techniques and matrices can be applied to the same bioactive compound, which presents challenges for the development of nanoencapsulation. The choice of nanocarrier should be controlled by the nature of the bioactive compound, the presence of surface groups, temperature and pH during processing, release rate, and degree of cellular uptake, as well as economic considerations [12].

Nanoparticles can be synthesized using various methods, including (a) physical methods, such as mechanical grinding or milling; (b) chemical methods, such as coprecipitation or microemulsion; and (c) biological methods, using microorganisms, plants, or enzymes. Recently, various eco-friendly technologies to produce environmentally benign, non-toxic products are being developed using green nanotechnology and biotechnological tools. Nanoparticles synthesized using biological methods, which is also called green technology, have diverse natures, with greater stability and appropriate dimensions because they are synthesized through a one-step procedure [31].

The use of bacteria for the synthesis of nanomaterial has emerged as a novel approach for the synthesis of metal nanoparticles. Bacteria are known to produce inorganic materials either intra cellularly or extra cellularly. Microorganisms are considered as a potential biofactory for the synthesis of nanoparticles such as gold, silver, and cadmium sulphide [32]. Jayaseelan *et al.* [33] studied zinc oxide nanoparticles using bacteria *Aeromonas hydrophila* as eco-friendly reducing and capping agent. The synthesized ZnONPs had average particle size of around 57 nm, hydrophilic nature, highly stability and had significant antimicrobial activity.

The fungal mediated nanoparticle synthesis is a relatively recent research area. Fungi could be used as a source for the production of large amount of nanoparticles [32]. Chan and Don [34] selected and compared five species of fungi for their capability to synthesize Ag nanoparticles (AgNPs). Among the five fungi tested, *Pycnoporus sanguineus* and *Schizophyllum commune* were capable in synthesizing AgNPs with an average particle size of range from 52.8 to 103.3 nm, respectively.

The use of algae for the biosynthesis of nanoparticles is also possible. The ability of algae to accumulate metals and reduce metal ions makes them a good contender for the biosynthesis of nanoparticles [35]. Azizi *et al.* [36] synthesized zinc oxide nanoparticles using the brown marine macroalgae *Sargassum muticum* (*S. muticum*) aqueous extract. The ZnO NPs had hexagonal structures and the particle size ranges from 3 nm to 57 nm with a mean size of 42 nm.

The use of plant extract for synthesizing nanoparticles could be advantageous compared to other biological methods, eliminating the elaborate process of maintaining cell cultures. While fungi and bacteria require a comparatively longer incubation time for the reduction of metal ions, water soluble phytochemicals do it in a much lesser time [32]. Extracts from plants may act as both reducing and stabilizing agents in the synthesis of nanoparticles (Table 2), perhaps due to the presence of phytochemicals. The main phytochemicals responsible have been identified as flavonoids, xanthophylls, carotenoids, anthocyanins, and phenolic acids [41].

Physical methods of synthesis frequently entail highly expensive instruments, high temperature and pressure, and high energy consumption. Chemical methods often require costly metal salts and toxic or hazardous solvents. Meanwhile, green methods synthesize nanoparticles through biological routes (microorganisms, plants, or viruses) or their byproducts (such as proteins and lipids) with the help of various biotechnological tools. Green nanotechnology has garnered wide interest due to its inherent features of rapidity, simplicity, eco-friendliness, and lower cost [31,46,47].

According to Srivastava and Bhargava [48], green nanotechnology has two key aspects. The first aspect involves nano-products that provide solutions for environmental challenges, and the second involves producing nanomaterials or products containing nanomaterials with an aim to minimize harm to human health or the environment. However, it is necessary to foster and promote the development of green nanotechnology for human and environmental sustainability. Its commercialization will require concerted effort from researchers, governments, and other stakeholders. The development of this environmentally friendly technology could greatly accelerate human welfare [32,48].

Toxicity of nanoparticles

Nanotechnology represents a very advanced field of research and application. Nanomaterials can be constituents in a wide range of manufactured commercial and domestic products [49–51]. Although beneficial, advances in nanotechnology are also associated with expectations of growing potential toxicity and ecotoxicity, largely due to nanoparticles' unusual properties (shape, small size, chemical composition, structure, and increased surface area) [7,51].

Considering their small size, nanoparticles can enter and translocate the circulatory and lymphatic systems, ultimately reaching bodily tissues and organs [52]. Some nanoparticles, depending on their composition and size, can irreversibly damage cells by oxidative stress or organelle injury. For example, small particle size and high specific surface area increases reactivity and facilitates their passage across biological barriers [51,52]. Understanding the impacts of nanomaterials for human health

Table 2

Nanoparticles synthesized using different plant extracts

Reducing/stabilizing agent	Product	Average sizes	Comments	References
Tecoma castanifolia leaf	Zinc oxide nanoparticles	72.5 nm	Zinc oxide nanoparticles had antibacterial, antioxidant, and anticancer activity.	[37•]
Mangifera indica leaves	Zinc oxide nanoparticles	47.7 nm	Zinc oxide nanoparticles exhibited cytotoxicity against lung cancer A549 cell lines and antioxidant activity.	[38]
Rosemary	Iron nanoparticles	50.5 nm	Iron nanoparticles showed potent cytotoxicity effects on 4T1 and C26 cancer cell lines.	[39]
Pistacia Atlantica	Gold nanoparticles	55.0 nm	Gold nanoparticles had great antibacterial and antioxidant effects.	[40]
Alternanthera bettzickiana	Gold nanoparticles	100.0 nm	Gold nanoparticles exhibited significant anti-microbial activity against *Bacillus subtilis, Staphylococcus aureus, Salmonella typhi, Pseudomonas aeroginosa, Micrococcus luteus*, and *Enterobacter aerogenes*.	[41]
Cinnamaldehyde	Nanoparticles	455.2 nm	Nanoparticles had high encapsulation efficiency and sustained release effect.	[42]
Turmeric	Silver nanoparticles	18.0 nm	Silver nanoparticles exhibited excellent antibacterial activity against foodborne pathogens (*Escherichia coli* O157:H7 and *Listeria* monocytogenes).	[43]
Olive leaf	Silver nanoparticles	30.0 nm	Silver nanoparticles significantly inhibited bacterial growth against multi-drug-resistant *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Escherichia coli*.	[44]
Viburnum opulus fruit	Silver nanoparticles	25.0 nm	Silver nanoparticles presented potent anti-inflammatory activity.	[45]

and the environment is an important aspect of the development of nanotechnology [50].

According to Sia [49], nanomaterials or nanoparticles released into the atmosphere, soil, and surface water can persist in the environment for a long time or be absorbed by organisms, offering an eco-toxicological risk and biodegrading or bioaccumulating across the food chain. Nanomaterials consumed by humans can have a biological fate in the gastrointestinal tract. Nanomaterials should be completely digested and absorbed; if partially digested, encapsulated compounds may be incompletely released or it are resistant to digestion and either the compounds themselves or the nanomaterials may be thrown from the digestive system or cross to the epithelium, entering the blood stream and resulting in immunological reactions [7]. Nanomaterials can also be absorbed through inhalation or through the skin. They can cause oxidative and inflammatory stresses and cardiovascular or extrapulmonary complications, generating bioaccumulation and toxicity to human health [49,53].

Exact quantification of the release of nanoparticles into the environment and occupational exposure is quite a challenge. There is an urgent need for information to better understand nanoparticle–biological interactions and processes [53,54]. While a steady stream of *in vitro* studies indicates promising directions, *in vivo* studies of nanoparticles have not yet established a unified system (Table 3). *In vivo* evaluation, one step closer to clinical application, more directly reflects organism's adaptation and injury. Despite some large-scale progress in the field of nanotoxicology, there remains a void between validation and evidence-based studies [53,54]. According to Naseer *et al.* [53], the current toxicological assays need to be revamped and new tools (such as proteomics, functional genomics, high-throughput screening, and metabolomics) should be increasingly incorporated into these studies. These advanced tools will minimize the number of false positives and accelerate and validate the evaluation of the toxicity of nanoparticles.

Nanomaterials and consumers

Currently, the use of nanomaterials is gaining increasing importance in industrial applications, including the food industry, as a way to provide new benefits to consumers and additional functionalities [63].

In 2011, according to the European Regulation Concerning the Provision of Consumer Food Information (Nº 1169/2011), the European Commission decided to label any ingredient containing nanomaterials as such, followed by the word 'nano' in parentheses. This regulation, which entered into force on 13 December 2014, seems to be difficult to apply in agreement with several consumer associations. Some French consumer associations, with the help of the French National Laboratory of Metrology and Testing (LNE), demonstrated the presence of nanomaterials in several products, with no labels on the list of ingredientes [64•].

Nongovernmental organizations from different countries have widely reported on research studies showing some negative effects of nanomaterials. Two of the latter are the study by Bettini *et al.* [65] and Schoepf *et al.* [66], among others, shown in Table 4, forcing the Australian

Table 3

Selected examples of nanoparticles assessment by *in vitro* and *in vivo* studies

Type of studied nanoparticle	Type of approach employed	Main conclusion	References
Dihydromyricetin nanocapsules (NC-DMY)	*In vitro* toxicity, cell viability assay by MTT (peripheral blood mononuclear cells) for genotoxic effects, comet assay for interaction with DNA	NC-DMY did not demonstrate cytotoxicity and genotoxicity, presenting high interaction with DNA *in vitro*, suggesting DNA sequestration.	[55•]
Pomegranate seed oil nanoemulsions (NE PSOB)	*In vitro* toxicity, cell viability assay by MTT (peripheral blood mononuclear cells) for genotoxic effects, comet assay	The results demonstrated no cytotoxicity or genotoxicity. NE PSOB increased cell viability by about 50%.	[56]
Kaempferol-loaded nanoemulsions (KPF-NE)	Isolated porcine nasal mucosa	KPF-NE caused no structural damage toward the nasal mucosa. The epithelial layer was intact, indicating that the formulation is biocompatible and safe for nasal administration.	[57]
Curcumin nanoemulsion (CNE) and curcumin mucoadhesive nanoemulsion (CMNE)	Human neuroblastoma cell line, SK-N-SH cells	The results suggest that CNE and CMNE did not inhibit growth and were non-toxic to the cells, suggesting they are safe for brain delivery.	[58]
Gold nanoparticles synthesized using leaf extract of *Alternanthera bettzickiana*	Zebrafish (*Danio rerio*) embryo model	Gold nanoparticles at 25 μm concentration caused low inhibition of hatching after exposing embryos. At 50 μm concentration, gold nanoparticles strongly inhibited hatching after treatment of 48 hour. The tail formation in treated zebra fish embryo was more affected than the control, and large amounts of dark material were found in the gut tract of treated embryos, but not in the control.	[41]
Chitosan-coated nanoliposome for encapsulating the flavonoid quercetin	*In vitro* toxicity in HepG2 cells, cell viability assay by MTT	At concentration of 10 mg/mL, the encapsulated quercetin exhibited comparatively lower cell viability (40.92%) than native quercetin (46.67%).	[59]
Silver nanoparticle synthesized using black tea extract	Cytotoxicity in human ovarian (A2780) and colorectal (HCT116) carcinoma cell lines	The AgNPs show higher cytotoxic activity against A2780 when compared to HCT116. The observed IC50 in A2780 cells is 10 times lower than that for normal human primary fibroblasts.	[60]
Nanosilver produced by two macroalgae: *Sargassum boveanum* and *Ulva flexuosa* extracts	*Daphnia magna*	AgNPs from *U. flexuosa* was less toxic and more environmental friendly than AgNPs synthesized from *S. boveanum*. Both showed significant lower toxicity than chemical nanosilver.	[61•]
Copper oxide nanoparticles synthesized using aqueous extract of *Pterospermum acerifolium* leaves	*Daphnia magna*	The results indicated that plant-synthesized CuO NPs are less toxic than engineered CuO NPs.	[62]

food safety agency and the French food safety agency to report on titanium dioxide and apatite (tricalcium phosphate), respectively. Unfortunately, non-governmental organizations did not report contradictory studies, leading to a climate of fear of nanotechnology by consumers and the implicit link between nanomaterial and toxicity, despite the message from the European Commission that explicitly stated that a nanomaterial is not intrinsically dangerous. Consumers are often unaware that food additives have been subjected to toxicological testing before food authorization and are regularly reassessed. As a consequence, several food industries have announced the ban on some food additives from their food products to maintain the confidence of their consumers [64•].

The reasons for this low market penetration are partly linked to the limited consumer acceptance and remaining uncertainties regarding safety aspects and legislation [63]. To assist in these studies the projective methods are welcome and essential, these constitute a group of qualitative sensory techniques, which are increasingly used in research to investigate the perception of consumers. This group of techniques allow a condition of free interpretation and response to an ambiguous stimulus, seeking to understand the motivations, feelings, beliefs, attitudes and the deep reasons to select alternatives and answers within a specific topic [72].

Giles *et al.* [73] carried out a systematic review methodology to synthesize current knowledge regarding the acceptance or rejection of society regarding nanotechnology applied to agri-food production. They found that nanotechnology is more likely to be accepted by consumers when applied to the development of packaging

Table 4

***In vitro* and *in vivo* toxicity studies of nanoparticles**

Nanoparticle	Model	Effect/conclusion	References
Silver nanoparticles (AgNPs)	*Drosophila melanogaster*	Lethal doses of dietary AgNPs caused developmental delays and profound lethality in developing animals and young adults. Sublethal doses were not deadly to developing animals but shortened the adult lifespan and compromised their tolerance to oxidative stress. Dietary AgNPs caused a variety of ROS-mediated stress responses, including apoptosis, DNA damage, and autophagy.	[67]
Zinc oxide (ZnO) and titanium dioxide (TiO_2) nanoparticles	Human lung epithelial cells (A549)	The results demonstrated that both materials generate reactive oxygen species (ROS), but nano-ZnO might also release zinc ions into the cell culture medium, potentially resulting in greater cell damage. Moreover, nano-ZnO has positive charge and can usually penetrate cells more readily and subsequently harm them than do negatively charged NPs, as nano-TiO2.	[68]
Titanium dioxide nanoparticles (TiO_2NPs)	L929 fibroblasts	TiO_2NPs decreased cell viability and GSH and SOD. Increased ROS production.	[69]
Gold/citrate nanoparticles	Human dermal fibroblasts	Nanoparticles decreased cell proliferation rate, adhesion, and motility.	[70]
Zinc oxide nanoparticles	Female Wistar rats	A single installation of ZnONP induced alveolar and interstitial inflammatory cell infiltration, including eosinophilia, airway epithelial cell injury, regenerative goblet cell hyperplasia, bronchocentric pulmonary fibrosis, and atelectasis.	[71]

with distinct benefits and not when integrated directly into agri-food products. Trust and confidence in agri-food nanotechnology and its governance must be fostered through transparent regulation and development of socially beneficial impacts to increase consumer acceptance. Providing information to consumers about the benefits of nanotechnology, and securing public information could help reduce consumer concern and inspire purchases of food nanotechnology. However, surveys are needed to understand what consumers perceive to be beneficial as well as how they interpret risks. Adoption of theoretically sustained approaches to understanding consumer perceptions and attitudes will facilitate comparative analysis across different consumer groups, different food applications of nanotechnology, and enable the assessment of trends, consumer priorities and concerns over time [73].

Regulatory aspects and future perspectives

Safety and health concerns, as well as regulatory policies, should be considered during the manufacture, processing, and active packaging and consumption of nanoprocessed food products [2]. Regarding the regulatory aspects of nanostructures in the food and medical sectors, no specific legislation is applied globally. Most countries still have no specific regulations for the risk assessment of encapsulated nano-products. The lack of consistency in the exchange of information among countries is a reported risk to human health and the environment and may limit the marketing of new and beneficial products around the world [74•]. Recently, European Union regulations have established that any food ingredient derived from nanotechnology applications must undergo a safety assessment before receiving approval for its use [75]. Some guidelines have also been released by the U.S. Food and Drug Administration regarding the use of nanotechnology in food [10•]. In an article published in the prestigious journal, *Nature Nanotechnology*, Associate Professor Steffen Foss Hansen [76•] from DTU Environment called for the adoption of a new regulatory framework for nanomaterials. This framework is named REACT NOW, standing for Registration, Evaluation, Authorisation, Categorisation and Tools to Evaluate Nanomaterials – Opportunities and Weaknesses. All uses of nanomaterials should be evaluated according to the NanoRiskCat methodology [76•].

Since there are no specified regulations for nanofoods, a database for those products currently released in the European market was established in 2012 [77]. To date, the database contains information about 3036 products, 128 of which concern nano-engineered food and beverages currently in the European market (www.nanodb.dk).

Further studies are needed to overcome the limitations of nanoencapsulation processes and to improve existing methods, formulations, and encapsulation systems, as well as to meet commercial demands and to explore the application of nano-products in food and gastrointestinal systems and their production at industrial scale. In addition, studies should emphasize the application of nanocarriers of bioactive compounds in food and biological systems, exploring their effects on cell viability and their adsorption, distribution, metabolism, and excretion

profiles in humans and other living systems [74•]. The prospects for the nanoencapsulation of bioactive compounds highlight the need for their worldwide regulation to facilitate their safe use and marketing. Various studies reporting the beneficial effects of nanostructured bioactive compounds support this trend and offer a promising future direction for research.

Conclusion

Nanotechnology applied to the encapsulation of bioactive compounds has several evident advantages from food processing to packing, such as improvements in stability and bioavailability. It also offers health benefits through the protection and controlled release of bioactive compounds. However, understanding the safety and toxicity of these nanomaterials requires more research, and global regulations must be drafted to promote the safe marketing of new nanotechnology products that are beneficial to health.

Conflict of interest statement

Nothing declared.

Acknowledgement

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest

1. Neethirajan S, Jayas DS: **Nanotechnology for the food and bioprocessing industries**. *Food Bioprocess Technol* 2011, **4**:39-47.

2. Bajpai VK, Kamle M, Shukla S, Mahato DK, Chandra P, Hwang SK, Kumar P, Huh YS, Y-K Han: **Prospects of using nanotechnology for food preservation, safety, and security**. *J Food Drug Anal* 2018, **26**:1201-1214.

3. Silva HD, Cerqueira MÂ, Vicente AA: **Nanoemulsions for food applications: development and characterization**. *Food Bioprocess Technol* 2012, **5**:854-867.

4. • Arancibia C, Riquelme N, Zúñiga R, Matiacevich S: **Comparing the effectiveness of natural and synthetic emulsifiers on oxidative and physical stability of avocado oil-based nanoemulsions**. *Innov Food Sci Emerg Technol* 2017, **44**:159-166.

O/W nanoemulsions were prepared with 10% avocado oil using natural (lecithin) and synthetic (Tween 80) emulsifiers at different concentrations (2.5–10%). The results showed that the nanoemulsions exhibited anionic lipid droplets (potential Z: −26 to −59 mV) with particle size between 103 and 249 nm. The type of emulsifier and concentration affected the properties and stability of the nanoemulsions. Tween 80 nanoemulsions showed the best physical and oxidative stability. Lecithin can be used to form healthy, natural nanoemulsions based on avocado oil.

5. Walker R, Decker E, McClements DJ: **Development of food-grade nanoemulsions and emulsions for delivery of omega-3 fatty acids: opportunities and obstacles in the food industry**. *Food Funct* 2015, **6**:41-54.

6. McClements DJ, Bai L, Chung C: **Recent advances in the utilization of natural emulsifiers to form and stabilize emulsions**. *Annu Rev Food Sci Technol* 2017, **8**:205-236.

7. Rezaei A, Fathi M, Jafari SM: **Nanoencapsulation of hydrophobic and low-soluble food bioactive compounds within different nanocarriers**. *Food Hydrocoll* 2019, **88**:146-162.

8. Martínez-Ballesta M, Gil-Izquierdo Á, García-Viguera C, Domínguez-Perles R: **Nanoparticles and controlled delivery for bioactive compounds: outlining challenges for new "smart-foods" for health**. *Foods* 2018, **7**:1-29.

9. Rastogi ID: **Nanotechnology: safety paradigms**. *J Toxicol Environ Health Sci* 2012, **4**:1-12.

10. • Pathakoti K, Manubolu M, Hwang HM: **Nanostructures: current uses and future applications in food science**. *J Food Drug Anal* 2017, **25**:245-253.

This review highlights the applications of nanostructured materials for their antimicrobial activity and possible mechanism of action against bacteria, including reactive oxygen species, membrane damage, and release of metal ions. In addition, an overview of nanostructured materials, and their current applications and future perspectives in food science are also presented.

11. Hamad AF, Han J-H, Kim B-C, Rather IA: **The intertwine of nanotechnology with the food industry**. *Saudi J Biol Sci* 2018, **25**:27-30.

12. Pisoschi AM, Pop A, Cimpeanu C, Turcuş V, Predoi G, Iordache F: **Nanoencapsulation techniques for compounds and products with antioxidant and antimicrobial activity - a critical view**. *Eur J Med Chem* 2018, **157**:1326-1345.

13. Neo YP, Ray S, Jin J, Gizdavic-Nikolaidis M, Nieuwoudt MK, Liu D, Quek SY: **Encapsulation of food grade antioxidant in natural biopolymer by electrospinning technique: a physicochemical study based on zein–gallic acid system**. *Food Chem* 2013, **136**:1013-1021.

14. Huang Q, Yu H, Ru Q: **Bioavailability and delivery of nutraceuticals using nanotechnology**. *J Food Sci* 2010, **75**:R50-R57.

15. Anandharamakrishnan C: **Nanoencapsulation of food bioactive compounds**. *Techniques for Nanoencapsulation of Food Ingredients. Springer Briefs in Food, Health, and Nutrition*. New York, NY: Springer; 2014.

16. Human C, De Beer D, Rijst M, Aucamp M, Joubert E: **Electrospraying as a suitable method for nanoencapsulation of the hydrophilic bioactive dihydrochalcone, aspalathin**. *Food Chem* 2019, **276**:467-474.

17. Jafari SM: **An introduction to nanoencapsulation techniques for the food bioactive ingredients**. In *Nanoencapsulation of Food Bioactive Ingredients*. Edited by Jafari SM. Academic Press; 2017:1-62.

18. Pérez-Masiá R, López-Nicolás R, Periago MJ, Ros G, Lagaron JM, López-Rubio A: **Encapsulation of folic acid in food hydrocolloids through nanospray drying and electrospraying for nutraceutical applications**. *Food Chem* 2015, **168**:124-133.

19. Fuciños C, Míguez M, Fuciños P, Pastrana LM, Rúa ML, Vicente AA: **Creating functional nanostructures: encapsulation of caffeine into α-lactalbumin nanotubes**. *Innov Food Sci Emerg Technol* 2017, **40**:10-17.

20. Cui H, Bai M, Lin L: **Plasma-treated poly(ethylene oxide) nanofibers containing tea tree oil/beta cyclodextrin inclusion complex for antibacterial packaging**. *Carbohydr Polym* 2018, **179**:360-369.

21. Yang H, Feng K, Wen P, Zong M-H, Lou W-Y, Wu H: **Enhancing oxidative stability of encapsulated fish oil by incorporation of ferulic acid into electrospun zein mat**. *LWT—Food Sci Technol* 2017, **84**:82-90.

22. Dasgupta N, Ranjan S, Mundra S, Ramalingam C, Kumar A: **Fabrication of food grade vitamin E nanoemulsion by low energy approach, characterization and its application**. *Int J Food Prop* 2016, **19**:700-708.

23. Wang T, Xue J, Hu Q, Zhou M, Luo Y: **Preparation of lipid nanoparticles with high loading capacity and exceptional gastrointestinal stability for potential oral delivery applications**. *J Colloid Interface Sci* 2017, **507**:119-130.

24. Basiri L, Rajabzadeh G, Bostan A: **α-Tocopherol-loaded niosome prepared by heating method and its release behavior**. *Food Chem* 2017, **221**:620-628.

25. Abd El-Fattah AI, Fathy MM, Ali ZY, El-Garawany AERA, Mohamed EK: **Enhanced therapeutic benefit of quercetin-loaded phytosome nanoparticles in ovariectomized rats**. *Chem Biol Interact* 2017, **271**:30-38.

26. Mohsenabadi N, Rajaei A, Tabatabaei M, Mohsenifard A: **Physical and antimicrobial properties of starch-carboxy methyl cellulose film containing rosemary essential oils encapsulated in chitosan nanogel**. *Int J Biol Macromol* 2018, **112**:148-155.

27. Arroyo-Maya IJ, McClements DJ: **Biopolymer nanoparticles as potential delivery systems for anthocyanins: fabrication and properties**. *Food Res Int* 2015, **69**:1-8.

28. Trucillo P, Campardelli R, Aliakbarian B, Perego P, Reverchon E: **Supercritical assisted process for the encapsulation of olive pomace extract into liposomes**. *J Supercrit Fluid* 2018, **135**:152-159.

29. Pushpalatha R, Selvamuthukumar S, Kilimozhi D: **Cross-linked, cyclodextrin-based nanosponges for curcumin delivery - physicochemical characterization, drug release, stability and cytotoxicity**. *J Drug Deliv Sci Technol* 2018, **45**:45-53.

30. Hu B, Liu X, Zhang C, Zeng X: **Food macromolecule based nanodelivery systems for enhancing the bioavailability of polyphenols**. *J Food Drug Anal* 2017, **25**:3-15.

31. Patra JK, Baek KH: **Green nanobiotechnology: factors affecting synthesis and characterization techniques**. *JNM* 2014:1-12.

32. Nath D, Banerjee P: **Green nanotechnology – a newhope for medical biology**. *Environ Toxicol Pharmacol* 2013, **36**:997-1014.

33. Jayaseelan C, Rahuman AA, Kirthi AV, Marimuthu S, Santhoshkumar T, Bagavan A, Gaurav K, Karthik L, Rao KVB: **Novel microbial route to synthesize ZnO nanoparticles using *Aeromonas hydrophila* and their activity against pathogenic bacteria and fungi**. *Spectrochim Acta A* 2012, **90**:78-84.

34. Chan YS, Don MM: **Biosynthesis and structural characterization of Ag nanoparticles from white rot fungi**. *Mater Sci Eng C* 2013, **33**:282-288.

35. Hassaan MA, Hosny S: **Green synthesis of Ag and Au nanoparticles from micro and macro algae - review**. *Int J Atmos Ocean Sci* 2018, **2**:10-22.

36. Azizi S, Ahmad MB, Namvar F, Mohamad R: **Green biosynthesis and characterization of zinc oxide nanoparticles using brown marine macroalga *Sargassum muticum* aqueous extract**. *Mater Lett* 2014, **116**:275-277.

37. • Sharmila G, Thirumarimurugan M, Muthukumaran C: **Green synthesis of ZnO nanoparticles using *Tecoma castanifolia* leaf extract: characterization and evaluation of its antioxidant, bactericidal and anticancer activities**. *Microchem J* 2019, **145**:578-587.

A green synthesis of zinc oxide nanoparticles (ZnO NPs) using *Tecoma castanifolia* leaf extract and a screening for its antibacterial, antioxidant and anticancer activity was proposed. The presence of bioactive phytochemical constituents in the methanolic extracts of *T. castanifolia* was identified by GC–MS. Totally, 54 compounds were identified, 8 major compounds having the highest composition peaks (1,15-Pentadecanediol; 1,10-Decanediol; *n*-Hexadecanoic acid, 9,12-Octadecadienoic acid (Z,Z), 9,12,15-Octadecatrienoic acid, (Z,Z,Z), Squalene, Octadecanoic acid, and dibutyl phthalate). GC–MS also reveals the presence of bioactive phytochemical constituents such as sugars, amides, alcohols, aldehydes ethers, ketones, carboxylic acids, amino acids, fatty acids, alkaloids, phenolic compounds, flavonoids, tannins, and terpenoids. *T. castanifolia* leaf extract derived ZnO NPs showed good antibacterial, antioxidant, and anticancer activities were due to the synergistic effect of bioactive phytochemical constituents.

38. Rajeshkumar S, Kumar SV, Ramaiah A, Agarwal H, Lakshmi T, Roopan SM: **Biosynthesis of zinc oxide nanoparticles using *Mangifera indica* leaves and evaluation of their antioxidant and cytotoxic properties in lung cancer (A549) cells**. *Enzyme Microb Technol* 2018, **117**:91-95.

39. Farshchi HK, Azizi M, Jaafari MR, Nemati SH, Fotovat A: **Green synthesis of iron nanoparticles by Rosemary extract and cytotoxicity effect evaluation on cancer cell lines**. *Biocatal Agric Biotechnol* 2018, **16**:54-62.

40. Hamelian M, Hemmati S, Varmira K, Veisi H: **Green synthesis, antibacterial, antioxidant and cytotoxic effect of gold nanoparticles using *Pistacia atlantica* extract**. *J Taiwan Inst Chem Eng* 2018, **19**:21-30.

41. Nagalingam M, Kalpana VN, Rajeswari VD, Panneerselvam A: **Biosynthesis, characterization, and evaluation of bioactivities of leaf extract-mediated biocompatible gold nanoparticles from *Alternanthera bettzickiana***. *Biotech Rep* 2018, **19**:e00268.

42. Ji M, Sun X, Guo X, Zhu W, Wu J, Chen L, Wang J, Chen M, Cheng C, Zhang O: **Green synthesis, characterization and in vitro release of cinnamaldehyde/sodium alginate/chitosan nanoparticles**. *Food Hydrocoll* 2019:515-522.

43. Alsammarraie K, Wang W, Zhou P, Mustapha A, Lin M: **Green synthesis of silver nanoparticles using turmeric extracts and investigation of their antibacterial activities**. *Colloid Surf B Biointerfaces* 2018, **171**:398-405.

44. Khalil MMH, Ismail EH, El-Baghdady KZ, Mohamed D: **Green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity**. *Arab J Chem* 2014, **7**:1131-1139.

45. Moldovan B, David L, Vulcu A, Olenic L, Perde-Schrepler M, Fischer-Fodor E, Baldea I, Clichici S, Filip GA: ***In vitro* and *in vivo* anti-inflammatory properties of green synthesized silver nanoparticles using *Vibumum opulus* L. fruits extract**. *Mater Sci Eng C* 2017, **79**:720-727.

46. Logeswari P, Silambarasan S, Abraham J: **Ecofriendly synthesis of silver nanoparticles from commercially available plant powders and their antibacterial properties**. *Sci Iran* 2013, **20**:1049-1054.

47. Shaik MR, Khan M, Kuniyil M, Al-Warthan A, Alkhathlan HZ, Siddiqui MRH, Shaik JP, Ahamed A, Mahmood A, Khan M, Adil SF: **Plant-extract-assisted green synthesis of silver nanoparticles using *Origanum vulgare* L. extract and their microbicidal activities**. *Sustainability* 2018, **10**:1-14.

48. Srivastava S, Bhargava A: **Green nanotechnology**. *J Nanotechnol Mater Sci* 2016, **3**:1-7.

49. Sia P: **Nanotechnology among innovation, health and risks**. *Procedia Soc Behav Sci* 2017, **237**:1076-1080.

50. Viswanath B, Kim S: **Influence of nanotoxicity on human health and environment: the alternative strategies**. *Rev Environ Contam Toxicol* 2016, **242**:61-104.

51. Walters C, Pool E, Somerset V: **Nanotoxicity in aquatic invertebrates**. In *Invertebrates Experimental Models in Toxicity Screening*. Edited by Larramendy ML. IntechOpen; 2016:13-34.

52. Buzea C, Blandino IIP, Robbie K: **Nanomaterials and nanoparticles: sources and toxicity**. *Biointerphases* 2007, **2**: MR17-MR172.

53. Naseer B, Srivastava G, Qadri OS, Faridi SA, Islam RU, Younis K: **Importance and health hazards of nanoparticles used in the food industry**. *Nanotechnol Rev* 2018, **7**:623-641.

54. Yang Y, Qin Z, Zeng W, Yang T, Cao Y, Mei C, Kuang Y: **Toxicity assessment of nanoparticles in various systems and organs**. *Nanotechnol Rev* 2017, **6**:279-289.

55. • Dalcin AJF, Vizzotto BS, Bochi GV, Guarda NS, Nascimento K, Sagrillo MR, Moresco RN, Schuch AP, Ourique AF, Gomes P: **Nanoencapsulation of the flavonoid dihydromyricetin protects against the genotoxicity and cytotoxicity induced by cationic nanocapsules**. *Colloids Surf B Biointerfaces* 2019, **173**:798-805.

The authors evaluated the influence of nanoencapsulation of the flavonoid dihydromyricetin (DMY) in reducing the genotoxicity and cytotoxicity induced by cationic nanocapsules produced with Eudragit RS100® in peripheral blood mononuclear cells. They evaluated the potential of protein corona formation, cytotoxicity, genotoxicity and the antioxidant capacity. Eudragit RS100® nanocapsules (NC-E) increased cytotoxicity and DNA damage formation. However, nanocapsules containing DMY (NC-DMY) do not induce cytotoxicity or genotoxicity, and demonstrates high antioxidant capacity. NC-DMY may decrease the cytotoxicity provided by the cationic polymer Eudragit RS100® due to its potential

antioxidant effect. Thereby DMY nano-encapsulated can be better exploited by nanomedicine for different biological applications.

56. Ferreira LM, Gehrcke M, Cervi VF, Bitencourt PER, Silveira EF, Azambuja JH, Ramos AP, Nascimento K, Moretto MB, Braganhol E, Sagrillo MR, Cruz L: **Pomegranate seed oil nanoemulsions with selective antiglioma activity: optimization and evaluation of cytotoxicity, genotoxicity and oxidative effects on mononuclear cells**. *Pharm Biol* 2016, **54**:2968-2977.

57. Colombo M, Figueiró F, Dias AF, Teixeira HF, Battastini AMO, Koester LS: **Kaempferol-loaded mucoadhesive nanoemulsion for intranasal administration reduces glioma growth *in vitro***. *Int J Pharm* 2018, **543**:214-223.

58. Sood S, Jain K, Gowthamarajan K: **Optimization of curcumin nanoemulsion for intranasal delivery using design of experiment and its toxicity assessment**. *Colloid Surf B Biointerfaces* 2014, **113**:330-337.

59. Hao J, Guo B, Yu S, Zhang W, Zhang D, Wang J, Wang Y: **Encapsulation of the flavonoid quercetin with chitosan-coated nanoliposomes**. *LWT—Food Sci Technol* 2017, **85**:37-44.

60. Ribeiro APC, Anbua S, Alegria ECBA, Fernandes AR, Baptista PV, Mendes R, Matias AS, Mendes M, Guedes da Silva MFC, Pombeiro AJL: **Evaluation of cell toxicity and DNA and protein binding of green synthesized silver nanoparticles**. *Biomed Pharmacother* 2018, **101**:137-144.

61. • Dezfuly ZT, Alishahi M, Aramoon A, Mashjoor S: **Evaluation of silver nanoparticles toxicity in *Daphnia magna*: comparison of chemical and green biosynthetic productions**. *IJAAH* 2017, **3**:74-85.

This study evaluated the toxicity of biosynthetic nanosilver produced by two macroalgae: *Sargassum boveanum* and *Ulva flexuosa* extracts and compared with commercial chemosynthetic nanosilver in *Daphnia magna*. All nanosilver were toxic in *D. magna*, regardless of their synthesis origin. The mortality rate enhanced in all tested groups, along with increasing nanosilver concentration and exposure time duration. Nanosilver from *U. flexuosa* was less toxic and more environmental friendly than AgNPs synthesized from *S. boveanum*. However, both biosynthesized AgNPs showed significant lower toxicity than chemical nanosilver, being recommended to produce biosynthesized nanoparticles from marine algae as a alternative to chemical nanosilver.

62. Saif S, Tahir A, Asim T, Chen Y: **Plant mediated green synthesis of CuO nanoparticles: comparison of toxicity of engineered and plant mediated CuO nanoparticles towards *Daphnia magna***. *Nanomaterials* 2016, **6**:205-220.

63. Dubascoux S, Wyser Y: **Nanomaterials in food: an overview**. *Encycl Food Chem* 2019:110-117.

64. • Ropers MH: **Nanomaterials and food security: the next challenge for consumers, food industries and policies**. *Food Sci Encycl Food Secur Sustain* 2019, **2**:575-581.

This paper reviewed current knowledge on nanomaterials and described the path the food science research community is taking to protect nanomaterials that contain food.

65. Bettini S, Boutet-Robinet E, Cartier C, Coméra C, Gaultier E, Dupuy J, Naud N, Taché S, Grysan P, Reguer S *et al.*: **Food-grade TiO_2 impairs intestinal and systemic immune homeostasis, initiates preneoplastic lesions and promotes aberrant crypt development in the rat colon**. *Sci Rep* 2017, **7**:40373.

66. Schoepf JJ, Bi Y, Kidd J, Herckes P, Hristovski K, Westerhoff P: **Detection and dissolution of needle-like hydroxyapatite nanomaterials in infant formula**. *Nanoimpact* 2017, **5**:22-28.

67. Mao BH, Chen XY, Wang YJ, Yan SJ: **Silver nanoparticles have lethal and sublethal adverse effects on development and longevity by inducing ROS-mediated stress responses**. *Sci Rep* 2018, **8**:2445-2461.

68. Hsiao IL, Huang YJ: **Effects of various physicochemical characteristics on the toxicities of ZnO and TiO2 nanoparticles toward human lung epithelial cells**. *Sci Tot Environ* 2011, **409**:1219-1228.

69. Jin CY, Zhu BS, Wang XF, Lu QH: **Cytotoxicity of titanium dioxide nanoparticles in mouse fibroblast cells**. *Chem Res Toxicol* 2008, **21**:1871-1877.

70. Pernodet N, Fang XH, Sun Y, Bakhtina A, Ramakrishnan A, Sokolov J, Ulman A, Rafailovich M: **Adverse effects of citrate/gold nanoparticles on human dermal fibroblasts**. *Small* 2006, **2**:766-773.

71. Cho WS, Duffin R, Howie SEM, Scotton CJ, Wallace WAH, MacNee W, Bradley M, Megson IL, Donaldson K: **Progressive severe lung injury by zinc oxide nanoparticles; the role of Zn^{2+} dissolution inside lysosomes**. *Part Fibre Toxicol* 2011, **8**:27-43.

72. Gambaro A: **Projective techniques to study consumer perception of food**. *Curr Opin Food Sci* 2018, **21**:46-50.

73. Giles EL, Kuznesof S, Beth Clark B, Hubbard C, Frewer LJ: **Consumer acceptance of and willingness to pay for food nanotechnology: a systematic review**. *J Nanopart Res* 2015, **17**:467.

74. • Shishir MRI, Xie L, Sun C, Zheng X, Chen W: **Advances in micro and nano-encapsulation of bioactive compounds using biopolymer and lipid-based transporters**. *Trends Food Sci Technol* 2018, **78**:34-60.

This review highlights the recent advances in micro and nano-encapsulation of bioactive compounds. They comprehensively discussed the importance of encapsulation, the application of biopolymer-based carrier agents and lipid-based transporters with their functionalities, suitability of encapsulation techniques in micro and nano-encapsulation, as well as different forms of improved and novel micro and nano-encapsulate systems.

75. Rauscher H, Rasmussen K, Sokull-Klüttgen B: **Regulatory aspects of nanomaterials in the EU**. *Chem Ing Tech* 2017, **89**:224-231.

76. • Hansen SF: **React now regarding nanomaterial regulation**. *Nat Nanotechnol* 2017, **12**:714-716.

They propose a new legislative framework that combines registration, evaluation, authorization, and categorization of nanomaterials, implementing a regulatory framework.

77. Hansen SF, Heggelund LR, Besora PR, Mackevica A, Boldrin A, Baun A: **Nanoproducts–what is actually available to European consumers?** *Environ Sci Nano* 2016, **3**:169-180.

5 ARTIGO 2 – DEVELOPMENT OF NANOEMULSIONS CONTAINING *Physalis peruviana* CALYX EXTRACT: A STUDY ON STABILITY AND ANTIOXIDANT CAPACITY

Artigo publicado na Revista **Food Research International, v. 125, p. 108645, 2019.**

Food Research International 125 (2019) 108645

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Development of nanoemulsions containing *Physalis peruviana* calyx extract: A study on stability and antioxidant capacity

Maiara Taís Bazana[a], Suelen Santos da Silva[a], Cristiane Franco Codevilla[a], Cassandra de Deus[a], Bruna Nichelle Lucas[a], Gustavo Andrade Ugalde[b], Marcio Antonio Mazutti[b], Erico Marlon Moraes Flores[c], Juliano Smanioto Barin[a], Cristiane de Bona da Silva[d], Cristiano Ragagnin de Menezes[a,*]

[a] Department of Food Science and Technology, Rural Science Center, Federal University of Santa Maria, Santa Maria, Brazil
[b] Department of Chemical Engineering, Technology Center, Federal University of Santa Maria, Santa Maria, Brazil
[c] Department of Chemistry, Center for Natural and Exact Sciences, Federal University of Santa Maria, Santa Maria, Brazil
[d] Departament of Industrial Pharmacy, Health Sciences Center, Federal University of Santa Maria, Santa Maria, Brazil

ARTICLE INFO

Keywords:
Phenolics
Antioxidant stability
Nanotechnology
Rutin
Temperature

ABSTRACT

The aim of this study was to develop and evaluate the physicochemical and antioxidant stability of nanoemulsions containing a *Physalis peruviana* calyx extract (CPp-NE) and free extracts under different storage conditions (7 and 25 °C) and with absence or incidence of light for 120 days. The calyx extracts were prepared with ethanol 60% and characterized for later preparation of the nanoemulsions by spontaneous emulsification. The formulations presented nanometric sizes, low polydispersity index, negative zeta potential, acid pH, rutin content (11 $\mu g \cdot mL^{-1}$), and encapsulation efficiency of 85%. Regarding the stability, the droplet size and PdI of the CPp-NE stored at refrigeration temperature in the dark, room temperature in the dark, and refrigeration temperature with light incidence were stable for 120 days and with no visible changes in the formulations. The antioxidant capacity was related to the reducing capacity, and the best results were found for nanoemulsions stored at room temperature and in absence of light. In addition, CPp-NE presented higher antioxidant and reducing capacity in relation to the free extracts.

1. Introduction

Physalis peruviana Linnaeus is a highly functional fruit belonging to the *Solanaceae* family and genus *Physalis*. Its cultivation has expanded in tropical and subtropical countries, with Colombia being the largest producer in the world followed by South Africa, countries of which export fruit to other countries of America and most importantly Europe (Rufato, Rufato, Lima, & Muniz, 2013). *P. peruviana* is an herbaceous, semi-shrub, vertical, and perennial plant that adapts in subtropical areas. It produces orange-yellow fruit with a juicy berry and has a calyx, which is a casing that naturally protects the fruit during its development and maturity, protecting it against insects, birds, diseases, and adverse climatic conditions. The calyx represents an essential source of carbohydrates during the first 20 days of fruit growth and development (Puente, Pinto-Muñoz, Castro, & Cortés, 2011). In addition, it is a residue rich in bioactive compounds, such as flavonols and hydroxycinnamic acid derivatives (Gironés-Vilaplana et al., 2014).

P. peruviana calyces are widely used in folk medicine due to their anticancer, antimicrobial, antipyretic, diuretic, immunomodulatory, and anti-inflammatory properties (Franco, Matiz, Calle, Pinzón, & Ospina, 2007), which are well established and reported in scientific studies using in vivo models (Castro, Ocampo, & Franco, 2015; Franco et al., 2007; Franco et al., 2014). Moreover, Toro, Aragón, Ospina, Ramos, and Castellanos (2014) verified that the crude ethanolic extract and some calyx fractions demonstrated promising anti-inflammatory and antioxidant activity.

According to Cömert and Gökmen (2018), food antioxidants have been extensively studied in food science because they provide health benefits in addition to the preservative effects on food, as there is growing interest among food scientists in developing in vitro and in vivo assays to improve knowledge on the physiological effects of antioxidants in the human body. The authors also reported that the

* Corresponding author at: Departamento de Tecnologia e Ciência dos Alimentos, Programa de Pós-Graduação em Ciência e Tecnologia dos Alimentos, Centro de Ciências Rurais, Universidade Federal de Santa Maria, Av. Roraima, 1000, CEP 97105-900 Santa Maria, RS, Brazil.
E-mail addresses: juliano@ufsm.br (J.S. Barin), csbona@smail.ufsm.br (C. de Bona da Silva), cristiano.ufsm@gmail.com (C.R. de Menezes).

https://doi.org/10.1016/j.foodres.2019.108645
Received 9 April 2019; Received in revised form 10 July 2019; Accepted 23 August 2019
Available online 24 August 2019

evolution of research on antioxidants by covering their occurrence, roles in various foods, and analytical methods for determining their antioxidant capacity and physiological effects.

The use of plant extracts or their derivatives is a growing trend for application in food and beverages in the food industry (Perumalla & Hettiarachchy, 2011; Valduga, Gonçalves, Magri, & Finzer, 2019). Oftentimes, these extracts are obtained by plant residues, which is a relevant factor for their use, as they are by-products and may present high nutritional values and economically viable reutilization. Therefore, the importance of these products is highlighted since they often represent a varied source of compounds with biological activity, therefore, being beneficial to health (Bilal & Iqbal, 2019).

However, these substances with excellent functional properties have several limiting elements that hinder their application in food for oral administration, including poor water solubility, low absorption, and bioavailability (Shin, Kim, & Park, 2015). Among these substances, rutin is one of the flavonoid compounds present in the highest quantity in the *P. peruviana* calyx in addition to having these disadvantages (Gullón, Lú-Chau, Moreira, Lema, & Eibes, 2017). Moreover, the inclusion of some bioactive compounds in food products may cause quality defects, such as astringent taste. Furthermore, they can act as substrates for darkening reactions, degradation, and/or deterioration by food processing and storage operations, such as heating, acidification, light, and oxygen (Codevilla, Bazana, Silva, Barin, & Ragagnin de Menezes, 2015). As a result, some extracts are not used due to such obstacles (Bonifácio et al., 2014).

To overcome the limitations of adding plant extracts or bioactive compounds to food products, innovative technologies, such as nanotechnology, become a promising alternative. In the food industry, the use of nanostructured systems can contribute to the processing, packaging, and preservation of food, in addition to providing longer shelf life to most products (Hamad, Han, Kim, & Rather, 2018). Nanoemulsions are one of the most interesting delivery systems in food industry. They are emulsions that have very small particle sizes, increased surface area, and less sensitivity to physical and chemical changes. These phenomena are thermodynamically unstable and can be kinetically stable over long time scales. Furthermore, nanoemulsion droplet sizes (O/W and W/O) are reported to be 20–500 nm, which is variable based on the composition of the system (surfactants/co-surfactants, oil, and water) and homogenization method used (Salem & Ezzat, 2018).

Nanoemulsions stand out among nanocarriers as a promising tool to develop delivery systems of lipophilic active compounds with the aim of facilitating their incorporation into food. Encapsulating compounds of natural origin in nanoemulsions may improve their stability and functional performance in either food matrices or gastrointestinal conditions (Acevedo-Fani, Soliva-Fortuny, & Martín-Belloso, 2017). Furthermore, nanoemulsions containing plant extracts and/or oils have shown advantages such as increased solubility, antioxidant efficiency, and slower phenolic compound release (Rezaei, Fathi, & Jafari, 2019). Other benefits also include increased stability and protection against degradation (Silva Junior et al., 2013), better antimicrobial activity (Paudel, Bhargava, & Kotturi, 2019), and improved stability through simulated gastrointestinal fluids (Li, Cai, Yang, & Sun, 2019).

In this context, the aim of this study was to develop nanoemulsions containing the *Physalis peruviana* calyx extract and evaluate extract and nanoemulsion stability under different storage conditions.

2. Material and methods

2.1. Material

The *P. peruviana* calyces were obtained from Italbraz (Vacaria, Brazil - 28:0:44 S and 50:56:02 W) in the 2016/2017 harvest. Sorbitan monooleate (Span 80®) was purchased from Fluka (São Paulo, Brazil), medium chain triglycerides (MCT) from Delaware (Porto Alegre, Brazil), polysorbate 80 (Tween® 80) from Neon Comercial Ltda (São Paulo, Brazil), and Folin-Ciocalteu was purchased from Cromoline Química Fina Ltda (São Paulo, Brazil). Fluorescein sodium was purchased from Synth (São Paulo, Brazil), peroxyl radical generator (AAPH: 2,2′-azobis-2-methyl-amidinopropane dihydrochloride) and standard antioxidant (Trolox: 6-hydroxy-2,5,7–8-tetramethylchromo-2-carboxylic acid 97%) from Sigma-Aldrich (USA). The other reagents were of PA grade for spectrophotometer analysis and HPLC grade for high performance liquid chromatography analyses.

2.2. Methods

2.2.1. Extraction

The calyx extracts were prepared following a modified version of the methodology proposed by Codevilla et al. (2018). The *P. peruviana* calyces (3 g) were separated from the fruit, submitted to knife milling (Mill MA 630/1-Marconi) (speed = 5 rpm, time = 10 s), added with 50 mL of 60% ethanol (v/v), and submitted to stirring in a homogenizer (Shaker model TE 421- Tecnal, Piracicaba, SP, Brazil) at a speed of 200 rpm for 2 h. Afterwards, the extracts were filtered in a qualitative filter (Fitec) for coarse separation of the calyx. Subsequently, the extract was subjected to filtration in a polypropylene syringe (0.22-μm pore size) (Chromfilter) and kept in a refrigerated environment.

2.2.2. Characterization of the P. peruviana calyx extract

The extract was characterized in terms of phenolic compounds via quantification by high performance liquid chromatography (HPLC), where the standards of caffeic acid, chlorogenic acid, rutin, quercetin, gallic acid, catechin, coumarin, luteolin, and kaempferol were tested. In addition, the reduction capacity by Folin-Ciocalteu and antioxidant capacity by Oxigen Radical Absorbance Capacity (ORAC) were evaluated.

2.2.3. Nanoemulsion preparation

Nanoemulsions were prepared in triplicate by spontaneous emulsification based on the technique of Bouchemal, Briançon, Perrier, and Fessi (2004). The organic phase was composed of sorbitan monooleate (Span 80® - 0.1925 g), medium chain triglycerides (MCT - 0.400 g), *P. peruviana* calyx extract (2.083 mL), and acetone (65 mL) was stirred in a magnetic stirrer until complete solubilization of the components. The organic phase was injected, under stirring, into the aqueous phase consisting of polysorbate 80 (Tween 80® - 0.1925 g) and ultrapure water (135 mL). Magnetic stirring was maintained for 10 min. Then, the formulation was taken to the rotary evaporator at 40 °C to remove the organic solvent and adjusting of the extract concentration to 5 $mg.mL^{-1}$ and final volume to 25 mL. For comparison purposes, the formulations without the extract were also prepared and evaluated (blank nanoemulsions - NE).

2.2.4. Physicochemical characterization of nanoemulsions

2.2.4.1. PH determination. The pH determination was performed directly on the nanoemulsions using a potentiometer (MS Tecnopon) previously calibrated with buffer solutions pH (4.0 and 7.0). Droplet size distribution was evaluated by water dispersion of the samples until the obscuration index was around 13% using the refractive index of the MCT = 1.461 and density 0.98 $g.cm^{-3}$ by laser diffraction (Mastersizer® 3000E, Malvern, UK). Determination of the mean droplet diameter and polydispersity index (PdI) was performed by Zetasizer® (Nano series, ZEN 3600, Malvern Instruments, UK) using photometric correlation spectroscopy. For this, the samples were diluted in ultrapure water in the proportion of 1:500 (v/v).

2.2.4.2. Determination of the zeta potential. the nanoemulsions were dispersed in a 10-mM NaCl solution (1:500, v/v) for further analysis of the zeta potential by electrophoretic mobility in the same equipment used to determine the mean diameter (Zetasizer®).

2.2.4.3. Morphological analysis. The morphology of the nanodroplets was evaluated by scanning electron microscopy (SEM) (Sigma 300 VP, Zeiss, Germany). For this, the sample was homogenized with 10% trehalose (cryoprotectant), frozen in a freezer at −18 °C (Electrolux FE 26) for 24 h, and then lyophilized (LIOTOP L101, Brazil) for 24 h. The lyophilized nanoemulsion sample was stuck with a double-sided tape in aluminum stubs and covered by a thin layer of carbon. The shape and surface of the droplets were analyzed.

2.2.4.4. Extract content and encapsulation efficiency determination (EE). The content of the extract was determined by rutin analysis because, among the tested standards, this was the major compound according to high performance liquid chromatography (HPLC) following a modified version of the methodologies proposed by Gironés-Vilaplana et al. (2014) and Medina et al. (2019). For this, an analytical curve with the rutin standard of 0.1 to 5 μg.mL^{-1} was performed. Shimadzu Nexera XR chromatograph equipped with a SPD-M20A UV-DAD detector set at 370 nm, LC-20 CE pump module, and CTO-20A column oven (Shimadzu, Kyoto, JP) was used. As the stationary phase, the C18 Luna®, 5 μm, 100 Å reverse phase column (4.6 i.d. x 250 mm) (Phenomenex, Torrance, CA, USA) was used. The injection volume was 50 μL and the elution gradient was composed of 0.45% formic acid in water (solvent A) and acetonitrile (solvent B) with the mobile phase at a constant flow rate (0.6 mL.min^{-1}). The linear gradient for solvent B was 0 min, 5%, 15 min, 100%, and 3 final min of column conditioning. For rutin extraction, 2-mL aliquots of nanoemulsions and 0.167-mL of extracts were diluted in 10 mL of methanol (1 mg.mL^{-1} of calyx extract) and submitted to 20 min of ultrasound and 10 min of centrifugation (48,000 *g*, 10 °C). Afterwards, the solutions were filtered in a 0.22-μm membrane and injected into the chromatograph. Encapsulation efficiency was determined by a centrifugation-ultrafiltration technique (AMICON 10000 MW Ultra, Millipore, Bedford, USA). An aliquot of nanocapsules (400 μL) was used and submitted to centrifugation-ultrafiltration at 2200 *g*. Rutin concentration in the ultrafiltrate was determined and encapsulation efficiency was calculated through the difference between total rutin concentration and ultrafiltrate concentration (free concentration).

2.2.5. Antioxidant capacity

2.2.5.1. Reducing capacity. The *P. peruviana* calyx extract and nanoemulsions were diluted in water to a concentration of 2 mg.mL^{-1}. Afterwards, they were subjected to ultrasound for 20 min and centrifugation for 10 min at 48000 *g*. Subsequently, 1 mL of the sample was pipetted, followed by 0.250 mL of the Folin-Ciocalteu reagent, followed by waiting for 5 min for the reaction. Next, 2 mL of 20% sodium carbonate was added and another 10 min was waited. The nanoemulsion samples were centrifuged for 10 min at 48000 *g* in order to eliminate turbidity. Subsequently, spectrophotometer reading was performed at a wavelength of 730 nm. A standard curve prepared with gallic acid was used to quantify the reducing capacity (mechanism based on electron transfer) and the results are expressed in mg gallic acid Equivalent per 100 g of sample (mgGAEq.100 g^{-1}) (Chandra & Mejia, 2004).

2.2.5.2. Oxigen radical absorbance capacity (ORAC). The antioxidant capacity was evaluated by the peroxyl radical deactivation capacity method via the Oxigen Radical Absorbance Capacity (ORAC) method described by Ou, Hampsch-Woodill, and Prior (2001). The capturing capacity of the *P. peruviana* calyx (present in the extracts and nanoemulsions) was verified against the formation of a peroxyl radical induced by 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) at 37 °C. The samples were diluted in phosphate buffer with pH 7.4 (75 mmol L^{-1}) before being added to the microplate, subjected to ultrasound for 20 min, and centrifuged for 10 min at 48000 *g*. In a 96-well microplate, 25 μL of diluted *P. peruviana* extract and nanoemulsion (300 mg.L^{-1}) in phosphate buffer pH 7.4 (75 mmol.L^{-1}) were added, followed by incubation in a microplate, SpectraMax M5 (Molecular Devices, CA USA) for 10 min at 37 °C with 150 μL fluorescein working solution (81 nmol.L^{-1}). After incubation, 25 μL of AAPH (152 mmol.L^{-1}) was added to form peroxyl radicals. Fluorescence was monitored every minute (excitation and emission wavelengths of 485 and 528 nm, respectively) for 120 min at 37 °C. Antioxidant capacity was determined using the area under the curve (AUC) for decay of sample fluorescence, standard, and blank versus time and the results were compared with a standard Trolox curve (0–96 μmol.L^{-1}) and expressed as μmol Trolox.g^{-1} calyx. The AUC was calculated as follows:

where

$$AUC = 1 + {f1}/{f0} + {f2}/{f0} + {f3}/{f0} + \ldots {fn}/{f0}$$

f 1…fn: determined fluorescence every minute.f0: fluorescence at time zero.

2.2.6. Stability study

The stability study was carried out following a modified version of the methodologies proposed by Pedreño & Escribano, 2001, Xiu-li, Xue-li, Yuan-ping, & Qiang, 2015, Marques da Silva et al., 2018 and Nunes et al., 2018. The nanoemulsions and extracts from the *P. peruviana* calyx were packed in transparent flasks (exposed to light) and amber flasks wrapped in aluminum foil (absence of light). In addition, they were stored at room temperature (25 ± 0.5 °C) or refrigerated (7 ± 0.5 °C). Regarding the presence of light, a cold white lamp (luminous flux of 343 lm, power of 7 W, and light efficiency of 49 lm.W^{-1}) was placed about 40 cm above the samples. Stability evaluation was performed shortly after preparation (time zero) and over a period of 120 days (15, 30, 45, 60, 90, and 120 days). The parameters evaluated were: pH, mean droplet diameter, zeta potential, rutin content, reducing capacity, and antioxidant capacity.

2.2.7. Statistical analyses

The experiments were performed in triplicate. The results were evaluated by analysis of variance (ANOVA) and the means were compared by the Tukey test considering the level of significance of 5% ($p < 0.05$).

3. Results and discussion

3.1. Characterization of the P. peruviana calyx extract

The *P. peruviana* calyx extracts were characterized after preparation. Regarding the reducing capacity, the result was 610 ± 20 mg gallic acid Eq.100 g^{-1} calyx. Regarding the antioxidant capacity by ORAC, they presented 137.93 ± 2.10 μmol of Trolox.g^{-1} of calyx.

According to Ballesteros-Vivas, Alvarez-Rivera, Ibánez, Parada-Alfonso, and Cifuentes (2019), the *P. peruviana* calyx extract was obtained under optimum conditions (at 125 °C and 75% EtOH v/v) and exhibited moderate antioxidant activity (EC_{50} of 77.18 g mL^{-1} and 1.08 mM trolox g^{-1}). Analysis of LC-q-TOF-MS/MS from the extract allowed the quantification of 4-hydroxywithanolide E and withanolide E.

For analysis by HPLC, the major compound found was rutin with a content of 11.38 ± 0.98 μg.mL^{-1} and rutin retention time of 9.45 min. The analytical method was linear in the concentration range of 0.1 to 5 μg.mL^{-1} ($y = 93{,}497\times + 4979.8$; $r = 0.9978$), specific, accurate (DPR = 1.96%) and with a detection limit of 0.01 μg rutin. mL^{-1} and 0.02 μg rutin quantification mL^{-1}, according to the analytical conditions used.

According to Medina et al. (2019), ultra-high performance liquid chromatography triple quadrupole tandem mass spectrometry (UHPLC-QqQ-MS/MS) revealed novel active oxylipins denominated phytoprostanes in the *P. peruviana* calyces. In addition, the profile of phenolic

Table 1
Physicochemical characteristics of the nanoemulsions containing the *P. peruviana* calyx extract (CPp-NE- 5 mg.mL^{-1} extract) and blank nanoemulsions (NE).

Formulation	Rutin concentration (μg.mL^{-1})	Droplet size (nm)	PdI[a]	Zeta potencial (mV)	pH	EE[b]
CPp-NE	10.70 ± 0.10	182 ± 1	0.14 ± 0.03	−10.7 ± 0.5	5.49 ± 0.08	84.13%
NE	–	165 ± 3	0.10 ± 0.02	−8.0 ± 1.2	6.33 ± 0.02	–

[a] PdI: polydispersity index.
[b] EE: Encapsulation efficiency.

compounds was characterized using LC-IT-DAD-MS/MS, where six phenolic derivatives were first described, of which the authors highlighted five cinnamoyl acid derivatives and one flavonoid (quercetin-3-O-glucoside). Among the compounds already identified, rutin was also the major phenolic compound.

It should be noted that there is a range of compounds in *P. peruviana* calyces, and Ballesteros-Vivas et al. (2019) reported a total of fifty-six phytochemicals, including major phenolic components, several withanolides (C28-isoprenoids) with a variety of biological activities, and a large family of anti-inflammatory sucrose esters tentatively identified, including rutin and quercetin as the major flavonoids found. These studies corroborate with the major compound found in our work because our focus was to find a marker for stability analyses.

3.2. Physicochemical characterization of nanoemulsions

The nanoemulsions presented homogeneous macroscopic appearance similar to a milky opalescent liquid with blueish reflex (Tyndall effect), which is typical of colloidal systems. The physicochemical characteristics of nanoemulsions are described in Table 1.

Nanoemulsions presented nanometric droplet sizes of 160–180 nm and polydispersity index below 0.15, which shows adequate homogeneity of the system. These values agree with other studies that characterize nanoemulsions because they have droplet sizes ranging between 20 and 300 nm (Komaiko & McClements, 2015; Yildirim, Oztop, & Soyer, 2017). In addition, the laser diffraction size results demonstrated nanometric distribution (D [4; 3]: 0.48 ± 0.01 and 0.44 ± 0.01 μm for CPp-NE and NE, respectively; and Span values of 0.62 ± 0.01 for both formulations, with values of Span ≤1 characterizing good formulation homogeneity), with a unimodal peak in this range and without the presence of micrometric drops. It is possible to observe that adding the extract to the formulations increased droplet sizes as demonstrated by Zorzi, Caregnato, Moreira, Teixeira, and Carvalho (2016) in the nanoemulsions containing the hydroalcoholic extract of macela (*Achyrocline satureioides*) in relation to the blank nanoemulsions. Furthermore, a more acidic pH was observed in the CPp-NE due to the extract acidity (CPp pH = 5.51 ± 0.01). In addition, among the phenolic compounds analyzed by HPLC, from the method used, the identified compound was rutin (concentration of 10.7 μg·mL^{-1}). According to the literature, the main disadvantage of the molecule is its poor solubility in the aqueous medium, which explains its low oral or topical bioavailability (Mauludin, Müller, & Keck, 2009) and being detrimental to its conversion into suitable dosage forms. The importance of nanoencapsulation in this case is emphasized, since it may make this compound have better apparent solubility in the aqueous medium, improving its bioaccessibility. The encapsulation efficiency obtained was around 85%, which is considered satisfactory, since other studies have reported lower efficiencies for bioactive compounds or plant extracts. For example, for exopolysaccharides extracted from brown algae (*Sargassum longifolium*) in nanoemulsions of orange oil, the EE found was 67% (Shofia, Jayakumar, Mukherjee, & Chandrasekaran, 2018). In another study, the authors obtained an EE of 68% for nanoemulsions containing phenolic constituents of the *Phyllanthus emblica* branch extract with 50% ethanol (Chaiittianan & Sripanidkulchai, 2014).

The nanoencapsulation of bioactive compounds has been a matter of great interest due to its exclusive functionalities compared to other techniques, such as high encapsulation efficiency and loading capacity, enhanced bioavailability, improved stability, sustained release profile, and masking undesirable flavors (Shishir, Xie, Sun, Zheng, & Chen, 2018).

The zeta potential reflects the surface potential of the particles, which is influenced by changes in the interface with the environment-dispersing agent due to the dissociation of functional groups on the surface of the particle or the adsorption of ionic species present in the aqueous dispersion medium (Schaffazick, Guterres, Freitas, & Pohlmann, 2003). Regarding the zeta potential, the samples presented negative values. The presence of the calyx extract in the formulations increased the zeta potential in modulus, which may predict a greater physicochemical stability of the nanoemulsions, since large repulsive forces tend to avoid aggregation between droplets (Kovalchuk & Starov, 2012).

As for droplet morphology, the nanoemulsion micrographs can be observed at different magnifications in Fig. 1. The produced CPp-NE had a spherical shape and smooth surface. In addition, they are in agreement with the size data obtained by photon correlation spectroscopy (PCS), confirming the size in the nano range.

3.3. Stability study

In the stability study, the formulations were maintained under different conditions: Refrigeration temperature (7 °C) in the dark (CPp-NE_{RE}), room temperature (25 °C) in the dark (CPp-NE_{AE}), refrigeration temperature (7 °C) with light incidence (CPp-NE_{RL}), room temperature (25 °C) with light incidence (CPp-NE_{AL}), and analyzed for 120 days at pre-established time intervals. In relation to pH, all formulations presented acidic pH (5.49 ± 0.08) with variations occurring over time and the CPp-NE_{RL} sample being stable at the end of the study (5.39 ± 0.02). Meanwhile, the CPp-NE_{AE} and CPp-NE_{AL} formulations showed a significant reduction in pH ($p < 0.05$), being more pronounced in the last formulation that was exposed to light and at room temperature (supplementary material), which may be due to oxidations in the formulation components.

Droplet size and PdI of the CPp-NE_{RE}, CPp-NE_{AE}, and CPp-NE_{RL} nanoemulsions did not show significant variations ($p > 0.05$) in 120 days (Fig. 2), remaining stable for these parameters and without any visible changes in the formulations. In addition, the same nanoemulsions presented PdI values below 0.25 at all analysis times, indicating good system homogeneity. However, in the CPp-NE_{AL} sample, a significant increase in size was observed at 15 days. In addition, after 90 days of analysis, a destabilization of this formulation was verified occurring the separation of phases. This reflected in a significant increase in droplet size and PdI ($p < 0.05$) at 90 and 120 days, even reaching the micrometer scale (data not shown). From this, this sample was excluded from the other analyses. Among the mechanisms of destabilization, such as flocculation, coalescence, Ostwald ripening, and creming, the Ostwald ripening is the dominant mechanism of destabilization for nanoemulsions, however, other phenomena may occur after, leading to the separation of phases. Several parameters can be related to these phenomena of instability, such as component structure and chemical properties, the concentration of emulsifiers and additives, and temperature, these of which cause changes in properties such as

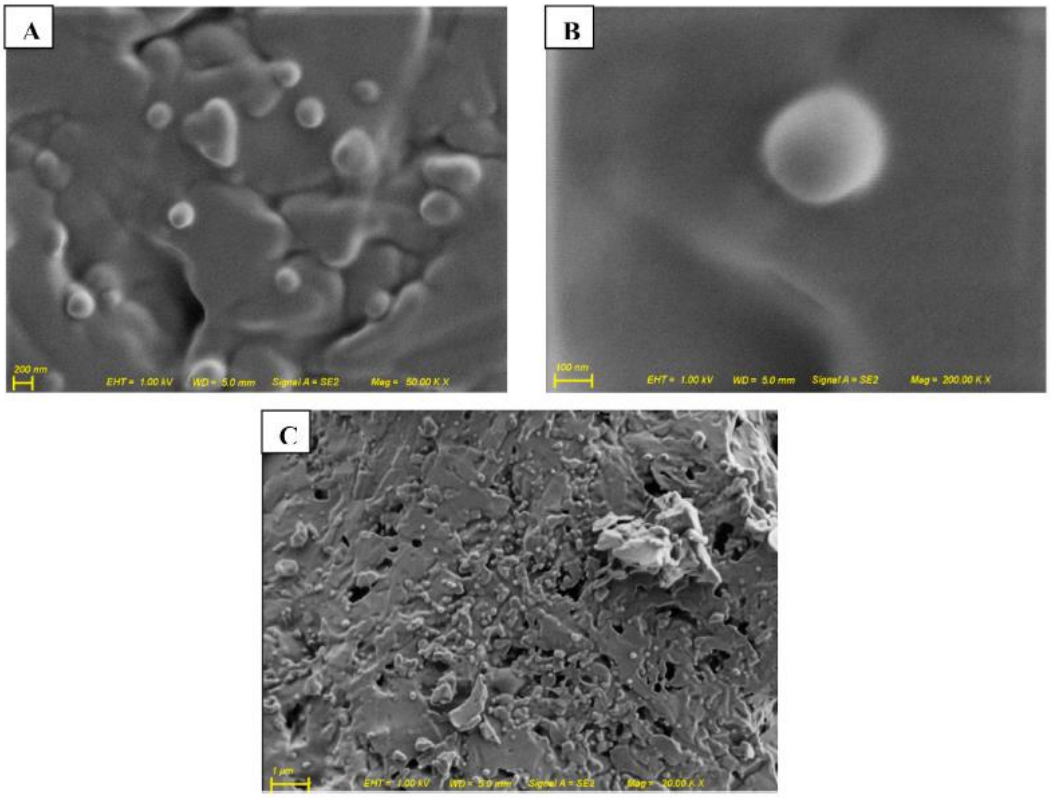


Fig. 1. Scanning electron microscopy of the nanoemulsions containing 5 mg.mL^{-1} of the *P. peruviana* calyx extract in different increases. (A) 50×, (B) 200×, and (C) 20×.

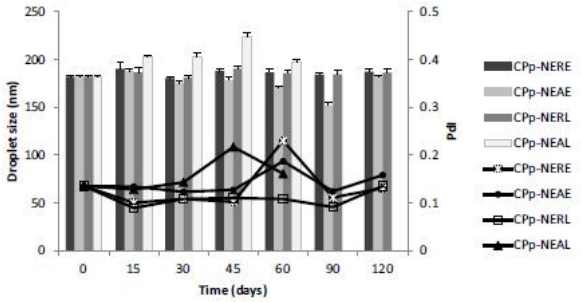


Fig. 2. Droplet size and polydispersity index (PdI) of the nanoemulsions containing the *P. peruviana* calyx extract (CPp-NE - 5 mg.mL^{-1} extract) in different storage conditions: R: refrigeration (7 °C); A: room (25 °C); E: dark; L: presence of light.

interfacial tension, droplet elasticity, and potential droplet interaction (Gupta, Eral, Hatton, & Doyle, 2016). According to Godoi et al. (2017), the nanoemulsions containing *Eucalyptus globulus* oil and stored at room temperature (25 °C) at 90 days showed increased sizes and reduced pH near 3, as verified in our study (sample CPp-NE$_{AL}$). Therefore, high temperatures or room temperatures and the incidence of light have been shown to be factors already reported to cause changes in nanoemulsion stability (Rebolleda et al., 2015).

Analyses of the zeta potential showed that the negative values remained stable during the study, regardless of the treatment. However, some modifications were observed over time. The samples CPp-NE$_{RE}$ and CPp-NE$_{RL}$ at the end of the experiment were stable with values of -10.46 ± 1.74 and -9.83 ± 1.45, respectively, and similar to the initial ones ($-11.23 \pm 0, 49$) ($p > 0.05$). In the samples at 25 °C with light incidence (CPp-NE$_{AL}$), a significant reduction in modulus was observed for -3.19 ± 0.29 ($p < 0.05$) at the zeta potential values at the end of the study, which is in agreement with the previous analyses that indicated instability in this formulation at 90 and 120 days. According to Giongo et al. (2016), the nanoemulsions containing the geranium oil presented phase separation at room temperature at 90 days, as observed in our study. In addition, both the nanoemulsions stored at 25 ± 2 °C and at 45 ± 2 °C showed increased droplet sizes and polydispersity index, which is expected since the temperature increases the kinetic energy of the system, increasing the possibility of occurring an instability phenomenon.

3.4. Antioxidant capacity

3.4.1. Reducing capacity

The values of the reducing capacity can be observed in Table 2. The CPp-NE$_{RE}$ formulation remained stable up to 90 days ($p > 0.05$). Afterwards, a reduction was observed and the experiment finished at around 85.22%. In contrast, the CPp-NE$_{AE}$ samples had a significant increase ($p < 0.05$) at 45 days of analysis, as well as the extract that was stored under the same conditions, which showed this increase after 90 days. Severo et al. (2010) already verified these changes in the reducing capacity in fruit stored at 23 °C, which increased from 210 to 360 mg gallic acid Eq.100 g^{-1}. On the other hand, at refrigeration temperature (4 °C), the increase was lower, reaching an amount of 300 mg gallic acid Eq.100 g^{-1}. Furthermore, in the same study, the

Table 2
Reducing capacity (mg gallic acid Eq. 100 g^{-1} calyx) of the nanoemulsions containing the *P. peruviana* calyx extract (CPp-NE - 5 mg.mL^{-1} extract) and CPp extracts under different storage conditions.

Samples	0 days	15 days	30 days	45 days	60 days	90 days	120 days
$CPp\text{-}NE_{RE}$	575 ± 28^{bAB}	535 ± 9^{cdBC}	619 ± 2^{aA}	619 ± 12^{bA}	615 ± 32^{bA}	614 ± 56^{cA}	490 ± 26^{cC}
$CPp\text{-}NE_{AE}$	575 ± 28^{bDE}	584 ± 12^{aD}	500 ± 7^{dE}	804 ± 24^{aC}	1696 ± 14^{aB}	1670 ± 19^{aB}	1814 ± 68^{aA}
$CPp\text{-}NE_{RL}$	575 ± 28^{bA}	534 ± 23^{cdAB}	523 ± 24^{cdBC}	520 ± 4^{dBC}	517 ± 3^{dBC}	514 ± 5^{dBC}	489 ± 15^{cC}
$CPp\text{-}NE_{AL}$	575 ± 28^{bA}	573 ± 9^{abA}	572 ± 1^{bA}	567 ± 4^{cA}	564 ± 17^{cA}	459 ± 11^{eB}	245 ± 29^{eC}
$CPp\text{-}_{RE}$	610 ± 20^{aA}	569 ± 6^{abcB}	549 ± 10^{bcC}	535 ± 4^{dCD}	522 ± 1^{dDE}	515 ± 1^{dE}	375 ± 9^{dF}
$CPp\text{-}_{AE}$	610 ± 20^{aB}	557 ± 6^{abcdC}	552 ± 4^{bcC}	522 ± 10^{dD}	466 ± 11^{efE}	754 ± 8^{bA}	611 ± 13^{bB}
$CPp\text{-}_{RL}$	610 ± 20^{aA}	523 ± 11^{dB}	524 ± 24^{cdB}	519 ± 8^{dBC}	485 ± 10^{deCD}	479 ± 8^{eD}	379 ± 14^{dE}
$CPp\text{-}_{AL}$	610 ± 20^{aA}	541 ± 19^{bcdB}	523 ± 18^{cdB}	481 ± 6^{eC}	441 ± 1^{fD}	386 ± 7^{fE}	382 ± 1^{dE}

Data were expressed as mean ± standard deviation (n = 3). Means with the same letter (lowercase in the column and uppercase in the row) do not differ statistically from each other by the Tukey test at 5% significance.
$CPp\text{-}NE_{RE}$: nanoemulsions containing the *P. peruviana* calyx extract maintained at refrigeration temperature (7 °C) in the dark.
$CPp\text{-}NE_{AE}$: nanoemulsions containing the *P. peruviana* calyx extract maintained at room temperature (25 °C) in the dark.
$CPp\text{-}NE_{RL}$: nanoemulsions containing the *P. peruviana* calyx extract maintained at refrigeration temperature (7 °C) with light incidence.
$CPp\text{-}NE_{AL}$: nanoemulsions containing the *P. peruviana* calyx extract maintained at room temperature (25 °C) with light incidence.
$CPp\text{-}_{RE}$: *P. peruviana* calyx extract maintained at refrigeration temperature (7 °C) in the dark.
$CPp\text{-}_{AE}$: *P. peruviana* calyx extract maintained at room temperature (25 °C) in the dark.
$CPp\text{-}_{RL}$: *P. peruviana* calyx extract maintained at refrigeration temperature (7 °C) with light incidence.
$CPp\text{-}_{AL}$: *P. peruviana* calyx extract maintained at room temperature (25 °C) with light incidence.

Table 3
Antioxidant capacity by ORAC (μmol Trolox.g^{-1} calyx) of nanoemulsions (NE) containing the *P. peruviana* calyx extract (CPp-NE - 5 mg.mL^{-1} extract) and CPp extracts under different storage conditions.

Samples	0 days	15 days	30 days	45 days	60 days	90 days	120 days
$CPp\text{-}NE_{RE}$	112.93 ± 2.85^{bA}	109.67 ± 1.53^{cdB}	111.29 ± 1.65^{abA}	110.80 ± 2.43^{aC}	110.13 ± 3.27^{aD}	110.52 ± 3.78^{abD}	111.77 ± 3^{bA}
$CPp\text{-}NE_{AE}$	112.93 ± 2.85^{bB}	111.24 ± 1.05^{cdBC}	105.04 ± 3.00^{bcBC}	104.34 ± 4.23^{abcBC}	103.09 ± 4.79^{abBC}	102.26 ± 3.77^{bcC}	131.66 ± 4.14^{aA}
$CPp\text{-}NE_{RL}$	112.93 ± 2.85^{bA}	108.52 ± 5.99^{cdA}	105.51 ± 6.48^{bcA}	87.27 ± 2.41^{dB}	74.35 ± 6.46^{cBC}	67.49 ± 3.90^{dC}	65.6 ± 1.22^{eC}
$CPp\text{-}NE_{AL}$	112.93 ± 2.85^{bA}	107.13 ± 1.20^{dAB}	94.65 ± 0.60^{cBC}	91.03 ± 8.11^{cdC}	74.05 ± 8.55^{cD}	14.54 ± 0.53^{eE}	–
$CPp\text{-}_{RE}$	137.93 ± 2.10^{aA}	140.54 ± 0.41^{aA}	117.17 ± 2.84^{abB}	108.77 ± 3.42^{abBC}	98.28 ± 10.67^{abC}	93.98 ± 8.49^{cC}	17.78 ± 0.49^{dD}
$CPp\text{-}_{AE}$	137.93 ± 2.10^{aA}	134.49 ± 11.83^{bAB}	125.11 ± 4.38^{aBC}	110.36 ± 5.58^{abD}	103.53 ± 7.53^{abD}	115.06 ± 0.96^{aCD}	67.05 ± 2.89^{cE}
$CPp\text{-}_{RL}$	137.93 ± 2.10^{aA}	118.63 ± 4.36^{bcB}	104.32 ± 8.57^{bcC}	96.99 ± 6.74^{bcdC}	95.26 ± 5.61^{abC}	77.30 ± 0.64^{dD}	8.29 ± 0.59^{eE}
$CPp\text{-}_{AL}$	137.93 ± 2.10^{aA}	110.87 ± 4.88^{cdB}	107.93 ± 7.56^{bcB}	105.35 ± 1.87^{abB}	88.19 ± 4.24^{bcC}	67.39 ± 3.34^{dD}	2.95 ± 0.14^{eE}

Data were expressed as mean ± standard deviation (n = 3). Means with the same letter (lowercase in the column and uppercase in the row) do not differ statistically from each other by the Tukey test at 5% significance.
$CPp\text{-}NE_{RE}$: nanoemulsions containing the *P. peruviana* calyx extract maintained at refrigeration temperature (7 °C) in the dark.
$CPp\text{-}NE_{AE}$: nanoemulsions containing the *P. peruviana* calyx extract maintained at room temperature (25 °C) in the dark.
$CPp\text{-}NE_{RL}$: nanoemulsions containing the *P. peruviana* calyx extract maintained at refrigeration temperature (7 °C) with light incidence.
$CPp\text{-}NE_{AL}$: nanoemulsions containing the *P. peruviana* calyx extract maintained at room temperature (25 °C) with light incidence.
$CPp\text{-}_{RE}$: *P. peruviana* calyx extract maintained at refrigeration temperature (7 °C) in the dark.
$CPp\text{-}_{AE}$: *P. peruviana* calyx extract maintained at room temperature (25 °C) in the dark.
$CPp\text{-}_{RL}$: *P. peruviana* calyx extract maintained at refrigeration temperature (7 °C) with light incidence.
$CPp\text{-}_{AL}$: *P. peruviana* calyx extract maintained at room temperature (25 °C) with light incidence.

antioxidant capacity remained stable at room temperature and decreased at 4 °C from 1.55 to 1.3 mmol TE.g^{-1}, as assessed by the ABTS-2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid). Based on this, it is emphasized that the storage temperature can influence reducing capacity values and consequently antioxidant capacity. The reducing capacity of the active substances present in the nanoemulsions with presence of light ($CPp\text{-}NE_{RL}$) remained for 45 days at 90%. Afterwards, it declined, finishing with 85.04% at 120 days. The $CPp\text{-}NE_{AL}$ sample remained stable for 60 days ($p > 0.05$). Another relevant factor is the high phenolic compound content present in the calyx. Gironés-Vilaplana et al. (2014) analyzed separately *P. peruviana* calyces and fruit from Colombia and, by comparing them, found that more compounds and high levels of non-red polyphenols were observed in the calyces (195.44 ± 3.88 mg.100 g^{-1} dry weight) than in the fruit (2.18 ± 0.71 mg.100 g^{-1} dry weight). This stresses the important source of bioactive compounds present in the calyx, making it of great interest for new food products. In addition, the presence of phenolic compounds in food products is extremely important since they provide numerous health benefits and act as anticancer, antithrombotic, antiulcerative, antiarterogenic, antiallergic, anti-inflammatory, antioxidant, immunomodulatory, antimicrobial and cardioprotective agents, vasodilators, and analgesics (Singh, Singh, Kaur, & Singh, 2017).

Altogether, the nanoemulsions had greater reducing capacity than the free extracts. These data are in agreement with those observed in the antioxidant capacity (Table 3). It should be noted that the $CPp\text{-}NE_{AE}$ samples had the antioxidant capacity potentiated and the $CPp\text{-}NE_{RE}$ samples at 120 days maintained their antioxidant capacity ($p > 0.05$). These results demonstrate that nanostructured systems can protect the bioactive compounds present in the extract against degradation, which favors prolonged shelf-life of nanoencapsulated extracts. The flavonoid mechanism of antioxidant action is determined by its structure, in particular by hydroxyls that can donate electrons and support delocalization around the aromatic system (Dornas, Oliveira, Rodrigues-das-Dores, Santos, & Nagem, 2007).

In the case of nanoemulsions that were subjected to light, $CPp\text{-}NE_{RL}$ samples were stable for 30 days ($p > 0.05$) and the samples at room temperature ($CPp\text{-}NE_{AL}$) showed significant reduction ($p < 0.05$), however, they still had approximately 84% of the initial antioxidant capacity at 30 days.

Table 4
Rutin content (μg.mL^{-1}) of nanoemulsions (NE) containing the *P. peruviana* calyx extract (CPp-NE - 5 mg.mL^{-1} extract) and CPp extracts under different storage conditions.

Samples	0 days	15 days	30 days	45 days	60 days	90 days	120 days
CPp-NE_{RE}	10.7 ± 0.1^{aA}	10.10 ± 0.39^{dB}	4.58 ± 0.14^{eC}	0.64 ± 0.12^{eDE}	0.65 ± 0.01^{cD}	0.38 ± 0.10^{dDE}	0.10 ± 0.02^{eE}
CPp-NE_{AE}	10.7 ± 0.1^{aA}	11.15 ± 0.21^{abA}	10.80 ± 0.04^{abcA}	8.62 ± 0.13^{abB}	8.03 ± 0.21^{aBC}	7.71 ± 1.14^{aBC}	7.05 ± 0.85^{aC}
CPp-NE_{RL}	10.7 ± 0.1^{aA}	9.51 ± 0.17^{cA}	9.50 ± 0.08^{dA}	6.84 ± 0.12^{dB}	6.12 ± 0.78^{bB}	5.73 ± 0.13^{bB}	5.59 ± 0.95^{bB}
CPp-NE_{AL}	10.7 ± 0.1^{aA}	9.28 ± 0.18^{cB}	10.29 ± 0.08^{bcdA}	7.05 ± 0.04^{cdC}	6.08 ± 0.60^{bD}	4.37 ± 0.15^{cE}	2.57 ± 0.09^{dF}
CPp-$_{RE}$	11.38 ± 0.98^{aA}	10.77 ± 0.16^{bcA}	11.74 ± 0.02^{aA}	9.11 ± 0.06^{abB}	8.95 ± 0.14^{aB}	6.22 ± 0.01^{bC}	5.42 ± 0.02^{bC}
CPp-$_{AE}$	11.38 ± 0.98^{aA}	10.44 ± 0.02^{cdAB}	11.27 ± 1.16^{abA}	9.84 ± 0.48^{aAB}	9.03 ± 0.74^{aB}	5.92 ± 0.34^{bC}	5.38 ± 0.16^{bC}
CPp-$_{RL}$	11.38 ± 0.98^{aA}	11.42 ± 0.01^{aA}	9.57 ± 0.03^{dB}	8.59 ± 0.39^{abBC}	8.3 ± 0.50^{aC}	7.63 ± 0.11^{aC}	6.08 ± 0.19^{abD}
CPp-$_{AL}$	11.38 ± 0.98^{aA}	11.16 ± 0.10^{abA}	10.07 ± 0.01^{cdAB}	8.39 ± 1.24^{bcB}	6.55 ± 0.29^{bC}	5.39 ± 0.07^{bcCD}	4.01 ± 0.06^{cD}

Data were expressed as mean ± standard deviation (n = 3). Means with the same letter (lowercase in the column and uppercase in the row) do not differ statistically from each other by the Tukey test at 5% significance.
CPp-NE_{RE}: nanoemulsions containing the *P. peruviana* calyx extract maintained at refrigeration temperature (7 °C) in the dark.
CPp-NE_{AE}: nanoemulsions containing the *P. peruviana* calyx extract maintained at room temperature (25 °C) in the dark.
CPp-NE_{RL}: nanoemulsions containing the *P. peruviana* calyx extract maintained at refrigeration temperature (7 °C) with light incidence.
CPp-NE_{AL}: nanoemulsions containing the *P. peruviana* calyx extract maintained at room temperature (25 °C) with light incidence.
CPp-$_{RE}$: *P. peruviana* calyx extract maintained at refrigeration temperature (7 °C) in the dark.
CPp-$_{AE}$: *P. peruviana* calyx extract maintained at room temperature (25 °C) in the dark.
CPp-$_{RL}$: *P. peruviana* calyx extract maintained at refrigeration temperature (7 °C) with light incidence.
CPp-$_{AL}$: *P. peruviana* calyx extract maintained at room temperature (25 °C) with light incidence.

3.4.2. Oxigen Radical Absorbance Capacity (ORAC)

The antioxidant capacity of the *P. peruviana* calyx was also evaluated by ORAC according to Gironés-Vilaplana et al. (2014). The authors observed that the *P. peruviana* calyces presented higher antioxidant capacity than the fruit, which highlights the importance of working with this residue. In addition, the values found for the calyx extract samples analyzed in our study presented higher antioxidant capacity values than those observed in the previously mentioned study, which may be due to the samples being from another location, growing season, and different methodology to obtain the extract.

3.5. Rutin content

Regarding the rutin content found in the samples, the nanoemulsions maintained at room temperature in the dark remained stable ($p > 0.05$) for 30 days and at 90 days presented a loss of 28% of rutin (Table 4). According to Yuan, Gao, Zhao, and Mao (2008), β-carotene nanoemulsions exhibited good physical stability but were chemically degraded during storage and at the end of the four-week period (30 days), with about 14–25% of β-carotene being lost, but with higher degradation at 25 °C than at 4 °C. On the other hand, NE stored at room temperature in the dark (CPp-NE_{AE}) were more stable for rutin content than the refrigerated ones in our study. As for the free extracts, the treatments submitted to the dark were stable for 30 days, and those stored with light incidence were stable for 15 days. In this manner, it is possible to infer that the rutin extracted from the *P. peruviana* calyx extract did not degrade on the first days of analysis, as the free extracts were expected to be more unstable in adverse temperatures and light conditions. In this case, rutin was found to have a positive point of not instantly degrading.

Rutin was also found in fruit of the same species, varying according to maturation rates (5.89 μg rutin. g^{-1} for beginning and 6.90 μg rutin. g^{-1} fruit at the end of maturation) (Licodiedoff, Koslowski, & Ribani, 2013). According to Gironés-Vilaplana et al. (2014), it was only possible to identify trace amounts of rutin (quercetin 3-O-rutinoside) and kaempferol 3-O-rutinoside in the fruit.

In the same study, two different quercetin glycosides were found in the *P. peruviana* calyx (among them rutin in greater quantity), two different kaempferol glycosides, 3-O-caffeoylquinic, and 5-O-caffeoylquinic acids. The calyx presented the highest concentrations of flavonols and derivatives of hydroxycinnamic acid among all the Latin American fruit analyzed (Gironés-Vilaplana et al., 2014). In addition, Toro et al. (2014) also found bioactive compounds, such as rutin and nicotoflorin (kaempferol-3-O-rutinoside) in the *P. peruviana* chalices, corroborating with the previous studies and the major compound (rutin) found in the present study.

In the literature, other encapsulation techniques have already been performed with the *P. peruviana* plant, as the fruit juice was microencapsulated to increase its stability in simulated digestion fluids (Dag, Kilercioglu, & Oztop, 2017) and development and optimization of the microencapsulated butanolic fraction of the *P. peruviana* calyces to improve its hypoglycemic activity (Echeverry, Valderrama, Costa, Ospina- Giraldo, & Aragón, 2018). Future trends point towards evaluating extracts and nanoemulsions against the release of bioactive compounds in simulated gastrointestinal fluid and toxicity in vitro and in vivo models.

4. Conclusion

Different storage conditions, such as the incidence or absence of light and the temperature, influence stability, especially in relation to bioactive compound content, reducing capacity, and antioxidant capacity. In this study, the best storage conditions of the samples were at both temperatures in the dark, and the highest stability was observed for the nanoemulsions containing the *P. peruviana* calyx extract stored at room temperature and in the absence of light. These results demonstrate that developed nanoemulsions have potential use in preserving the antioxidant capacity of *P. peruviana* calyx extract, thus favoring its reuse, as they avoid waste and have bioactive compounds that are beneficial to health. Therefore, the use of nanotechnology together with plant residues is of great interest for the science of food and development of new food products.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2019.108645.

Acknowledgements

This study was financed in part by the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* - Brasil (CAPES) - Finance Code 001".

References

Acevedo-Fani, A., Soliva-Fortuny, R., & Martín-Belloso, O. (2017). Nanoemulsions as edible coatings. *Current Opinion in Food Science, 15*(supplement C), 43–49.
Ballesteros-Vivas, D., Alvarez-Rivera, G., Ibánez, E., Parada-Alfonso, F., & Cifuentes, A. (2019). A multi-analytical platform based on pressurized-liquid extraction, in vitro

assays and liquid chromatography/gas chromatography coupled to high resolution mass spectrometry for food by-products valorisation. Part 2: characterization of bioactive compounds from goldenberry (Physalis peruviana L.) calyx extracts using hyphenated techniques. *Journal of Chromatography A, 1584*, 144–154.

Ballesteros-Vivas, D., Alvarez-Rivera, G., Sánchez-Camargo, A., Ibánez, E., Parada-Alfonso, F., & Cifuentes, A. (2019). A multi-analytical platform based on pressurized-liquid extraction, in vitro assays and liquid chromatography/gas chromatography coupled to high resolution mass spectrometry for food by-products valorisation. Part 1: Withanolides-rich fractions from goldenberry (Physalis peruviana L.) calyces obtained after extraction optimization as case study. *Journal of Chromatography A, 1584*, 155–164.

Bilal, M., & Iqbal, H. M. N. (2019). Sustainable bioconversion of food waste into high-value products by immobilized enzymes to meet bio-economy challenges and opportunities–A review. *Food Research International, 123*, 226–240.

Bonifácio, B. V., da Silva, P. B., Ramos, M. A. D. S., Negri, K. M. S., Bauab, T. M., & Chorilli, M. (2014). Nanotechnology-based drug delivery systems and herbal medicines: A review. *International Journal of Nanomedicine, 9*, 1–15.

Bouchemal, K., Briançon, S., Perrier, E., & Fessi, H. (2004). Nano-emulsion formulation using spontaneous emulsification: Solvent, oil and surfactant optimisation. *International Journal of Pharmaceutics, 280*(1), 241–251.

Castro, J., Ocampo, Y., & Franco, L. (2015). Cape gooseberry [Physalis peruviana L.] calyces ameliorate TNBS acid-induced colitis in rats. *Journal of Crohn's and Colitis, 9*(11), 1004–1015.

Chaiittianan, R., & Sripanidkulchai, B. (2014). Development of a nanoemulsion of Phyllanthus emblica L. branch extract. *Drug Development and Industrial Pharmacy, 40*(12), 1597–1606.

Chandra, S., & Mejia, E. G. (2004). Polyphenolic compounds, antioxidant capacity, and Quinone reductase activity of an aqueous extract of Ardisia compressa in comparison to mate (Ilex paraguariensis) and green (Camellia sinensis) teas. *Journal of Agricultural and Food Chemistry, 52*(11), 3583–3589.

Codevilla, C., Bazana, M., Silva, C., Barin, J., & Ragagnin de Menezes, C. (2015). Nanoestruturas contendo compostos bioativos extraídos de plantas. Ciência e Natura. 37, Ed *Especial-nano e microencapsulação de compostos bioativos e probióticos em alimentos* (pp. 142–151). .

Codevilla, C. F., Tischer, B., Gindri, A. L., Nogueira-Librelotto, D. R., Barin, J. S., Silva, C. B., ... Menezes, C. R. (2018). Cytotoxicity and antioxidant activity of goldenberry extracts obtained with high intensity ultrasound. *Ciência Rural, 48*(2), e20170362.

Cömert, E. D., & Gökmen, V. (2018). Evolution of food antioxidants as a core topic of food science for a century. *Food Research International, 105*, 76–93.

Dag, D., Kilercioglu, M., & Oztop, M. H. (2017). Physical and chemical characteristics of encapsulated goldenberry (Physalis peruviana L.) juice powder. *LWT - Food Science and Technology, 83*(suppl. C), 86–94.

Dornas, W. C., Oliveira, T. T., Rodrigues-das-Dores, R. G., Santos, A. F., & Nagem, T. J. (2007). Flavonóides: potencial terapêutico no estresse oxidativo. *Revista de Ciências Farmacêuticas Básica e Aplicada, 28*(3), 241–249.

Echeverry, S. M., Valderrama, I. H., Costa, G. M., Ospina- Giraldo, L. F., & Aragón, D. M. (2018). Development and optimization of microparticles containing a hypoglycemic fraction of calyces from Physalis peruviana. *Journal of Applied Pharmaceutical Science, 8*(05), 10–18.

Franco, L. A., Matiz, G. E., Calle, J., Pinzón, R., & Ospina, L. F. (2007). Actividad antinflamatoria de extractos y fracciones obtenidas de cálices de Physalis peruviana L. *Biomédica, 27*, 110–115.

Franco, L. A., et al. (2014). Sucrose esters from Physalis peruviana calyces with anti-inflammatory activity. *Planta Medica, 80*(17), 1605–1614.

Giongo, J. L., et al. (2016). Development of nanoemulsion containing Pelargonium graveolens oil: Characterization and stability study. *International Journal of Pharmacy and Pharmaceutical Sciences, 8*(12), 271–276.

Gironés-Vilaplana, A., Baenas, N., Villaño, D., Speisky, H., García-Viguera, C., & Moreno, D. A. (2014). Evaluation of Latin-American fruits rich in phytochemicals with biological effects. *Journal of Functional Foods, 7*(supplement C), 599–608.

Godoi, S. N., et al. (2017). Evaluation of stability and in vitro security of nanoemulsions containing eucalyptus globulus oil. *BioMed Research International, 2017*, 1–10 Article ID 2723418.

Gullón, B., Lú-Chau, T. A., Moreira, M. T., Lema, J. M., & Eibes, G. (2017). Rutin: A review on extraction, identification and purification methods, biological activities and approaches to enhance its bioavailability. *Trends in Food Science & Technology, 67*, 220–235.

Gupta, A., Eral, H. B., Hatton, T. A., & Doyle, P. S. (2016). Nanoemulsions: formation, properties and applications. *Soft Matter, 12*(11), 2826–2841.

Hamad, A. F., Han, J.-H., Kim, B.-C., & Rather, I. A. (2018). The intertwine of nano-technology with the food industry. *Saudi Journal of Biological Sciences, 25*(1), 27–30.

Komaiko, J., & McClements, D. J. (2015). Low-energy formation of edible nanoemulsions by spontaneous emulsification: factors influencing particle size. *Journal of Food Engineering, 146*(supplement C), 122–128.

Kovalchuk, N. M., & Starov, V. M. (2012). Aggregation in colloidal suspensions: Effect of colloidal forces and hydrodynamic interactions. *Advances in Colloid and Interface Science, 179-182*(supplement C), 99–106.

Li, Z.-H., Cai, M., Yang, K., & Sun, P.-L. (2019). Kinetic study of d-limonene release from finger citron essential oil loaded nanoemulsions during simulated digestion in vitro. *Journal of Functional Foods, 58*, 67–73.

Licodiedoff, S., Koslowski, L. D., & Ribani, R. H. (2013). Flavonols and antioxidant activity of Physalis peruviana L. fruit at two maturity stages. *Acta Scientiarum Technology, 35*(2), 393–399.

Marques da Silva, T., et al. (2018). Development and characterization of microcapsules containing Bifidobacterium Bb-12 produced by complex coacervation followed by freeze drying. *LWT-Food Science and Technology, 90*, 412–417.

Mauludin, R., Müller, R. H., & Keck, C. M. (2009). Development of an oral rutin nanocrystal formulation. *International Journal of Pharmaceutics, 370*(1), 202–209.

Medina, S., et al. (2019). Potential of Physalis peruviana calyces as a low-cost valuable resource of phytoprostanes and phenolic compounds. *Journal of the Science of Food and Agriculture, 99*, 2194–2204.

Nunes, G. L., et al. (2018). Inulin, hi-maize, and trehalose as thermal protectants for increasing viability of Lactobacillus acidophilus encapsulated by spray drying. *LWT-Food Science and Technology, 89*, 128–133.

Ou, B., Hampsch-Woodill, M., & Prior, R. L. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry, 49*(10), 4619–4626.

Paudel, S. K., Bhargava, K., & Kotturi, H. (2019). Antimicrobial activity of cinnamon oil nanoemulsion against Listeria monocytogenes and Salmonella spp. on melons. *LWT-Food Science and Technology, 111*, 682–687.

Pedreño, M. A., & Escribano, J. (2001). Correlation between antiradical activity and stability of betanine from Beta vulgaris L roots under different pH, temperature and light conditions. *Journal of the Science of Food and Agriculture, 81*, 627–631.

Perumalla, A. V. S., & Hettiarachchy, N. S. (2011). Green tea and grape seed extracts-potential applications in food safety and quality. *Food Research International, 44*, 827–839.

Puente, L. A., Pinto-Muñoz, C. A., Castro, E. S., & Cortés, M. (2011). Physalis peruviana Linnaeus, the multiple properties of a highly functional fruit: A review. *Food Research International, 44*(7), 1733–1740.

Rebolleda, S., Sanz, M. T., Benito, J. M., Beltrán, S., Escudero, I., & González San-José, M. (2015). Formulation and characterisation of wheat bran oil-in-water nanoemulsions. *Food Chemistry, 167*(supplement C), 16–23.

Rezaei, A., Fathi, M., & Jafari, S. M. (2019). Nanoencapsulation of hydrophobic and low-soluble food bioactive compounds within different nanocarriers. *Food Hydrocolloids, 88*, 146–162.

Rufato, A. R., Rufato, L., Lima, C. S. M., & Muniz, J. (2013). A cultura da physalis. In A. A. Kretzschmar, L. Rufato, & T. R. Pelizza (Eds.). *Pequenas Frutas* (pp. 172–238). Florianópolis: UDESC 2013. ((Série Fruticultura, 2), 2013).

Salem, M. A., & Ezzat, S. M. (2018). Nanoemulsions in food industry. In J. M. Milani (Ed.). *Some new aspects of colloidal systems in foods* (pp. 31–51). IntechOpen. https://doi.org/10.5772/intechopen.79447. chapter 3. Retrieved from https://www.intechopen.com/books/some-new-aspects-of-colloidal-systems-in-foods/nanoemulsions-in-food-industry.

Schaffazick, S. R., Guterres, S. S., Freitas, L. L., & Pohlmann, A. R. (2003). Caracterização e estabilidade físico-química de sistemas poliméricos nanoparticulados para administração de fármacos. *Química Nova, 26*(5), 726–737.

Severo, J., Lima, C. S. M., Coelho, M. T., Rufato, A. R., Rombaldi, C. V., & Silva, J. A. (2010). Atividade antioxidante e fitoquímicos em frutos de Physalis (Physalis peruviana, L.) durante o amadurecimento e o armazenamento. *Revista Brasileira de Agrociência, 16*(1–4), 77–82.

Shin, G. H., Kim, J. T., & Park, H. J. (2015). Recent developments in nanoformulations of lipophilic functional foods. *Trends in Food Science & Technology, 46*(1), 144–157.

Shishir, M. R. I., Xie, L., Sun, C., Zheng, X., & Chen, W. (2018). Advances in micro and nano-encapsulation of bioactive compounds using biopolymer and lipid based transporters. *Trends in Food Science & Technology, 78*, 34–60.

Shofia, S. I., Jayakumar, K., Mukherjee, A., & Chandrasekaran, N. (2018). Efficiency of brown seaweed (Sargassum longifolium) polysaccharides encapsulated in nanoemulsion and nanostructured lipid carrier against colon cancer cell lines HCT 116. *Royal Society of Chemistry Advances, 8*, 15973–15984.

Silva Junior, E., et al. (2013). Formação de nanoemulsões do tipo óleo em água contendo óleo de semente de romã. *Disciplinarum Scientia. Série: Ciências Naturais e Tecnológicas, 14*(1), 115–122.

Singh, B., Singh, J. P., Kaur, A., & Singh, N. (2017). Phenolic composition and antioxidant potential of grain legume seeds: a review. *Food Research International, 101*(supplement C), 1–16.

Toro, R. M., Aragón, D. M., Ospina, L. F., Ramos, F. A., & Castellanos, L. (2014). Phytochemical analysis, antioxidant and anti-inflammatory activity of calyces from Physalis peruviana. *Natural Product Communications, 9*(11), 1573–1575.

Valduga, A. T., Gonçalves, I. L., Magri, E., & Finzer, J. R. D. (2019). Chemistry, pharmacology and new trends in traditional functional and medicinal beverages. *Food Research International, 120*, 478–503.

Xiu-li, H., Xue-li, L., Yuan-ping, L., & Qiang, H. (2015). Composition and color stability of anthocyanin-based extract from purple sweet potato. *Food Science and Technology, 35*(3), 468–473.

Yildirim, S. T., Oztop, M. H., & Soyer, Y. (2017). Cinnamon oil nanoemulsions by spontaneous emulsification: Formulation, characterization and antimicrobial activity. *LWT-Food Science and Technology, 84*(supplement C), 122–128.

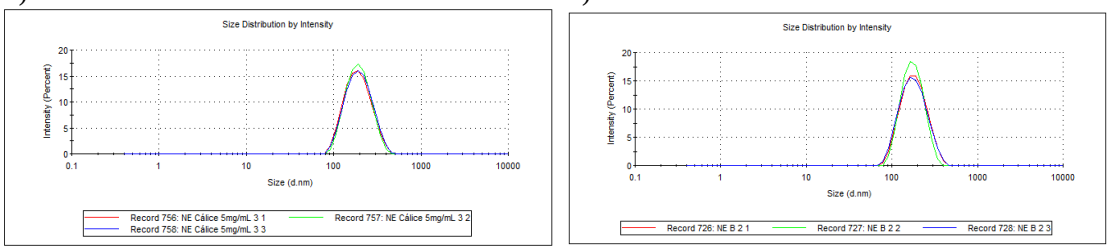
Yuan, Y., Gao, Y., Zhao, J., & Mao, L. (2008). Characterization and stability evaluation of β-carotene nanoemulsions prepared by high pressure homogenization under various emulsifying conditions. *Food Research International, 41*(1), 61–68.

Zorzi, G. K., Caregnato, F., Moreira, J. C. F., Teixeira, H. F., & Carvalho, E. L. S. (2016). Antioxidant effect of nanoemulsions containing extract of Achyrocline satureioides (Lam) D.C.—Asteraceae. *AAPS PharmSciTech, 17*(4), 844–850.

MATERIAL SUPLEMENTAR DO ARTIGO 2

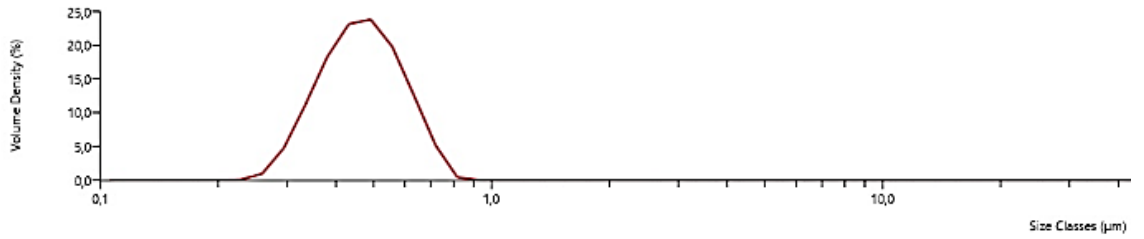
Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2019.108645.

A) B)

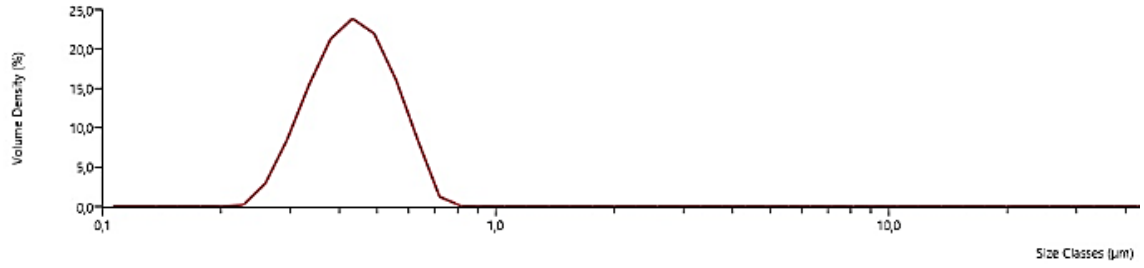


Supplementary Fig. S1. Nanoemulsion droplet size distribution containing 5 mg.mL^{-1} of the *P. peruviana* calyx extract (A) and blank nanoemulsion (B) analyzed by photon correlation spectroscopy.

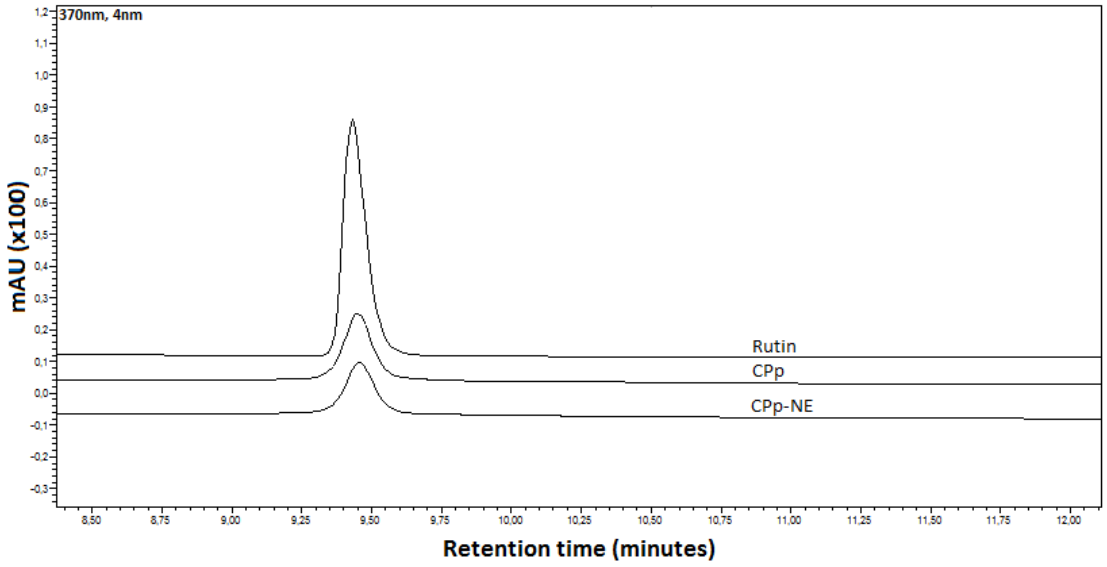
A)



B)



Supplementary Fig. S2. Nanoemulsion droplet size distribution containing 5 mg.mL^{-1} of the *P. peruviana* calyx extract (A) and blank nanoemulsion (B) analyzed by laser diffraction.



Supplementary Fig. S3. Overlapping chromatograms obtained by rutin standard HPLC from the *P. peruviana* calyx extract (CPp) and the nanoemulsions containing the *P. peruviana* calyx extract (CPp-NE).

Supplementary Table S1. pH measurements of the nanoemulsions containing the *P. peruviana* calyx extract (CPp-NE - 5 mg.mL^{-1} extract) under different storage conditions.

Time (days)	CPp-NE$_{RE}$	CPp-NE$_{AE}$	CPp-NE$_{RL}$	CPp-NE$_{AL}$
0	5.49 ± 0.08^{cA}	5.49 ± 0.08^{aA}	5.49 ± 0.08^{cA}	5.49 ± 0.08^{aA}
15	5.26 ± 0.02^{dB}	5.34 ± 0.01^{bA}	5.33 ± 0.01^{dA}	3.16 ± 0.02^{cC}
30	5.23 ± 0.04^{dA}	4.95 ± 0.01^{cB}	5.35 ± 0.02^{dA}	3.32 ± 0.11^{bC}
45	5.46 ± 0.02^{cA}	4.65 ± 0.01^{dC}	5.37 ± 0.02^{dB}	3.24 ± 0.01^{bcD}
60	5.67 ± 0.01^{bA}	4.55 ± 0.01^{eC}	5.59 ± 0.02^{aB}	3.25 ± 0.03^{bcD}
90	5.89 ± 0.02^{aA}	4.38 ± 0.01^{fC}	5.53 ± 0.02^{abB}	3.25 ± 0.02^{bcD}
120	5.67 ± 0.01^{bA}	4.35 ± 0.02^{fC}	5.39 ± 0.02^{cdB}	3.28 ± 0.01^{bcD}

Data were expressed as mean ± standard deviation (n = 3). Means with the same letter (lowercase in the column and uppercase in the row) do not differ statistically from each other by the Tukey test at 5% significance.
CPp-NE$_{RE}$: nanoemulsions containing the *P. peruviana* calyx extract maintained at refrigeration temperature (7 °C) in the dark
CPp-NE$_{AE}$: nanoemulsions containing the *P. peruviana* calyx extract maintained at room temperature (25 °C) in the dark
CPp-NE$_{RL}$: nanoemulsions containing the *P. peruviana* calyx extract maintained at refrigeration temperature (7 °C) with light incidence
CPp-NE$_{AL}$: nanoemulsions containing the *P. peruviana* calyx extract maintained at room temperature (25 °C) with light incidence
CPp-$_{RE}$: *P. peruviana* calyx extract maintained at refrigeration temperature (7 °C) in the dark
CPp-$_{AE}$: *P. peruviana* calyx extract maintained at room temperature (25 °C) in the dark
CPp-$_{RL}$: *P. peruviana* calyx extract maintained at refrigeration temperature (7 °C) with light incidence
CPp-$_{AL}$: *P. peruviana* calyx extract maintained at room temperature (25 °C) with light incidence

6 MANUSCRITO 1 – ANTIBACTERIAL AND ANTIBIOFILM ACTIVITY OF *Physalis peruviana* CALYX EXTRACT

Manuscrito submetido a Revista Ciência Rural.

Antibacterial and antibiofilm activity of *Physalis peruviana* calyx extract

Atividade antibacteriana e antibiofilme do extrato do cálice de *Physalis peruviana*

Maiara Taís Bazana[I] Marcos Vinícius Missel[II] Cassandra de Deus[I] Vandré Sonza Pinto[I] Suelen Santos da Silva[I] Cristiane Franco Codevilla[I] Roberto Christ Vianna Santos[II] Cristiane de Bona da Silva[III] Cristiano Ragagnin de Menezes[I*]

ABSTRACT

This study aimed to evaluate the antibacterial and antibiofilm activity of a *P. peruviana* calyx extract. Goldenberry calyx extracts were prepared with 60% (v/v) ethanol. Minimum inhibitory concentration (MIC) analyses were performed by the 96-well plate microdilution method together with the minimum bactericidal concentration (MBC). Biofilm inhibition and destruction was performed in microdilution plates. The *P. peruviana* calyx extract presented antibacterial activity against the pathogens analysed (*Enterobacter aerogenes, Klebsiella pneumoniae, Staphylococcus epidermidis, Streptococcus pneumoniae, Pseudomonas aeruginosa* PA01, *Enterococcus faecalis*, *Escherichia coli, Shigella sonnei, Acinetobacter baumannii, Streptococcus agalactie, Acinetobacter baumannii, Salmonella sp., Salmonella enteridis and Staphylococcus aureus*). MIC varied from 3.15 to 30 mg/mL extract and showed bacteriostatic activity against eight pathogens and bactericidal activity at 30 mg/mL concentration against six strains. Regarding the biofilm tests, there was inhibition of biofilm formation, but there was no destruction. According to these results, the potential antibacterial activity of *P. peruviana* calyx extract was verified. This will enable further studies to be carried out to contribute to its use in the food industry as a preservative of natural origin and other clinical applications.

Keywords: biofilm, minimum inhibitory concentration, minimum bactericidal concentration.

[I]Departamento de Tecnologia e Ciência dos Alimentos, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brasil

[II]Departamento de Microbiologia e Parasitologia, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brasil.

[III]Departamento de Farmácia Industrial, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brasil.

[I*] Departamento de Tecnologia e Ciência dos Alimentos, Universidade Federal de Santa Maria (UFSM), 97105-900, Santa Maria, RS, Brasil. E-mail: cristiano.ufsm@gmail.com Autor para correspondência.

RESUMO

Este estudo teve como objetivos avaliar a atividade antibacteriana e antibiofilme do extrato do cálice de *P. peruviana*. Os extratos do cálice de goldenberry foram preparados com etanol 60% (v/v). As análises de concentração inibitória mínima (CIM) foram realizadas pelo método de microdiluição em placas de 96 poços, juntamente com a concentração bactericida mínima (CBM). A inibição e destruição de biofilme foi realizada em placas de microdiluição. O extrato do cálice de *P. peruviana* apresentou atividade antibacteriana frente aos patógenos analisados (*Enterobacter aerogenes, Klebsiella pneumoniae, Staphylococcus epidermidis, Streptococcus pneumoniae, Pseudomonas aeruginosa* PA01, *Enterococcus faecalis, Escherichia coli, Shigella sonnei, Acinetobacter baumannii, Streptococcus agalactie, Acinetobacter baumannii, Salmonella sp., Salmonella enteridis and Staphylococcus aureus*). A CIM variou de 3,15 a 30 mg/mL de extrato, demonstrando atividade bacteriostática frente a oito patógenos e atividade bactericida na concentração de 30 mg/mL frente a seis cepas. Em relação aos testes com biofilmes, houve inibição da formação de biofilme, porém não houve destruição. De acordo com esses resultados, foi verificada a potencial atividade antibacteriana do extrato do cálice de *P. peruviana*. Isto possibilitará que novos estudos sejam realizados para contribuir na sua utilização na indústria de alimentos, como um conservante de origem natural.

Palavras-chave: biofilme, concentração inibitória mínima, concentração bactericida mínima.

INTRODUCTION

Among the new natural sources, *Physalis peruviana* has been studied for its antimicrobial potential. ERTÜRK et al. (2017) evaluated the antioxidant and antimicrobial activity of ethanolic extracts from different parts of *P. peruviana* (fruit, seed, root, body and leaf). Seeds and fruit were the most effective of the plant in terms of its antimicrobial activity. In another study, the dimethyl sulfoxide extract of *P. peruviana* fruit inhibited the growth of microorganisms used in the test (GÖZTOK; ZENGIN, 2013). All parts of the *P. peruviana* plant may be potential sources of antimicrobial agents, the most potent being leaf extracts in dichloromethane (KAMAU et al., 2017). In addition, the ripe berries and leaves of *P. peruviana* were evaluated and the ethanolic extracts were more effective than the aqueous ones (CUEVA et al., 2017).

P. peruviana fruit is usually marketed without its calyx; consequently, the waste generated by its trade is substantial. Colombia is the world's leading producer of *P. peruviana*, which represents the country's second largest exported product. However, waste generated by the agro-industrial sector, particularly fruit and vegetable waste, is a major economic and environmental issue for many companies, as it is up to them to assume the cost of their management (MEDINA et al., 2019).

Contamination of foods is usually found when they are damaged or contaminated by containing microorganisms such as bacteria, parasites or toxic substances that make them unfit for consumption (HUSSAIN, 2016). *P. aeruginosa* PA01 is a Gram-negative rod, considered a standard biofilm-producing strain and an important pathogen chosen for this study. A biofilm is a complex of bacterial cells lined with a polysaccharide layer that acts as a protective factor for bacteria against antimicrobial attack and the host immune system. The

formation of biofilms causes considerable problems in the medical and industrial area, as these structures cause greater resistance to treatment with antibiotics and biocides. Resistance to antibiotic therapy and the emergence of multiresistant strains is worrisome, and new therapeutic approaches are required (LIMA et al., 2018).

Several chemical preservatives are used to control these foodborne pathogens (BAI et al., 2016). However, due to the increased awareness of the side effects of these additives, there is a growing consumer demand for natural products in place of chemical preservatives. In recent years, research on natural antimicrobial compounds has increased at a rapid pace (LEE et al., 2019).

There are a variety of natural preservatives from different sources and with different preservation properties, such as plant extracts, essential oils, organic acids (acetic, ascorbic, tartaric, malic, and citric), lactic acid bacteria and bacteriocins from microbiological sources, and chitosan of animal origin (BAPTISTA; HORITA; ANA, 2019). Plants that possess these properties have bioactive compounds that act to protect plants from microbiological attacks, although they can also be exploited and employed by humans as sources for food and medicine (ANTOLAK; KREGIEL, 2017).

Thus, *P. peruviana* calyces are an important byproduct that deserves further study so that different sources of reuse can be explored and the database on their antimicrobial activity expanded. Therefore, the aim of this study was to evaluate, for the first time, the antibacterial activity against different microorganisms and antibiofilm activity against *Pseudomonas aeruginosa* of *P. peruviana* calyx extract for therapeutic and food application.

MATERIAL AND METHODS

Material: *P. peruviana* calyces were obtained from Italbraz (Vacaria, Brazil - 28:0:44 S and 50:56:02 W) in the 2016/2017 harvest. Mueller Hinton broth (Himedia®) and Mueller-Hinton agar (Sigma-Aldrich®) and crystal violet (Sigma Chemical®) were used.

Microorganisms and inoculum: American Type Culture Collection (ATCC) bacterial strains and clinical isolates provided by the UFSM Department of Microbiology and Parasitology were used [*Enterobacter aerogenes* ATCC 13048, *Klebsiella pneumonia* ATCC 1705, *Escherichia coli* (clinical isolate), *Staphylococcus epidermidis* (clinical isolate), *Streptococcus pneumoniae* ATCC 99619, *Shigella sonnei* (clinical isolate), *Acinetobacter baumannii* ATCC 19606, *Streptococcus agalactie* (clinical isolate), *Acinetobacter baumannii* (clinical isolate), *Pseudomonas aeruginosa* PA01, *Enterococcus faecalis* ATCC 29212, *Salmonella sp.* (clinical isolate), *Salmonella enteridis* (clinical isolate), *and Staphylococcus aureus* (clinical isolate)]. The bacterial inoculum sizes were standardized according to CLSI guidelines (2017). Isolated colonies were grown for 18 and 24 h in Mueller Hinton Agar and the suspension was prepared in saline solution (NaCl 0.85%) with density adjusted to 0.5 on the McFarland scale ($1.5x10^8$ CFU/mL).

Extraction: The calyx extracts were prepared following Bazana et al. (2019). The *P. peruviana* calyces (3 g) were separated from the fruit, submitted to knife milling (Mill MA 630/1-Marconi) (speed = 5 rpm, time = 10s), added with 50 mL of 60% ethanol (v/v), and submitted to stirring in a homogenizer (Shaker model TE 421- Tecnal, Piracicaba, SP, Brazil) at a speed of 200 rpm for 2 h. Afterwards, the extracts were filtered in a qualitative filter (Fitec) for coarse separation of the calyx. Subsequently, the extract was subjected to filtration in a polypropylene syringe (0.22-μm pore size) (Chromfilter) and kept in a refrigerated environment.

Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC): The MICs were determined by the microdilution method according to Clinical and Laboratory Standards Institute (CLSI, 2017). The assay was carried out in 96-well-microplates using Mueller Hinton broth (MHB). Several concentrations of *P. peruviana* calyx extract (60; 30; 15; 7.5; 3.75; and 1.87 mg/mL) was diluted in ethanol 60% (v/v) and added in wells with MHB and the suspension with microorganism (0.5 MacFarland scale). The positive control was considered the well with the bacterial suspension and MHB while the negative control was MHB and extract. A control with ethanol 60% (v/v) was performed to discard the diluent activity. The plates were incubated at 37°C and the minimum inhibitory concentration (MIC) was recorded after 24 h of incubation. 2,3,5-triphenyltetrazolium chloride was used as an indicator which develop a red colour in the microbial grown. The assays were performed in triplicate. The MICs were defined as the lowest concentration that inhibits visible bacterial growth. The determination of the MBC was performed in conjunction with the MIC, using the method proposed by Courvalin (1985). After the determination of MIC, wells containing visible or non-visible growth were transferred to Petri dishes containing the solid Mueller-Hinton agar, then incubated at 37°C for 18-24 hours. After this period, the number of colonies per plaque was determined and the MBC was defined as the lowest concentration of the extract that presented 0.01% viable bacteria.

Biofilm inhibition: For this analysis, 15 µL of inoculum were pipetted with 100 µL MHB and 100 µL of extract, in MIC and subinhibitory concentrations into four wells of a sterile flat-bottomed microtiter plate. After incubation for 24 h at 37°C, all of the planktonic microorganisms were removed and washed with distilled water three times. The biofilm was visualised by adding 200 µL of 0.1% crystal violet solution to each well. The microplates were washed with distilled water and air-dried. To solubilize the biofilm, 200 µL of 95%

ethanol was added. The solution was transferred to a new microtiter plate and the biofilm formation was revealed by measuring the absorbance at 570 nm in a microplate reader. For the control culture broth was used for negative control and only *P. aeruginosa PA01* inoculum for positive control (BONEZ et al., 2017).

Biofilm destruction: 15 µL of inoculum with 185 µL of MHB was pipetted into four wells of a sterile flat-bottomed microtiter plate. After incubation for 24 h at 37°C, the planktonic microorganisms were removed from the wells and the extracts were added in MIC and subinhibitory concentrations. After incubation for 24 h at 37°C, all of the planktonic microorganisms were removed and washed with distilled water three times. The biofilm was visualised by adding 200 µL of 0.1% crystal violet solution to each well. The microplates were washed with distilled water and air-dried. To solubilize the biofilm, 200 µL of 95% ethanol was added. The solution was transferred to a new microtiter plate and the biofilm formation was revealed by measuring the absorbance at 570 nm in a microplate reader. For the controls, culture broth was used as the negative control and only *P. aeruginosa PA01* inoculum was used as the positive control (BONEZ et al., 2017).

Statistical analyses: The experiments were performed in triplicate. The results were evaluated by analysis of variance (ANOVA), and the means were compared by the Tukey test, considering the level of significance at 5% ($p<0.05$).

RESULTS AND DISCUSSION

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

The antimicrobial activity of the ethanolic extracts of *P. peruviana* calyx is shown in Table 1. The lowest minimum inhibitory concentration observed was against *Enterococcus*

faecalis bacteria, in which the extract concentration of 3.75 mg/mL was able to inhibit growth of this bacterium. MBC = 30 mg/mL was found for *Enterobacter aerogenes, Klebsiella pneumoniae*, *Staphylococcus epidermidis, Streptococcus pneumoniae, Pseudomonas aeruginosa* PAO1e *Enterococcus faecalis,* indicating that for these bacteria the 30 mg/mL *P. peruviana* calyx extract has a bactericidal action. For the other microorganisms, MBC ≥ 30 mg/mL indicated that the extract has bacteriostatic action. These results suggest that the bioactive compounds of calyx are responsible for antimicrobial action. According to Ballesteros-Vivas et al. (2019), several compounds were identified in the *P. peruviana* calyx as phytochemicals (terpenoids, phytosterols and phytol derivatives), flavonoids, phenolic acids and withanolides. Phytosterols are reported as bioactive compounds of great interest due to their antioxidant capacity and health impact. They are referred to as anti-inflammatory, antitumor, antibacterial, antifungal and hypocholesterolemic compounds. Its presence in significant levels in oil extracted from the skin and pulp of *P. peruviana* has already been found (PUENTE et al., 2011). According to these values, the calyx extract can be considered promising for use as a preservative of natural origin or to be part of a sanitizer for use in the food industry.

Table 1. MIC and MBC values of *P. peruviana* calyx extract against bacterial strains

Bacterial strains	*** MIC (mg/mL)**	**** MBC (mg/mL)**
Enterobacter aerogenes ATCC 13048	30	30
Klebsiella pneumoniae ATCC 1705	30	30
Escherichia coli (Clinical isolate)	7.5	≥ 30
Staphylococcus epidermidis (Clinical isolate)	15	30
Streptococcus pneumoniae ATCC 99619	30	30
Shigella sonnei (Clinical isolate)	15	≥ 30
Acinetobacter baumannii ATCC 19606	7.5	≥ 30
Streptococcus agalactie (Clinical isolate)	7.5	≥ 30
Acinetobacter baumannii (Clinical isolate)	30	≥ 30
Pseudomonas aeruginosa PA01	7.5	30
Enterococcus faecalis ATCC 29212	3.75	30
Salmonella sp. (Clinical isolate)	15	≥ 30
Salmonella enteridis (Clinical isolate)	7.5	≥ 30
Staphylococcus aureus (Clinical isolate)	7.5	≥ 30

* Minimum Inhibitory Concentration
** Minimum Bactericidal Concentration

Biofilm inhibition and destruction

The results regarding biofilm are shown in Figure 1. The biofilm growth in our study was confirmed by the standard *P. aeruginosa* strain PA01 (control) with 100% growth. *P. aeruginosa* is known to be an opportunistic human pathogen capable of causing a wide range of acute and chronic infections that can be life-threatening, particularly in immunocompromised patients. It has been historically associated with lung infections in cystic fibrosis patients and is one of the major nosocomial pathogens affecting hospitalized patients, while being intrinsically resistant to a wide range of antibiotics (MORADALI; GHODS; REHM, 2017). In addition, the presence of *Pseudomonas* biofilms is also found in

food production and its facilities, causing food spoilage and generating various problems (MELIANI; BENSOLTANE, 2016).

P. peruviana calyx extract concentrations of 3.75 and 7.5 mg/mL inhibited biofilm formation in the early stages (Figure 1A). In Figure 1B, where the biofilm destruction was tested, it was observed that there was no condition that led to total destruction of the microorganism. The lowest extract concentration (1.87 mg/mL) did not have the ability to inhibit biofilm formation, i.e. the most effective concentrations to inhibit *P. aeruginosa* PA01 biofilm formation were 3.75 and 7.5 mg/mL of calyx extract.

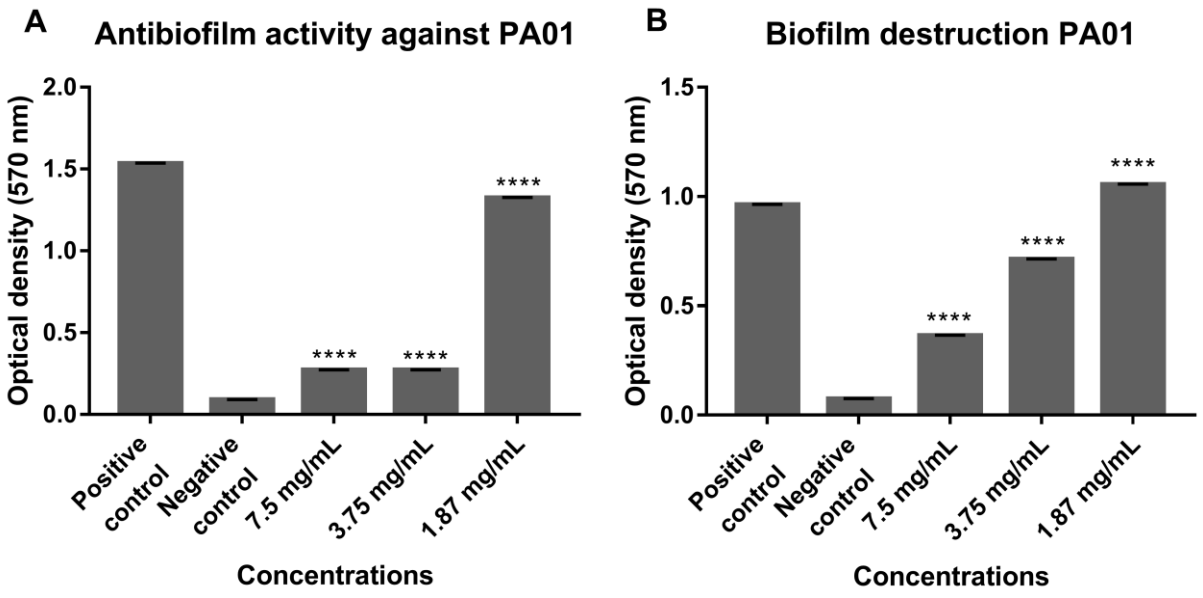


Figure 1. A: Inhibition of *P. aeruginosa* PA01 biofilm formation. B: Destruction of *P. aeruginosa* biofilm. *Error bars* are shown as one standard deviation and differences were considered statistically significant when $p < 0.0001$ between the positive control and the corresponding concentration. Each experiment was carried out three times, yielding similar results.

CONCLUSION

The *P. peruviana* calyx extract showed antibacterial activity against the pathogens analysed. The minimum inhibitory concentration ranged from 3.15 to 30 mg/mL extract. The highest activity demonstrated was bacteriostatic, but also had bactericidal activity at a concentration of 30 mg/mL against *Enterobacter aerogenes, Klebsiella pneumoniae, Staphylococcus epidermidis, Streptococcus pneumoniae*, *Pseudomonas aeruginosa* PA01 and *Enterococcus faecalis*, indicating that, at this concentration, it acts as a bactericidal agent against these pathogens. Regarding the biofilm tests, there was inhibition of biofilm formation at the concentrations of 3.75 and 7.5 mg/mL of the calyx extract and a partial destruction at the same concentrations. These results may be used in future research with an emphasis on the use of *P. peruviana* calyx extract in therapeutic approaches and in the food industry as a preservative of natural origin.

ACKNOWLEDGEMENTS

This study was financed in part by the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES)* - Finance Code 001 and Fundação de Amparo a Pesquisa do Rio Grande do Sul (FAPERGS).

DECLARATION OF CONFLICTING INTERESTS

We have no conflicts of interest to declare.

REFERENCES

ANTOLAK, H.; KREGIEL, G. Food preservatives from plants. Karunaratne, D.N.; Pamunuwa, G. (Eds.). In: **Food additives**. [1st ed.]. p. 45–48. IntechOpen (2017). Available from: <http://dx.doi.org/10.5772/intechopen.70090>. Accessed: Nov. 25, 2019. doi: 10.5772/intechopen.70090.

BAI, J. et al. Biocontrol and rapid detection of food-borne pathogens using bacteriophages and endolysins. **Frontiers in Microbiology**, v. 7, n. 474, p. 1-15, 2016. Available from: <http://doi.org/10.3389/fmicb.2016.00474>. Accessed: July. 11, 2019. doi: 10.3389/fmicb.2016.00474

BALLESTEROS-VIVAS, D. et al. A multi-analytical platform based on pressurized-liquid extraction, in vitro assays and liquid chromatography/gas chromatography coupled to high resolution mass spectrometry for food by-products valorisation. Part 2: Characterization of bioactive compound. **Journal of Chromatography A**, v. 1584, p. 144-154, 2019.Available from:< https://doi.org/10.1016/j.chroma.2018.11.054>. Accessed: July. 19, 2019. doi: 10.1016/j.chroma.2018.11.054.

BAPTISTA, R. C.; HORITA, C. N.; ANA, A. S. S. Natural products with preservative properties for enhancing the microbiological safety and extending the shelf-life of seafood: a review. **Food Research International**, v. 127, p. 108762, 2019. Available from: <https://doi.org/10.1016/j.foodres.2019.108762>. Accessed: Nov. 25, 2019. doi: 10.1016/j.foodres.2019.108762.

BAZANA, M. T. et al (2019). Development of nanoemulsions containing *Physalis peruviana* calyx extract: A study on stability and antioxidant capacity. **Food Research International**, v. 125, 108645, 2019. Available from:<https://doi.org/10.1016/j.foodres.2019.108645>. Accessed: Aug. 29, 2019. doi: 10.1016/j.foodres.2019.108645.

BONEZ, P. C. et al. Anti-biofilm activity of A22 ((S-3,4-dichlorobenzyl) isothiourea

hydrochloride) against *Pseudomonas aeruginosa*: Influence on biofilm formation, motility and bioadhesion. **Microbial Pathogenesis**, v. 111, p. 6-13, 2017. Available from: <http://doi.org/10.1016/j.micpath.2017.08.008>. Accessed: May. 06, 2019. doi:10.1016/j.micpath.2017.08.008.

CLSI. **Performance standards for antimicrobial susceptibility testing. 27th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute**. [s.l: s.n.]. Available from:< https://clsi.org/media/1469/m100s27_sample.pdf>. Accessed: Jun. 10, 2019.

CODEVILLA, C. F. et al. Cytotoxicity and antioxidant activity of goldenberry extracts obtained with high intensity ultrasound. **Ciência Rural**, v. 48, n. 2, p. e20170362, 2018. Available from:<http://doi.org/10.1590/0103-8478cr20170362>. Accessed: May. 08, 2019. doi:10.1590/0103-8478cr20170362.

COURVALIN, P. L'ant biogramme. MPC - Videom, Paris. 1985. Accessed: May. 10, 2019.

CUEVA, M. B. R. et al. Antibacterial effects of uvilla (*Physalis peruviana* L.) extracts against *Listeria spp.* isolated from meat in Ecuador. **International Journal of Current Microbiology and Applied Sciences**, v. 6, n. 4, p. 1146-1153, 2017. Available from: <http://doi.org/10.20546/ijcmas.2017.604.141>. Accessed: July. 14, 2019. doi:10.20546/ijcmas.2017.604.141.

ERTÜRK, Ö. et al. Antioxidant, antimicrobial activities and phenolic and chemical contents of *Physalis peruviana* L. from Trabzon, Turkey. **Indian Journal of Pharmaceutical Education and Research**, v. 51, n. 3, p. S213-S216, 2017. Available from: <http://doi.org/10.5530/ijper.51.3s.15>. Accessed: July. 11, 2019. doi:10.5530/ijper.51.3s.15.

GÖZTOK, F.; ZENGIN, F. The antimicrobial activity of *Physalis peruviana* L. **Bitlis Eren University Journal of Science and Technology**, v. 3, n. 1, p. 15-17, 2013. Available from: <http://doi.org/10.17678/beujst.51277>. Accessed: July. 12, 2019. doi:10.17678/beujst.51277.

HUSSAIN, M. Food contamination: major challenges of the future. **Foods**, v. 5, n. 2, p. 21,

2016. Available from:<http://doi.org/10.3390/foods5020021>. Accessed: July. 11, 2019. doi:10.3390/foods5020021.

KAMAU, P. K. et al. Antimicrobial evaluation and phytochemical screening of aqueous and dichloromethane crude extracts of Kenyan *Physalis peruviana* L (Cape Gooseberry). v. 16, n. 5, p. 101-109, 2017. Available from:<http://doi.org/10.9790/0853-160509101109>. Accessed: July. 9, 2019. doi:10.9790/0853-160509101109.

LEE, K. H. et al. Preparation, characterization, and food application of rosemary extract-loaded antimicrobial nanoparticle dispersions. **LWT**, v. 101, p. 138-144, 2019. Available from:<http://doi.org/10.1016/j.lwt.2018.10.072>. Accessed: July. 11, 2019. doi:10.1016/j.lwt.2018.10.072.

LIMA, J. L. DA C. et al. Biofilm production by clinical isolates of *Pseudomonas aeruginosa* and structural changes in LasR protein of isolates non biofilm-producing. **Brazilian Journal of Infectious Diseases**, v. 22, n. 2, p. 129-136, 2018. Available from: <https://doi.org/10.1016/j.bjid.2018.03.003>. Accessed: Jun. 17, 2019. doi:10.1016/j.bjid.2018.03.003.

MEDINA, S. et al. Potential of Physalis peruviana calyces as a low-cost valuable resource of phytoprostanes and phenolic compounds. **Journal of the Science of Food and Agriculture**, v. 99, n. 5, p. 2194-2204, 2019. Available from:<https://doi.org/10.1002/jsfa.9413>. Accessed: July. 22, 2019. doi: 10.1002/jsfa.9413.

MELIANI, A.; BENSOLTANE, A. Review of *Pseudomonas* attachment and biofilm formation in food industry. **Poultry, Fisheries & Wildlife Sciences**, v. 03, n. 01, p. 1-7, 2016. Available from:< https://www.longdom.org/open-access/review-of-pseudomonas-attachment-and-biofilm-formation-in-food-industry-2375-446X-1000126.pdf >. Accessed: July. 24, 2019. doi: 10.4172/2375-446x.1000126.

MORADALI, M. F.; GHODS, S.; REHM, B. H. A. *Pseudomonas aeruginosa* lifestyle: a

paradigm for adaptation, survival, and persistence. **Frontiers in Cellular and Infection Microbiology**, v. 7, n. 39, p. 1-29, 2017. Available from: <https://doi.org/10.3389/fcimb.2017.00039>. Accessed: Jun. 17, 2019. doi: 10.3389/fcimb.2017.00039

PUENTE, L. A. et al. *Physalis peruviana* Linnaeus, the multiple properties of a highly functional fruit: A review. **Food Research International**, v. 44, n. 7, p. 1733-1740, 2011. Available from:<https://doi.org/10.1016/j.foodres.2010.09.034>. Accessed: July. 19, 2019. doi:10.1016/j.foodres.2010.09.034.

7 MANUSCRITO 2 – *IN VITRO* AND *IN VIVO* TOXICITY ASSESSMENT AND RELEASE IN SIMULATED GASTROINTESTINAL CONDITIONS OF *P. peruviana* CALYX EXTRACT-LOADED NANOEMULSIONS

Manuscrito formatado de acordo com as normas para submissão da revista **Journal of Functional Foods**

In vitro and *in vivo* toxicity assessment and release in simulated gastrointestinal conditions of *P. peruviana* calyx extract-loaded nanoemulsions

Maiara Taís Bazana[1], Suelen Santos da Silva[1], Cassandra de Deus[1], Marina Lopes Machado[2], Larissa Marafiga Cordeiro[2], Félix Alexandre Antunes Soares[2], Daniele Rubert Nogueira-Librelotto[3], Clarice Madalena Bueno Rolim[3], Cristiane Franco Codevilla[1], Cristiane de Bona da Silva[3], Cristiano Ragagnin de Menezes[1].

[1]Departamento de Tecnologia e Ciência dos Alimentos, Universidade Federal de Santa Maria, 97105-900, Santa Maria, RS, Brasil. E-mail: cristiano.ufsm@gmail.com *Corresponding author

[2]Departamento de Bioquímica e Biologia Molecular, Universidade Federal de Santa Maria, Santa Maria, RS, Brasil.

[3]Departamento de Farmácia Industrial, Universidade Federal de Santa Maria, Santa Maria, RS, Brasil.

ABSTRACT

The potential toxic effects of nanoemulsions containing *P. peruviana* calyx extract was evaluated *in vitro* using tumor and non-tumor cell lines and *in vivo* using a *Caenorhabditis elegans* model. Cytotoxicity tests employing non-tumor cells indicated that the extract associated or not with nanoemulsions maintained cell viability at different concentrations. In the tests using the tumor cell line, greater cytotoxicity was observed after each treatment with the nanoemulsions. Regarding the *in vivo* tests, there was no change in the survival rate of the

worms, and the formulations at low concentrations showed better results against resistance to oxidative stress. In addition, release under simulated gastrointestinal conditions demonstrated that nanoemulsions containing the extract protected rutin from degradation and showed controlled release. Therefore, the nanoemulsions containing *P. peruviana* calyx extract revealed slight to moderate *in vitro* cytotoxicity, which depends on the cell line, and no *in vivo* toxicity under the evaluated conditions. Moreover, the proposed formulations protected the bioactive compound against gastrointestinal conditions, allowing new studies to enable applicability in the food industry.

Keywords: cytotoxicity, *C. elegans*, goldenberry, nanotoxicology, *in vitro* release

1. INTRODUCTION

Physalis peruviana L., which is also known as cape gooseberry, is an herbaceous plant native to the Andes that belongs to the Solanaceae family (Álvarez-Flórez, López-Cristoffanini, Jáuregui, Melgarejo, & López-Carbonell, 2017). The calyx grows into a bladder-like organ during development of the fruit and surrounds it, providing protection against insects, birds, diseases, and adverse weather conditions. In addition, this structure represents an essential carbohydrate source during the first 20 days of fruit development (Puente, Pinto-Muñoz, Castro, & Cortés, 2011).

The cape gooseberry fruit is usually marketed without the calyx, generating large quantities of waste (Medina et al., 2019). However, this byproduct has the attracted interest of scientific studies and several bioactive compounds have already been identified as phytochemicals (terpenoids, phytosterols, and phytol derivatives), flavonoids, phenolic acids, and withanolides (Gironés-Vilaplana et al., 2014; Ballesteros-Vivas, Álvarez-Rivera, Del

Pilar Sánchez-Camargo, et al., 2019; Ballesteros-Vivas, Álvarez-Rivera, Ibáñez, Parada-Alfonso, & Cifuentes, 2019; Medina et al., 2019). These compounds frequently have some limitations, including low stability against light, heat, oxygen, low water solubility, low absorption, and low bioavailability. Based on these factors, our research group developed nanoemulsions containing *P. peruviana* calyx extract and evaluated the stability and antioxidant capacity (Bazana et al., 2019). Despite advances in nanotechnology, the safety of these systems has not yet been fully proven. As a result, the number of studies on nanoparticle biosafety evaluation using different biological models has risen (Wu, Xu, Liang, & Tang, 2019).

Cytotoxicity assays are widely used in preliminary investigations of nanomaterial toxicity (Hillegass et al., 2010). Established cell lines are useful alternative test systems for these studies (Crespi, 1995). The degree of cell damage is measured by several assays that evaluate aspects of cell viability, such as metabolic activity and plasma membrane integrity. Among the most widely used tests is the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium) reduction assay, which determines cellular metabolic activity. This method measures the reduction of MTT salt to a colored insoluble formazan in active mitochondria in viable cells and, in certain cases, outside the mitochondria. For reliable data from cytotoxicity studies, comparative sensitivity analyses with more than one cell type are strongly recommended (Nogueira, Mitjans, Infante, & Vinardell, 2011). Additionally, it is recommended to consider the properties of the nanosystem and critical issues that may impact its behavior *in vitro*, such as the concentration of components, population, and nanoparticle structure/type (Mendes et al., 2015).

The *Caenorhabditis elegans* nematode (*C. elegans*) is a complete model and important *in vivo* assay system for evaluating nanotoxicity (Ávila, Roncato, & Jacques, 2018; Velasques et al., 2018; Silva et al., 2019). The worm has excellent features of a typical model system,

such as a short life cycle (2 to 3 weeks), a tiny and transparent body, easy cultivation, low maintenance costs, and no bioethical concerns (Wu et al., 2019).

Finally, the gastrointestinal behavior of nanoemulsion-based delivery systems for controlled or targeted release of bioactive compounds should also be considered when designing nanoemulsions with oral administration purposes. Exposure to these different regions may cause considerable changes in nanoemulsion properties or encapsulated bioactive compounds (Zhang & McClements, 2018). In this context, this study aimed to evaluate nanoemulsions containing *P. peruviana* calyx extract for cytotoxicity, toxicity in the *C. elegans* model, and release in the simulated gastrointestinal tract.

2. MATERIAL AND METHODS

2.1 Material

P. peruviana calyces were obtained from Italbraz (Vacaria, Brazil - 28:0:44 S and 50:56:02 W). Ethanol and acetone were purchased from Dynamics (São Paulo, Brazil), polysorbate 80 (Tween® 80) from Neon Comercial Ltda (São Paulo, Brazil), sorbitan monooleate (Span® 80) from Fluka (São Paulo, Brazil), and medium chain triglycerides (MCT) from Delaware (Porto Alegre, Brazil). The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium), trypsin-EDTA solution, and DMSO were obtained from Sigma-Aldrich (St. Louis, USA). Fetal bovine serum (FBS) and Dulbecco's Modified Eagle's Medium (DMEM), which were supplemented with L-glutamine (584 mg/L) and antibiotic/antimicotic (50 mg/mL gentamicin sulfate and 2 mg/L amphotericin B), were purchased from Vitrocell (Campinas, SP, Brazil). The wild type strain of *C. elegans* N2 (var. Bristol) was supplied by

the Caenorhabditis Genetics Center (University of Minnesota, USA). The other reagents were HPLC grade for high performance liquid chromatography analysis. Hydroalcoholic extracts of the calyces and nanoemulsions with *P. peruviana* calyx extract (NE) and without (NB) were performed according to Bazana et al. (2019).

2.2 *In vitro* toxicity

The *in vitro* cytotoxicity of *P. peruviana* calyx extract (CE), blank nanoemulsions (NB), and nanoemulsions containing the extract (NE) was measured using the non-tumor cell line 3T3 (murine Swiss albino fibroblasts) and tumor cell line MCF-7 (human breast cancer cells). The 3T3 and MCF-7 cells were grown in DMEM medium supplemented with 10% (v/v) FBS at 37 °C and 5% CO_2. After reaching approximately 80% confluence, the cells were harvested using trypsin-EDTA and seeded into 96-well plates at a density of 1×10^5 cells/mL. After incubation for 24 h under 5% CO_2 at 37 °C, the spent medium was replaced with 100 μL of fresh medium containing the extract, nanoemulsion containing extract or blank nanoemulsion at the defined concentrations (10, 50, and 150 μg/mL) or with 100 μL medium only for untreated control cells. After 24 h, the sample-containing medium was removed and 100 μL of MTT diluted in medium without FBS (0.5 mg/mL) was added to the cells. The plates were further incubated for 3 h, after which the medium was removed, and 100 μL of DMSO were then added to each well to dissolve the purple formazan product. After 10 min of shaking, the absorbance of the resulting solutions was measured at 550 nm using a Multiskan FC (Thermo Fisher Scientific, Waltham, MA, EUA) microplate reader. The effect of each treatment was calculated as a percentage of cell viability inhibition against the untreated control cells (cells incubated with medium only).

2.3 *In vivo* toxicity

2.3.1 Worm maintenance

C. elegans strains N2 (wild-type) were manipulated and maintained at 20 °C in the nematode growth medium (NGM) fed with *Escherichia coli* OP50 (Brenner, 1974). Worms in the young adult stage used in all exposures were obtained by a synchronization process, which consists of exposing the pregnant worms to a lysis solution (1% NaOCl, 0.25 M NaOH) to separate the eggs from the worms. After 14 h, the eggs hatched and released the L1 larvae, which were added to the surface of the NGM plates containing *E. coli* OP50, where they remained at 20 ºC until reaching young adult stage.

2.3.2 Treatment

C. elegans at young adult stage were exposed to 0.5, 1.0 or 2.0 mg/mL of CE, NE, NB or vehicle for 2 h at 20 °C. The CE was diluted in ethanol (final concentration 1%) and the NE and NB nanoemulsions were diluted in water. Treatments were performed with about 1000 worms per group in M9 buffer (42 mM Na_2HPO_4, 22 mM KH_2PO_4, 8.6 mM NaCl, and 1 mM $MgSO_4$). After 2 h, the worms were washed 3 times and transferred to the NGM plates seeded with *E. coli* OP50. The evaluations were performed after 24 h at 20 ºC.

2.3.3 Survival

Survival assay was performed following the protocol previously described, with some modifications (Velasques et al., 2018). About 100 nematodes per group were evaluated for viability under a Nikon E200 microscope (Tokyo, Japan). Animals that reacted to a mechanical stimulus were classified as alive and non-responding animals were classified as

dead. Analyses were performed in three independent trials. Results were expressed as percentage of survival.

2.3.4 Pharyngeal pumping rate

The number of pharyngeal pumping over a range of 10 s (Huang, Xiong, & Kornfeld, 2004) was measured with a Nikon E200 microscope. The analyses were performed in three independent trials with ten worms per group. Results were expressed as pharyngeal beats/minute.

2.3.5 Defecation cycle

The defecation frequencies were performed by observing the duration between bupic steps (the contraction of the posterior body muscle) of three consecutive defecations (Migliori, Simonetta, Romanowski, & Golombek, 2011) in a Nikon E200 microscope. Analyses were performed in three independent trials with ten worms per group. Results were expressed as defecation cycle/s.

2.3.6 Oxidative stress resistance assay

To perform the acute oxidative stress resistance assay, worms previously exposed for 2 h to the extracts, the nanoemulsions or vehicle were removed from their treatments and immediately exposed for one hour to Juglone (5-hydroxy-1,4-naphthoquinone) at the final concentration of 100 µM (LC50) at 20 °C. Afterwards, the worms were washed from their treatments and transferred to *E. coli* OP50 seeded NGM plates and, after 24 h, 100 nematodes per group were evaluated for viability under a Nikon E200 microscope (Tokyo, Japan). Animals that reacted to a mechanical stimulus were classified as alive and non-responding

animals were classified as dead. Analyses were performed in three independent assays. Results were expressed as percentage of survivors.

2.4 Release under simulated gastrointestinal conditions

Evaluation of *in vitro* rutin release profile from calyx extracts (CE) and nanoemulsions containing *P. peruviana* calyx extract (NE) were evaluated (n=3) by the dialysis bag method and exposed to the simulated gastrointestinal conditions according to Madureira, Amorim, Gomes, Pintado, & Malcata (2011), with modifications. The dialysis bag (MWCO = 12,000 Da, Sigma-Aldrich Corporation, MO, EUA) containing 1 mL of the samples (5 mg/mL of calyx extract) was placed in an erlenmeyer containing 150 mL of dissolution medium. In the esophagus–stomach step, 50 µL of pepsin to 25 mg/mL (Sigma) was added. This solution was prepared in 0.1 N HCl and added in equal aliquots at each step during the whole gastric phase for a total of 90 min at 130 rpm agitation, with the pH being adjusted until 2, using 0.1 N HCl. In the duodenum stage, 250 µL of solution containing 2 g/L of pancreatin (Sigma) and 12 g/L of bovine bile salts (Sigma) were diluted in 0.1 M NaHCO3; this solution was added and the pH adjusted up to 5.0 for 20 minutes at agitation of 45 rpm. Finally, the ileum stage was performed for 90 minutes at 45 rpm agitation by raising the pH to 6.5. Analysis was conducted in a refrigerated shaker incubator (TE-421, Tecnal, Brazil) maintained at 37 °C to simulate human body temperature and mechanical agitation was used in parallel to simulate intestinal peristaltic movements. At the end of each stage, aliquots were withdrawn and filtered through a 0.22 µm membrane for HPLC analyses. Rutin samples were evaluated by HPLC according to the modified and validated method by Bazana et al. (2019).

2.5 Statistics

Statistical analyses were performed using *GraphPad Prism*® (Version 7.0, San Diego, CA). All experiments were independently replicated and the results were plotted as the mean ± SEM of at least three individual experiments. Significance was determined using analysis of variance (ANOVA) followed by Tukey post-hoc test. Values of $p < 0.05$ were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 In vitro toxicity

The evaluation of *in vitro* cytotoxicity of a nanosystem is an important preliminary test, even when the purpose of its formulation application in food products, thus verifying the safety issues (Silva et al., 2018). In this study, two cell lines were chosen as model systems because the use of cells from different species and with different embryogenic origins is an important approach to understand the cell specific responses induced by nano-based materials (Fröhlich, Meindl, Roblegg, Griesbacher, & Pieber, 2012).

The viability results obtained from 3T3 cells (Figure 1A) remained without significant differences between the formulations tested, apart from NB at 150 µg/mL, which showed significant reduction in cell viability.

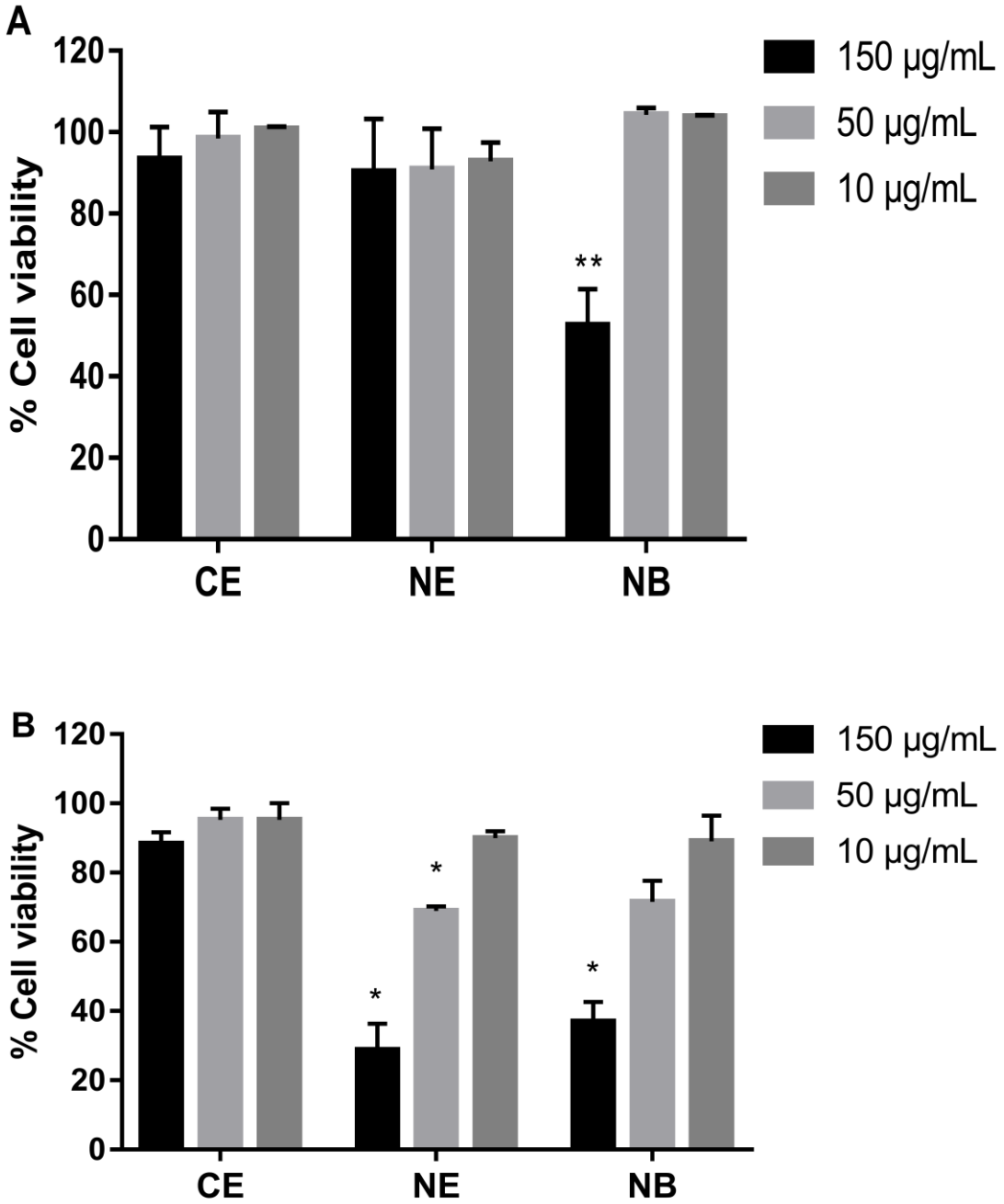


Figure 1 Cell viability after exposure of the cells with *P. peruviana* calyx extracts (CE), nanoemulsions containing *P. peruviana* calyx extract (NE), and blank nanoelmulsions (NB). (A) viability plot of 3T3 cells (non-tumor fibroblasts); (B) viability plot of MCF-7 cells (breast tumor). Each experiment was performed three times in triplicate. * means significant difference ($p < 0.05$) compared to the control after one-way ANOVA analysis followed by Tukey post-hoc test.

This cytotoxic response demonstrated that, at this concentration, NB was toxic to cells, as viability was reduced to about 50%. In order to be considered cytotoxic, viability must be below 75% (ISO 10993-5) (Wang et al., 2015). Previously, Codevilla et al. (2018) also observed that the hydroalcoholic extract of goldenberry fruit was non-cytotoxic. Likewise, studies conducted in our research group by Silva et al. (2019) reported that *P. peruviana* fruit extract and the nanoemulsion containing the extract and blank nanoemulsion at concentrations of 50, 200, and 400 µg/mL did not display cytotoxicity against 3T3 cells. Notably, the low cytotoxic potential of nanoemulsions has already been reported when associated with essential oils or plant extracts (Mendes et al., 2015).

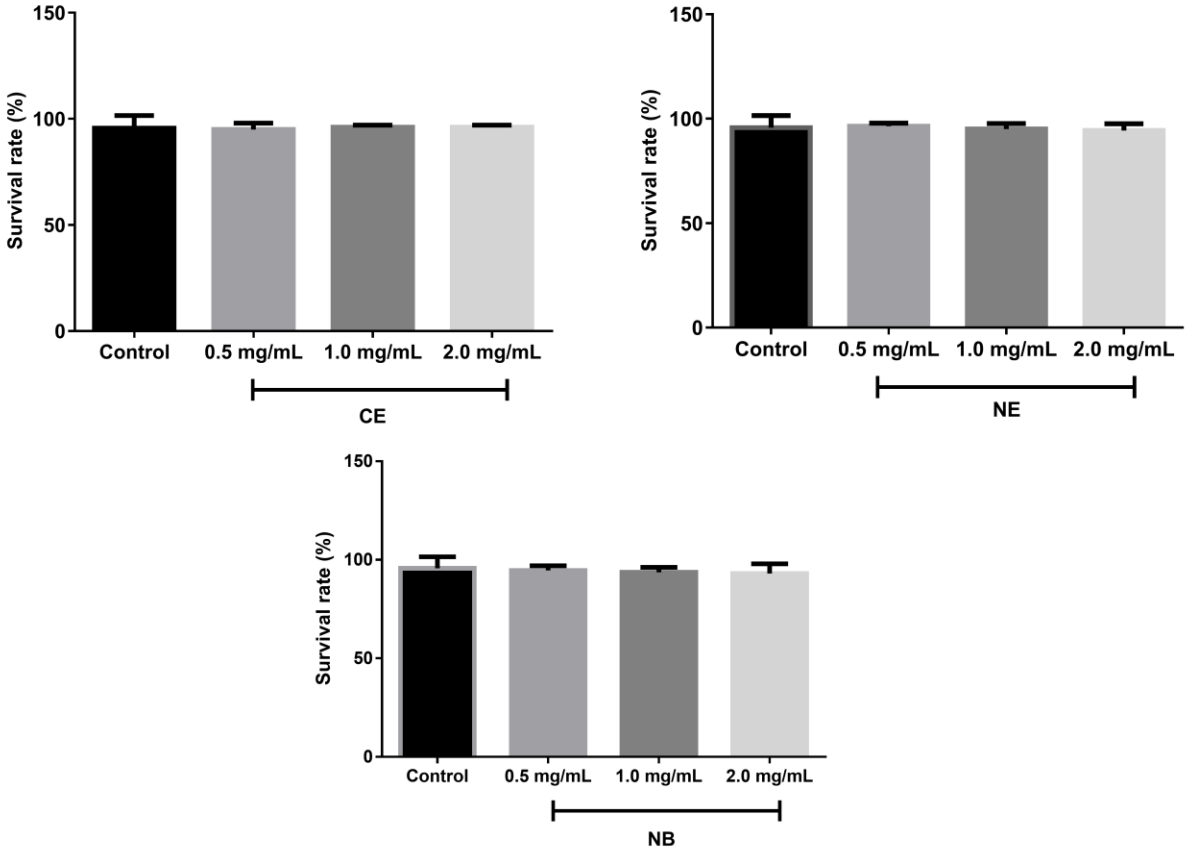
Regarding MCF-7 (breast tumor) cells, free extract (CE) was unable to reduce the viability of cancer cells (Figure 2B). Other studies also reported that the ethanolic extract of goldenberry fruit was ineffective, i.e. Demir (2014) using 80% (v/v) ethanol to make the extract, and Silva et al. (2019) at the concentrations of 50, 200, and 400 µg/mL extract made with 60% (v/v) ethanol. In contrast, NE and NB showed higher inhibition rates in the growth of these cells as the formulation concentrations increased (Figure 1B). According to Mendes et al. (2015), the type and amount of surfactant in the formulation may influence their cytotoxicity. Contri et al. (2016) reported decreased keratinocyte viability induced by blank nanocapsules, which was similar to the one induced by polysorbate 80 solution, indicating that nanocapsule toxicity is in fact linked to the nonionic surfactant used. In addition, the presence of the nonionic surfactant (polysorbate 80) has also been indicated as a substance that may cause toxicity in cell cultures, although it is better tolerated than ionic surfactants (especially cationic surfactants) (Contri et al., 2016). Nevertheless, polysorbate 80 has already been described as the least toxic surfactant for human fibroblasts compared to others, such as those tested in this study, which can be classified in the following order of increasing cytotoxicity: Tween 80 < Texapon N40 < Tween 60 < Texapon K1298 < Triton x100 <

benzethonium chloride (Arechabala, Coiffard, Rivalland, Coiffard, & De Roeck-Holtzhauer, 1999). Thus, cytotoxic responses have been observed even in blank nanoparticles during the design of *in vitro* experiments using different cell lines (Mendes et al., 2014; Mendes et al., 2015; Silva et al., 2019). Finally, it is worth mentioning that the different sensitivity of the tested cell lines to the nanoemulsion treatments may be partly explained by healthy and tumor cells having differences in their structures, functions, and more notably metabolic activity and molecular composition (Frey et al., 2007). Therefore, the characteristics of each cell line may lead to distinct mechanisms of defense and, consequently, different sensitivities to the toxic effects of the applied treatment (Nogueira et al., 2011). This evidence highlights the relevance of the combination of cell lines from different origins in order to obtain more information on the potential toxic effects of novel nano-based systems.

3.2 In vivo toxicity

The *in vivo* toxicity of the formulations was evaluated using the *C. elegans* model. After the 2h-treatment, the survival rate of all animals remained around 100% (Figure 2), regardless of the sample tested (CE, NE, and NB), indicating that exposure to the samples did not cause lethality.

After acute treatment with the formulations, some *C. elegans* behaviors were analyzed to evaluate the effects on toxicity. Animals exposed to the free extract (CE) at the concentrations of 1 and 2 mg/mL had reduced pharyngeal pumping rates, whereas nanoemulsions containing *P. peruviana* calyx extract (NE) did not cause any changes (Figure 3).



277

278 **Figure 2** Survival rate of young adult worms of N2 strain exposed for 2h to different
279 concentrations of *P. peruviana* calyx extracts (CE), nanoelmulsions containing *P. peruviana*
280 calyx extract (NE), and blank nanoelmulsions (NB). The data are expressed as percent of
281 control; each experiment was performed three times in triplicate. (mean, SEM, n =
282 approximately 300 worms per group).

283

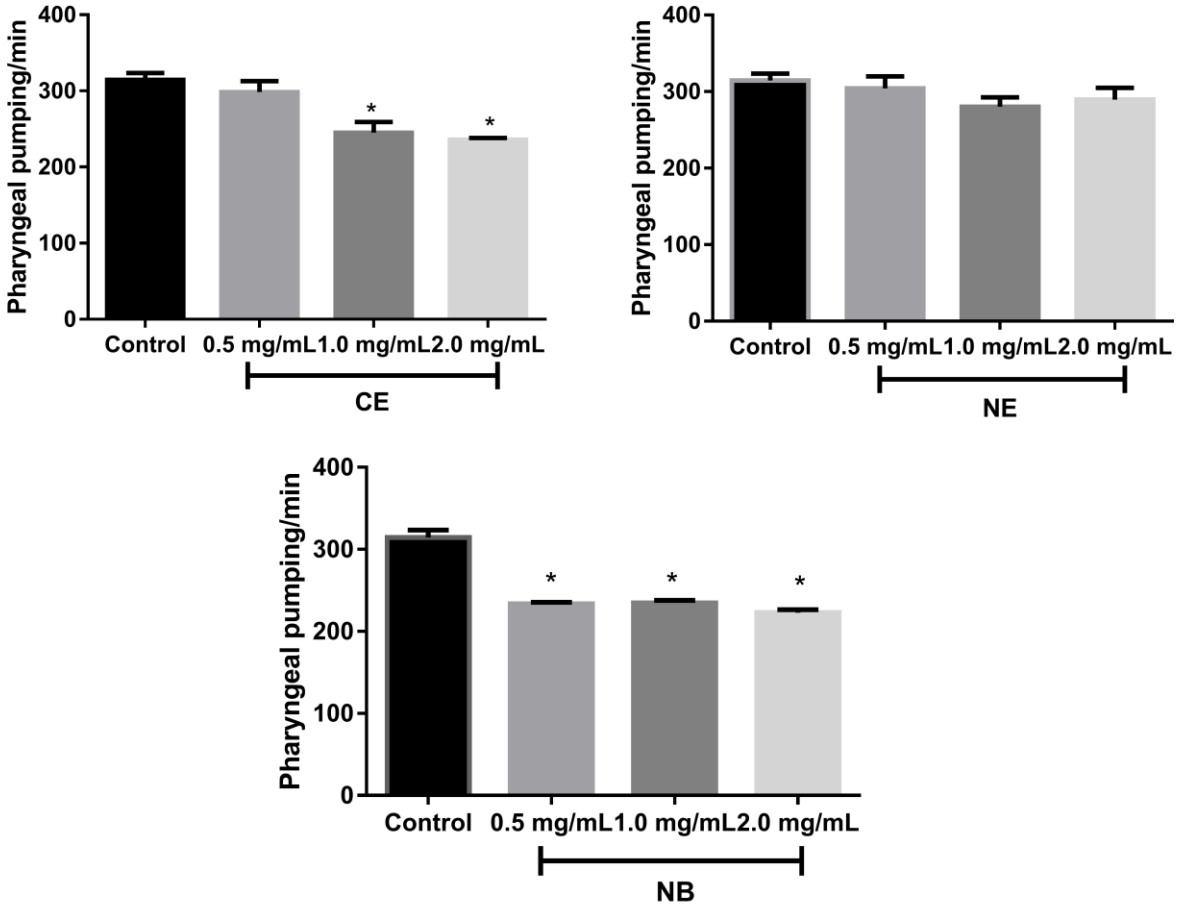


Figure 3 Pharyngeal pumping/min of young adult worms of the N2 strain exposed for 2 h at different concentrations of *P. peruviana* calyx extracts (calyx extract), blank nanoelmulsions (NB), and nanoelmulsions containing *P. peruviana* calyx extract (NE) in liquid medium. Each experiment was performed three times in triplicate. (mean, SEM, n = approximately 15 worms per group). * means significant difference ($p < 0.05$) compared to the control after one-way ANOVA analysis followed by Tukey post-hoc test.

However, NB showed a significant reduction in pharyngeal pumping rate, which has already been observed in the literature by other authors (Silva et al., 2019). Depending on the sample or concentration, there were changes in the feeding behavior of the worms in relation to the control group. According to You, Kim, Raizen, & Avery (2008), *C. elegans* varies its feeding rate by regulating the pumping rate, being a pharyngeal movement. Feeding rates are

usually high in the presence of food and low in their absence. However, the nutritional status of the animal, its feeding history, and food quality are influencing factors, just as the authors suggest that quiescence is a satiety behavior of worms. Therefore, the reduced pharyngeal pumping rates may have been influenced by these factors highlighted above and by the diversity of the samples, since the control animals consumed only M9 buffer. Pharyngeal pumping is an indicator of a healthy worm and is mainly controlled by cholinergic and glutamatergic innervation and dopamine and serotonin (Raizen, Song, Trojanowski, & You, 2012). The nanoemulsion containing *P. peruviana* calyx extract (NE) did not cause changes in the pharyngeal pumping of this organism. These findings are in agreement with the study by Moraes et al. (2016), who reported that polymeric nanocapsules decrease the toxic effects of clozapine in *C. elegans*, and with Pascoli et al. (2019), who demonstrated that zein nanoparticles decreased the toxicity of neem oil in this organism.

Defecation cycle frequency decreased after free extract (CE) and blank nanoemulsion (NB) treatments in some concentrations (Figure 4). This behavior is a stereotyped motor program that occurs in the nematode approximately every 50 s (Thomas, 1990). Each cycle begins with a posterior body contraction that directs the contents of the intestine to the anterior part of the worm and is followed by a contraction of the nematode. The anterior muscle pushes the bowel contents to the back of the animal, followed by a contraction of the enteric muscle that expels the bowel contents (Riddle, Blumenthal, Meyer, & Priess, 1997). The nanoemulsion containing *P. peruviana* calyx extract (NE) showed no change in the frequency of defecation cycles in relation to the control (Figure 4B), which is a satisfactory result for not presenting worm toxicity in this parameter.

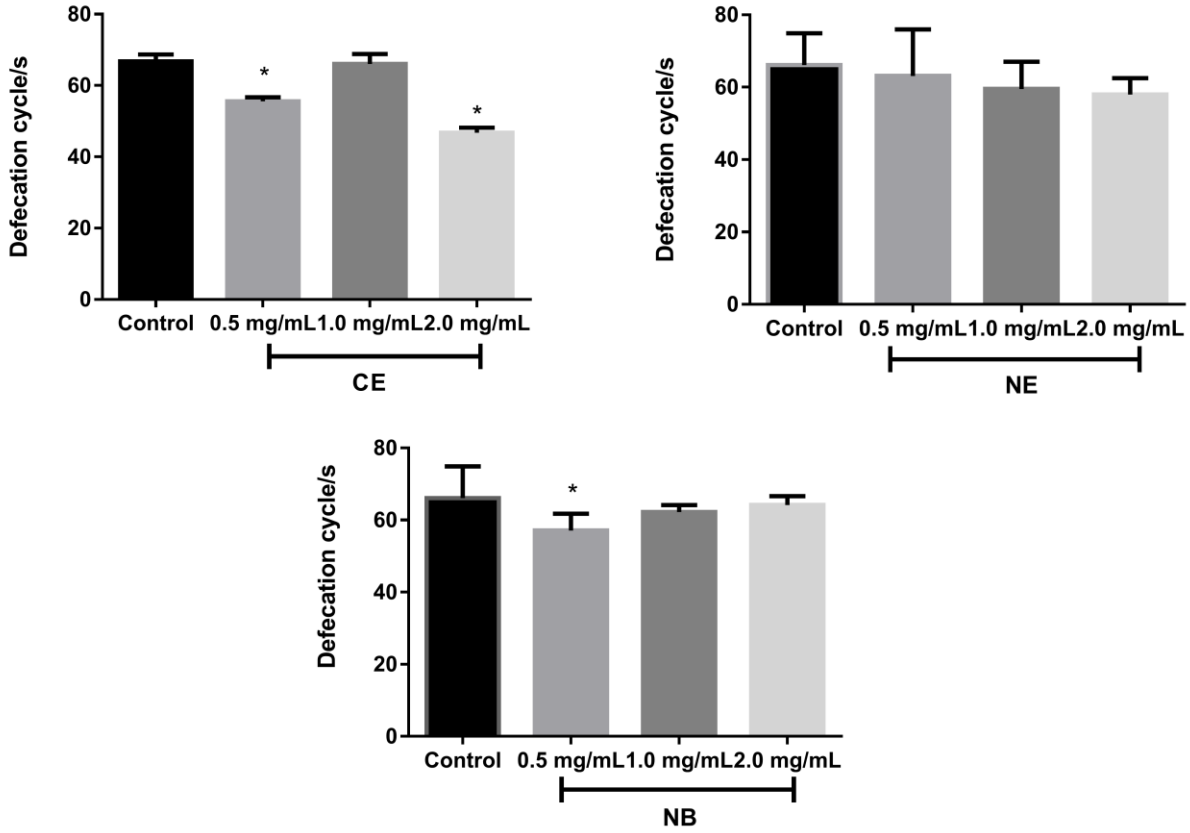


Figure 4 Defecation cycle / s of young adult worms of strain N2 exposed for 2h at different concentrations of *P. peruviana* calyx extracts (CE), nanoemulsions containing *P. peruviana* calyx extract (NE), and blank nanoelmulsions (NB) in liquid medium. Each experiment was performed three times in triplicate. (mean, SEM, n = approximately 15 worms per group). * means significant difference ($p < 0.05$) compared to the control after one-way ANOVA analysis followed by Tukey post-hoc test.

We therefore used a pro-oxidant juglone to evaluate the potential antioxidant effects of the samples in the nematode *C. elegans*. Juglone can induce oxidative stress through various mechanisms, such as superoxide (O_2 $^{-\bullet}$), hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2) formation, triggering toxic effects by protein modifications (Inbaraj & Chignell, 2004; Stadtman, 2006), lipid oxidation (Fong, McCay, Poyer, Keele, & Misra, 1973), and nuclear DNA damage (Bjelland & Seeberg, 2003).

Both nanoemulsions reduced juglone toxicity, increasing the survival rate relative to the control group or remaining similar to the juglone control group levels (Figure 5). The best response to oxidative stress was found in the group treated with 0.5 mg/mL of nanoemulsion containing *P. peruviana* calyx extract (NE), showing an increase in survival rate of 24.3% compared to the juglone control group. For blank nanoemulsions (NB), concentrations of 0.5 and 1 mg/mL showed a significant increase in survival.

On the other hand, in the treatment with the calyx extract (CE), only 0.5 mg/mL protected against juglone-induced oxidative stress, in addition to the concentrations of 1 and 2 mg/mL increasing juglone toxicity, which reduced the survival rate. This effect is likely due to the vehicle used to obtain the extracts (ethanol) because a larger volume was used at these concentrations. At higher concentrations, ethanol may have exacerbated the toxic effects of juglone to *C. elegans*. These findings indicate that the formulations were ingested by the worms, do not have toxic effects, and most likely exerted their pharmacological effects at low concentrations *in vivo*.

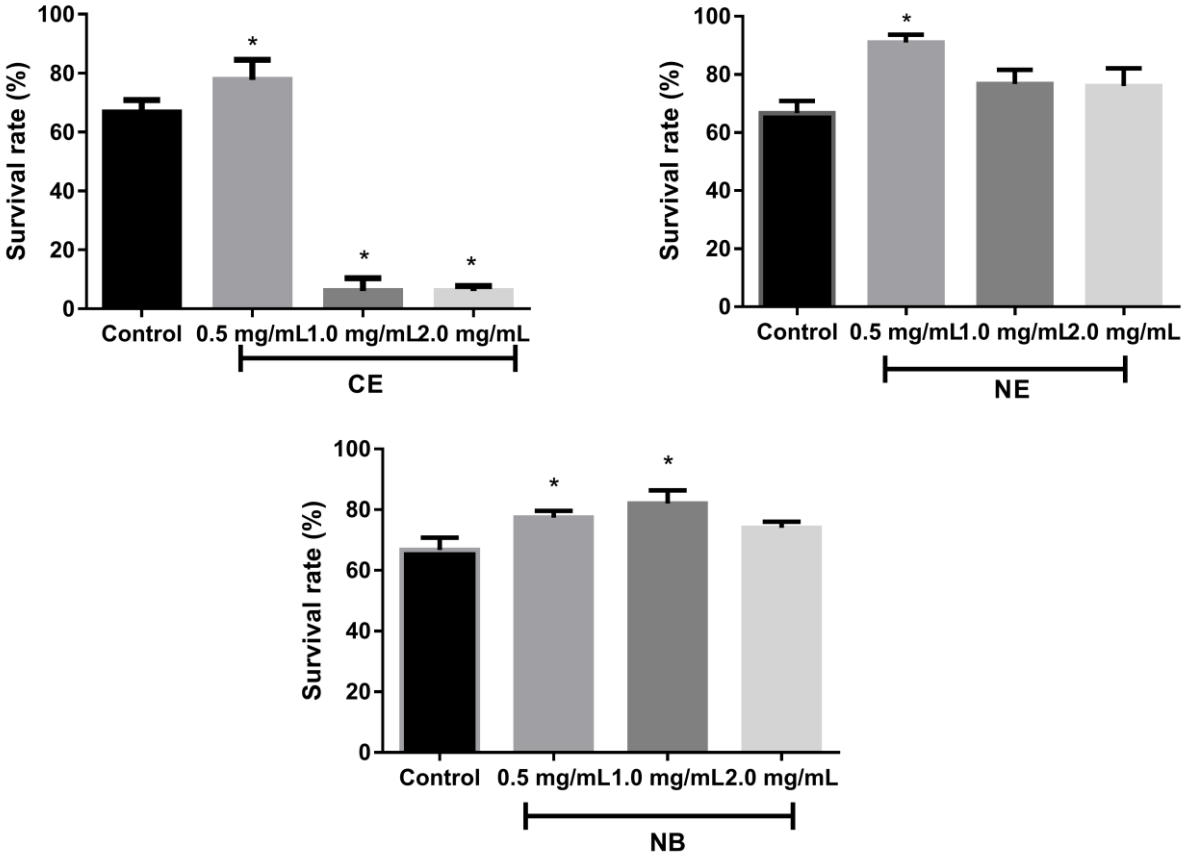


Figure 5 Survival of young adult worms of N2 strain exposed for 2h to different concentrations of *P. peruviana* calyx extracts (CE), nanoemulsions containing *P. peruviana* calyx extract (NE), blank nanoelmulsions (NB), and then exposed for 1h to 100 µM juglone. The data are expressed as percent of control; each experiment was performed three times in triplicate (mean, SEM, n = approximately 300 worms per group). * means significant difference ($p < 0.05$) compared to the control after one-way ANOVA analysis followed by Tukey post-hoc test.

3.3 Release under simulated gastrointestinal conditions

The results obtained are expressed in Figure 6. The free calyx extract (CE) released 45% of rutin in the oesophagus/stomach stage and showed no statistical difference regarding the concentrations detected in the duodenum and ileum stages. Thus, these results indicate

that all rutin content present in the extracts was released in the gastric phase and that 55% of the bioactive compound degraded due to acidic pH conditions.

On the other hand, nanoemulsions (NE) were able to protect the *P. peruviana* calyx extract, which can be observed by the % cumulative rutin release. Initially, around 65% of rutin was released under gastric conditions. However, nanoemulsions continued to control the release in the next steps and increased rutin contents was observed in the duodenum and ileum, ending the test with 80% of rutin released and available for absorption in the ileum. The remaining 20% may have been degraded during the process, as there was release in the oesophagus/stomach stage. Furthermore, another hypothesis is that the bioactive compound was not fully released by the end of the test and may slowly release the rutin from the NEs for a longer time.

Wang, Xue, Hu, Zhou, & Luo (2017) evaluated the release rates of free and encapsulated (solid lipid nanoparticles crosslinked with pectin - PSLN) curcumin in simulated gastrointestinal conditions and found that curcumin release was better controlled by PSLN than in the free form. About 20-40% of curcumin from nanoparticles was detected in release medium after 2 h, and only about 10-20% more curcumin released during the next 4 h stage, demonstrating that, in this study, not all content was released.

Note that the oral stage was not included, although there may be changes in the size and interfacial characteristics of nanoemulsions, influencing their fate in the gastrointestinal tract (McClements & Xiao, 2012) as the sample is liquid (and therefore chewing is not relevant and residence time in the mouth is very short) and contains no starch (i.e. the primary enzyme present in saliva, amylase would not act) (Silva et al., 2018).

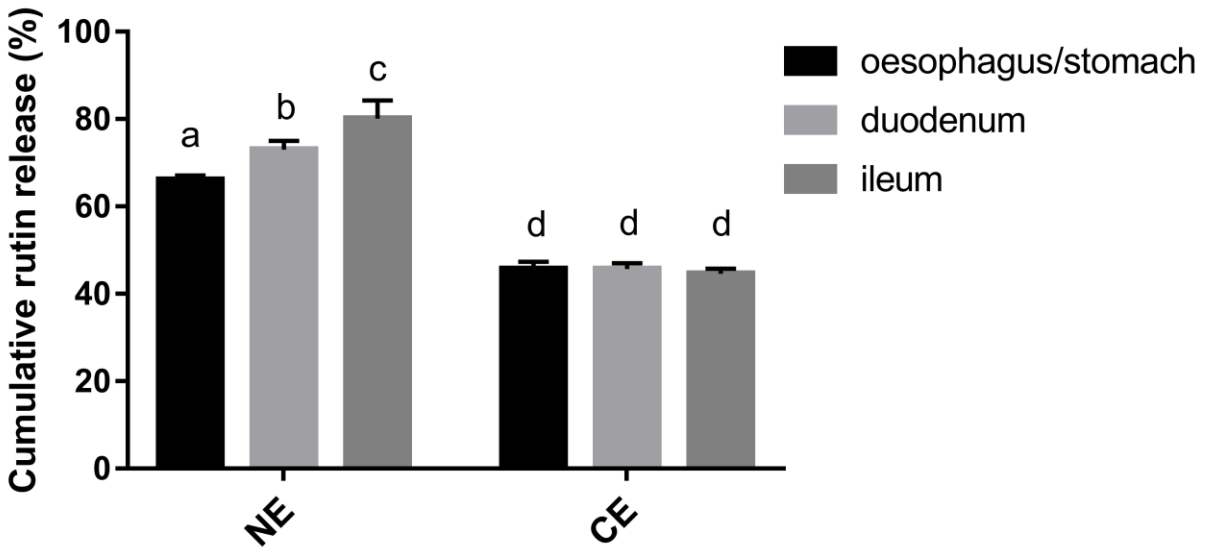


Figure 6 *In vitro* rutin release profile of calyx extracts (CE) and nanoemulsions containing *P. peruviana* calyx extract (NE) using dialysis bag method exposed to the simulated gastrointestinal conditions throughout incubation time and pH values. Esophagus/stomach: 90 min/pH=2.0; duodenum: 20 min/pH=5.0; ileum: 90 min/pH=6.5. Bars represent the mean ± SD, n = 3. Two-way ANOVA - Tukey's multiple comparisons test ($p < 0.05$), same letters mean no statistically significant difference.

4. CONCLUSION

In vitro and *in vivo* tests were fundamental to evaluate the possible toxicity of nanoemulsions and *P. peruviana* calyx extract. The extracts and nanoemulsions containing the extract (NE) showed no toxicity in the non-tumor cell line. However, as the concentration of NE and blank nanoemulsions (NB) increased, they showed cytotoxicity against the tumor cell line. In the *in vivo* model, all samples caused no toxicity to *C. elegans* in the survival test. The pharyngeal pumping rate and worm defecation cycle in animals treated with NE were not altered, the other formulations caused a slight change in diet, which reduced some rates.

Regarding oxidative stress resistance, the formulations exerted their antioxidant effects at low concentrations *in vivo*. In addition, the NE was able to protect the *P. peruviana* calyx extract against degradation of gastrointestinal conditions, controlling rutin release and increasing the levels of bioactive compound released in the duodenum and ileum, which is where absorption occurs. Thus, future food applications of nanoemulsions containing calyx extract can be further studied and explored.

ACKNOWLEDGMENTS

This study was financed in part by the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil* (CAPES) - Finance Code 001. We would like to thank Gustavo A. Ugalde and Prof. Marcio A. Mazutti for the chromatographic analysis and Reneu Morais for the English revision of this study. Cristiane de Bona da Silva would like to thank CNPq/Brazil for the financial support.

REFERENCES

Álvarez-Flórez, F., López-Cristoffanini, C., Jáuregui, O., Melgarejo, L. M., & López-Carbonell, M. (2017). Changes in ABA, IAA and JA levels during calyx, fruit and leaves development in cape gooseberry plants (*Physalis peruviana* L.). *Plant Physiology and Biochemistry*, *115*, 174–182. https://doi.org/10.1016/j.plaphy.2017.03.024

Arechabala, B., Coiffard, C., Rivalland, P., Coiffard, L. J. M., & De Roeck-Holtzhauer, Y. (1999). Comparison of cytotoxicity of various surfactants tested on normal human fibroblast cultures using the neutral red test, MTT assay and LDH release. *Journal of Applied Toxicology*, *19*(3), 163–165. https://doi.org/10.1002/(SICI)1099-1263(199905/06)19:3<163::AID-JAT561>3.0.CO;2-H

Ávila, D. S., Roncato, J. F., & Jacques, M. T. (2018). Nanotoxicology assessment in complementary/alternative models. *Energy, Ecology and Environment*, *3*(2), 72–80. https://doi.org/10.1007/s40974-018-0086-y

Ballesteros-Vivas, D., Álvarez-Rivera, G., Del Pilar Sánchez-Camargo, A., Ibáñez, E., Parada-Alfonso, F., & Cifuentes, A. (2019). A multi-analytical platform based on pressurized-liquid extraction, *in vitro* assays and liquid chromatography/gas chromatography coupled to high resolution mass spectrometry for food by-products valorisation. Part 1: Withanolides-rich fractions from goldenberry (*Physalis peruviana* L.) calyces obtained after extraction optimization as case study. *Journal of Chromatography. A*, *1584*, 155–164. https://doi.org/10.1016/j.chroma.2018.11.055

Ballesteros-Vivas, D., Álvarez-Rivera, G., Ibáñez, E., Parada-Alfonso, F., & Cifuentes, A. (2019). A multi-analytical platform based on pressurized-liquid extraction, *in vitro* assays and liquid chromatography/gas chromatography coupled to high resolution mass spectrometry for food by-products valorisation. Part 2: Characterization of bioactive compound. *Journal of Chromatography A*, *1584*, 144–154. https://doi.org/10.1016/j.chroma.2018.11.054

Bazana, M. T., da Silva, S. S., Codevilla, C. F., de Deus, C., Lucas, B. N., Ugalde, G. A., … de Menezes, C. R. (2019). Development of nanoemulsions containing *Physalis peruviana* calyx extract: A study on stability and antioxidant capacity. *Food Research International*, *125*, 108645. https://doi.org/10.1016/j.foodres.2019.108645

Bjelland, S., & Seeberg, E. (2003). Mutagenicity, toxicity and repair of DNA base damage induced by oxidation. *Mutation Research*, *531*(1–2), 37–80. https://doi.org/10.1016/j.mrfmmm.2003.07.002

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics*, *77*(1), 71–94. https://doi.org/10.1111/j.1749-6632.1999.tb07894.x

Codevilla, C. F., Tischer, B., Gindri, A. L., Nogueira-Librelotto, D. R., Barin, J. S., Silva, C. de B. da, … Menezes, C. R. de. (2018). Cytotoxicity and antioxidant activity of goldenberry extracts obtained with high intensity ultrasound. *Ciência Rural*, *48*(2), e20170362. https://doi.org/10.1590/0103-8478cr20170362

Crespi, C. L. (1995). Xenobiotic-metabolizing human cells as tools for pharmacological and toxicological research. *Advances in Drug Research*, *26*, 179–235. https://doi.org/10.1016/S0065-2490(05)80006-1

Demir, T. (2014). Antioxidant and cytotoxic activity of *Physalis peruviana*. *Medicinal Plant Research*, *4*(4). https://doi.org/10.5376/mpr.2014.04.0004

Fong, K. L., McCay, P. B., Poyer, J. L., Keele, B. B., & Misra, H. (1973). Evidence that peroxidation of lysosomal membranes is initiated by hydroxyl free radicals produced during flavin enzyme activity. *The Journal of Biological Chemistry*, *248*(22), 7792–7797. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/4147983

Frey, C., Pavani, M., Cordano, G., Muñoz, S., Rivera, E., Medina, J., … Ferreira, J. (2007). Comparative cytotoxicity of alkyl gallates on mouse tumor cell lines and isolated rat hepatocytes. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology*, *146*(4), 520–527. https://doi.org/10.1016/j.cbpa.2006.03.007

Fröhlich, E., Meindl, C., Roblegg, E., Griesbacher, A., & Pieber, T. R. (2012). Cytotoxity of nanoparticles is influenced by size, proliferation and embryonic origin of the cells used for testing. *Nanotoxicology*, *6*(4), 424–439. https://doi.org/10.3109/17435390.2011.586478

Gironés-Vilaplana, A., Baenas, N., Villaño, D., Speisky, H., García-Viguera, C., & Moreno, D. A. (2014). Evaluation of Latin-American fruits rich in phytochemicals with biological effects. *Journal of Functional Foods*, *7*(1), 599–608. https://doi.org/10.1016/j.jff.2013.12.025

Hillegass, J. M., Shukla, A., Lathrop, S. A., MacPherson, M. B., Fukagawa, N. K., & Mossman, B. T. (2010, May). Assessing nanotoxicity in cells *in vitro*. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*, Vol. 2, pp. 219–231. https://doi.org/10.1002/wnan.54

Huang, C., Xiong, C., & Kornfeld, K. (2004). Measurements of age-related changes of physiological processes that predict lifespan of *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences*, *101*(21), 8084–8089. https://doi.org/10.1073/pnas.0400848101

Inbaraj, J. J., & Chignell, C. F. (2004). Cytotoxic action of juglone and plumbagin: a mechanistic study using HaCaT keratinocytes. *Chemical Research in Toxicology*, *17*(1), 55–62. https://doi.org/10.1021/tx034132s

Madureira, A. R., Amorim, M., Gomes, A. M., Pintado, M. E., & Malcata, F. X. (2011). Protective effect of whey cheese matrix on probiotic strains exposed to simulated gastrointestinal conditions. *Food Research International*, *44*(1), 465–470. https://doi.org/10.1016/j.foodres.2010.09.010

McClements, D. J., & Xiao, H. (2012). Potential biological fate of ingested nanoemulsions: influence of particle characteristics. *Food & Function*, *3*(3), 202–220. https://doi.org/10.1039/c1fo10193e

Medina, S., Collado-González, J., Ferreres, F., Londoño-Londoño, J., Jiménez-Cartagena, C., Guy, A., … Gil-Izquierdo, Á. (2019). Potential of *Physalis peruviana* calyces as a low-cost valuable resource of phytoprostanes and phenolic compounds. *Journal of the Science of Food and Agriculture*, *99*(5), 2194–2204. https://doi.org/10.1002/jsfa.9413

Mendes, L. P., Delgado, J. M. F., Costa, A. D. A., Vieira, M. S., Benfica, P. L., Lima, E. M., & Valadares, M. C. (2015). Biodegradable nanoparticles designed for drug delivery: The number of nanoparticles impacts on cytotoxicity. *Toxicology in Vitro*, *29*(6), 1268–1274.

https://doi.org/10.1016/j.tiv.2014.12.021

Mendes, L. P., Gaeti, M. P. N., De Ávila, P. H. M., De Sousa Vieira, M., Dos Santos Rodrigues, B., De Ávila Marcelino, R. I., … Lima, E. M. (2014). Multicompartimental nanoparticles for Co-encapsulation and multimodal drug delivery to tumor cells and neovasculature. *Pharmaceutical Research*, *31*(5), 1106–1119. https://doi.org/10.1007/s11095-013-1234-x

Migliori, M. L., Simonetta, S. H., Romanowski, A., & Golombek, D. A. (2011). Circadian rhythms in metabolic variables in *Caenorhabditis elegans*. *Physiology & Behavior*, *103*(3–4), 315–320. https://doi.org/10.1016/j.physbeh.2011.01.026

Moraes, B. K. S., Vieira, S. M., Salgueiro, W. G., Michels, L. R., Colomé, L. M., Avila, D. S., & Haas, S. E. (2016). Clozapine-loaded polysorbate-coated polymeric nanocapsules: Physico-chemical characterization and toxicity evaluation in *Caenorhabditis elegans* model. *Journal of Nanoscience and Nanotechnology*, *16*(2), 1257–1264. https://doi.org/10.1166/jnn.2016.11668

Nogueira, D. R., Mitjans, M., Infante, M. R., & Vinardell, M. P. (2011). Comparative sensitivity of tumor and non-tumor cell lines as a reliable approach for *in vitro* cytotoxicity screening of lysine-based surfactants with potential pharmaceutical applications. *International Journal of Pharmaceutics*, *420*(1), 51–58. https://doi.org/10.1016/j.ijpharm.2011.08.020

Pascoli, M., Jacques, M. T., Agarrayua, D. A., Avila, D. S., Lima, R., & Fraceto, L. F. (2019). Neem oil based nanopesticide as an environmentally-friendly formulation for applications in sustainable agriculture: An ecotoxicological perspective. *Science of the Total Environment*, *677*, 57–67. https://doi.org/10.1016/j.scitotenv.2019.04.345

Puente, L. A., Pinto-Muñoz, C. A., Castro, E. S., & Cortés, M. (2011). *Physalis peruviana* Linnaeus, the multiple properties of a highly functional fruit: A review. *Food Research*

International, *44*(7), 1733–1740. https://doi.org/10.1016/j.foodres.2010.09.034

Contri, R.V., Fiel, L.A., Alnasif, N., Pohlmann, A. R., Guterres, S.S., & Schäfer-Korting, M. (2016). Skin penetration and dermal tolerability of acrylic nanocapsules: Influence of the surface charge and a chitosan gel used as vehicle. *International Journal of Pharmaceutics*, *507*, 12–20. https://doi.org/10.1016/j.ijpharm.2016.03.

Raizen, D., Song, B. M., Trojanowski, N., & You, Y. J. (2012). Methods for measuring pharyngeal behaviors. *WormBook : The Online Review of C. Elegans Biology*, pp. 1–13. https://doi.org/10.1895/wormbook.1.154.1

Riddle, D. L., Blumenthal, T., Meyer, B. J., & Priess, J. R. (1997). Introduction to *C. elegans*. In *C. elegans II*. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21413243

Silva, S. S. da, Bazana, M. T., Deus, C. de, Machado, M. L., Cordeiro, L. M., Soares, F. A. A., … Codevilla, C. F. (2019). Physicochemical characterization and evaluation of *in vitro* and *in vivo* toxicity of goldenberry extract nanoemulsion. *Ciência Rural*, *49*(8), 1–7. https://doi.org/10.1590/0103-8478cr20190015

Silva, H. D., Poejo, J., Pinheiro, A. C., Donsì, F., Serra, A. T., Duarte, C. M. M., … Vicente, A. A. (2018). Evaluating the behaviour of curcumin nanoemulsions and multilayer nanoemulsions during dynamic *in vitro* digestion. *Journal of Functional Foods*, *48*, 605–613. https://doi.org/10.1016/j.jff.2018.08.002

Stadtman, E. R. (2006). Protein oxidation and aging. *Free Radical Research*, *40*(12), 1250–1258. https://doi.org/10.1080/10715760600918142

Thomas, J. H. (1990). Genetic analysis of defecation in *Caenorhabditis elegans*. *Genetics*, *124*(4), 855–872. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2323555

Velasques, K., Maciel, T. R., de Castro Dal Forno, A. H., Teixeira, F. E. G., da Fonseca, A. L., Varotti, F. de P., … Haas, S. E. (2018). Co-nanoencapsulation of antimalarial drugs increases their *in vitro* efficacy against Plasmodium falciparum and decreases their

toxicity to *Caenorhabditis elegans*. *European Journal of Pharmaceutical Sciences : Official Journal of the European Federation for Pharmaceutical Sciences*, *118*, 1–12. https://doi.org/10.1016/j.ejps.2018.03.014

Wang, J., Witte, F., Xi, T., Zheng, Y., Yang, K., Yang, Y., … Qin, L. (2015). Recommendation for modifying current cytotoxicity testing standards for biodegradable magnesium-based materials. *Acta Biomaterialia*, *21*, 237–249. https://doi.org/10.1016/j.actbio.2015.04.011

Wang, T., Xue, J., Hu, Q., Zhou, M., & Luo, Y. (2017). Preparation of lipid nanoparticles with high loading capacity and exceptional gastrointestinal stability for potential oral delivery applications. *Journal of Colloid and Interface Science*, *507*, 119–130. https://doi.org/10.1016/j.jcis.2017.07.090

Wu, T., Xu, H., Liang, X., & Tang, M. (2019). *Caenorhabditis elegans* as a complete model organism for biosafety assessments of nanoparticles. *Chemosphere*, *221*, 708–726. https://doi.org/10.1016/j.chemosphere.2019.01.021

You, Y. jai, Kim, J., Raizen, D. M., & Avery, L. (2008). Insulin, cGMP, and TGF-β Signals Regulate Food Intake and Quiescence in *C. elegans*: A Model for Satiety. *Cell Metabolism*, *7*(3), 249–257. https://doi.org/10.1016/j.cmet.2008.01.005

Zhang, R., & McClements, D. J. (2018). Characterization of Gastrointestinal Fate of Nanoemulsions. In *Nanoemulsions: Formulation, Applications, and Characterization* (pp. 577–612). https://doi.org/10.1016/B978-0-12-811838-2.00018-7

8 DISCUSSÃO GERAL

Os cálices são um importante subproduto do processamento do fruto de *goldenberry* e são uma promissora fonte de compostos bioativos, tais como o 4β-hidroxivitanolídeo E e vitanolídeo E (BALLESTEROS-VIVAS et al., 2019b), cinquenta e seis fitoquímicos, incluindo os principais componentes fenólicos, vários vitanolídeos (C28-isoprenóides) com uma variedade de atividades biológicas e uma grande família de anti-inflamatórios (BALLESTEROS-VIVAS et al., 2019a). Novas oxilipinas ativas denominadas fitoprostanos, também, foram identificados. Além disso, seis derivados fenólicos foram descritos pela primeira vez. Entre os compostos já identificados, a rutina também foi o principal composto fenólico encontrado (MEDINA et al., 2019). Assim, baseado nesses estudos podemos observar a variedade de compostos já conhecidos no cálice e ressaltar a importância da escolha desse resíduo para nossa pesquisa, corroborando com os nossos achados em que o composto majoritário foi a rutina.

O extrato do cálice de *P. peruviana* foi caracterizado quanto sua capacidade redutora (610 ± 20 mg Eq. de ácido gálico/100 g de cálice), capacidade antioxidante por ORAC (137,93 ± 2,10 μmol de Trolox/g de cálice) e composto majoritário quantificado por CLAE (rutina: 11,38 ± 0.98 μg/mL). Em relação à análise cromatográfica buscou-se por quantificar o composto predominante e usá-lo como marcador durante o estudo de estabilidade e liberação.

Estudos em relação ao potencial antimicrobiano de *P. peruviana* vêm sendo realizados. Extratos etanólicos de diferentes partes da planta (fruta, semente, raiz, corpo e folha) foram avaliados, sendo que semente e fruta foram as mais efetivas em termos de sua atividade antimicrobiana (ERTÜRK et al., 2017). Todas as partes da planta de *P. peruviana* podem ser fontes potenciais de agentes antimicrobianos, sendo o mais potente, os extratos das folhas em diclorometano (KAMAU et al., 2017). Além disso, os extratos etanólicos das bagas maduras e das folhas de *P. peruviana* foram mais efetivos do que os aquosos (CUEVA et al., 2017). Neste sentido, a atividade antibacteriana e antibiofilme do extrato do cálice de *P. peruviana*, também, foi avaliada.

De acordo com essas pesquisas até o momento nenhuma havia especificado a atividade antimicrobiana do extrato etanólico dos cálices. Assim, em nosso estudo verificamos que o extrato do cálice de *P. peruviana* apresentou atividade antibacteriana contra os patógenos analisados, possuindo atividade bactericida na concentração de 30 mg/mL frente a seis bactérias: *Enterobacter aerogenes, Klebsiella pneumoniae, Staphylococcus epidermidis, Streptococcus pneumoniae, Pseudomonas aeruginosa* PA01 e *Enterococcus*

faecalis. A CIM variou de 3,15 a 30 mg/mL de extrato e mostrou atividade bacteriostática frente a oito cepas: *Escherichia coli, Shigella sonnei, Acinetobacter baumannii* ATCC 19606, *Acinetobacter baumannii* (isolado clínico), *Streptococcus agalactie, Salmonella sp., Salmonella enteridis and Staphylococcus aureus*. Em relação à capacidade antibiofilme, houve inibição da formação de biofilme, mas não houve destruição.

De acordo com esses resultados, foi verificada a potencial atividade antibacteriana do extrato do cálice de *P. peruviana* e essa atividade não pode ser observada nas nanoemulsões, pois a maioria das atividades foram em concentrações de extrato acima de 7,5 mg/mL e a concentração de extrato usada nas nanoemulsões foi menor (5 mg/mL). Durante o desenvolvimento das NEs foram realizados testes com maior concentração de extrato, porém acima de 5 mg/mL havia a formação de gotículas no tamanho micrométrico e nosso objetivo era focar em uma formulação exclusivamente na escala nano. De qualquer forma, sabe-se que o extrato possui essa atividade e é de extrema importância, pois incentiva a realização de novos estudos para aplicabilidade de seu potencial antibacteriano na área de alimentos.

Em comparação com outras técnicas, a nanoencapsulação de compostos bioativos tem sido uma questão de grande interesse devido a suas funcionalidades exclusivas, tais como alta eficiência de encapsulamento, melhor biodisponibilidade, maior estabilidade, perfil de liberação sustentada e mascarar sabores indesejáveis (SHISHIR et al., 2018). Assim, com intuito de utilizar uma tecnologia inovadora, em nosso estudo aplicamos a nanotecnologia no extrato do cálice de *P. peruviana* desenvolvendo nanoemulsões.

As nanoemulsões obtidas apresentaram características físico-químicas satisfatórias com tamanho de gotículas nanométrico, baixo índice de polidispersão, potencial zeta negativo, pH ácido, rutina (11,38 ± 0.98 μg/mL) como composto bioativo majoritário com eficiência de encapsulamento de 85% e morfologicamente apresentaram forma esférica e superfície lisa. Quanto ao estudo de estabilidade frente a diferentes condições de armazenamento (7 e 25°C) com ausência ou incidência de luz por 120 dias, observou-se que a formulação armazenada a temperatura ambiente e com incidência de luz (CPp-NE_{AL}) foi a que apresentou maiores alterações e instabilidades como a redução drástica do pH, aumento do tamanho de gotícula, aumento do índice de polidispersão e redução em módulo significativa do potencial zeta. Esses fenômenos de instabilidade podem estar relacionados a vários parâmetros como as propriedades químicas e a estrutura dos componentes, concentração de emulsificantes e aditivos, e a temperatura, que causam alterações nas propriedades, como tensão interfacial, elasticidade da gota e potencial interação da gota (GUPTA et al., 2016). Portanto, alta temperatura ou temperatura ambiente e a incidência de

luz são fatores já relatados como causadores de alterações na estabilidade de nanoemulsões (Rebolleda et al., 2015).

As melhores condições de armazenamento das amostras foram em ambas temperaturas no escuro, e a maior estabilidade foi observada para as nanoemulsões contendo extrato do cálice de *P. peruviana* armazenadas à temperatura ambiente e com ausência de luz. Além disso, a NE apresentou maior capacidade antioxidante e redutora em relação aos extratos livres. Esses resultados demonstram que as nanoemulsões desenvolvidas têm potencial para preservar a capacidade antioxidante do extrato do cálice de *P. peruviana*.

Entender a segurança e toxicidade dos nanomateriais é um grande desafio e requer muitas pesquisas, pois para promover a comercialização segura de novos produtos nanotecnológicos que sejam benéficos para a saúde, deveria existir um regulamento mundial (BAZANA; CODEVILLA; MENEZES, 2019). Com o intuito de estudar os potencias de toxicidade das nanoemulsões desenvolvidas e do extrato, realizou-se testes *in vitro* e *in vivo*. Como investigação preliminar da toxicidade de um nanomaterial, os ensaios de citotoxicidade são amplamente utilizados (HILLEGASS et al., 2010).

No ensaio de citotoxicidade, quanto à viabilidade das células 3T3 (não tumorais) o extrato e as nanoemulsões contendo extrato não apresentaram toxicidade, somente a nanoemulsão branca na maior concentração (150 µg/mL) mostrou uma redução significativa na viabilidade celular. Em relação às células MCF-7 (tumorais), o extrato livre não reduziu a viabilidade das células cancerígenas. Já, as NEs e as NBs à medida que a concentração da formulação aumentou, demonstraram altos valores de inibição no crescimento dessas células (Figura 1, manuscrito 2).

Neste estudo, duas linhagens celulares foram escolhidas como sistemas modelo, porque o uso de células de diferentes espécies e com diferentes origens embriogênicas é uma abordagem importante para entender as respostas específicas das células induzidas por materiais nano-baseados (FRÖHLICH et al., 2012). A NB provavelmente exerceu esse efeito devido à presença do polissorbato 80, conforme já relatado por (CONTRI et al., 2016), pois o tipo e a quantidade de tensoativo na formulação podem influenciar sua citotoxicidade (MENDES et al., 2015). Assim, respostas citotóxicas foram observadas mesmo em nanopartículas brancas durante a realização de experimentos *in vitro* utilizando diferentes linhagens celulares (MENDES et al., 2014; MENDES et al., 2015; SILVA et al., 2019).

Portanto, vale ressaltar que a diferente sensibilidade das linhagens celulares testadas aos tratamentos de nanoemulsões pode ser parcialmente explicada pelo fato de que células

saudáveis e tumorais apresentam diferenças em sua estrutura e função, especialmente em sua atividade metabólica e composição molecular (FREY et al., 2007).

O modelo *C. elegans* foi utilizado para as análises de toxicidade realizadas *in vivo*. A taxa de sobrevivência de todos os vermes permaneceu em torno de 100% (Figura 2, manuscrito 2), independentemente da amostra testada (CE, NE e NB), indicando que a exposição às amostras não causou letalidade. A nanoemulsão contendo o extrato do cálice de *P. peruviana* (NE) não demonstrou alterações quanto aos demais parâmetros analisados (bombeamento faríngeo, ciclo defecação e resistência ao estresse oxidativo).

Além disso, a NE protegeu o extrato do cálice de *P. peruviana* frente à degradação das condições gastrointestinais simuladas, controlando a liberação de rutina e aumentando os níveis do composto bioativo liberado no duodeno e íleo, onde ocorre a absorção. Assim, futuras aplicações alimentares utilizando a nanotecnologia aliada aos resíduos da fruta de *P. peruviana* (cálice) podem ser mais e explorados pela ciência e tecnologia dos alimentos.

9 CONCLUSÃO

- As nanoemulsões contendo extrato do cálice de *P. peruviana* apresentaram características físico-químicas adequadas (tamanho nanométrico de gotículas, baixo índice de polidispersão, potencial zeta negativo, pH ácido, rutina como composto majoritário, eficiência de encapsulamento de 85% e morfologia esférica e lisa).
- Quanto ao estudo de estabilidade as melhores condições de armazenamento das amostras foram nas temperaturas de 7° C e 25 °C no escuro, sendo que a maior estabilidade foi observada para as nanoemulsões contendo extrato do cálice de *P. peruviana* armazenadas à temperatura ambiente e com ausência de luz.
- O extrato do cálice de *P. peruviana* apresentou atividade antibacteriana contra os patógenos analisados, demonstrando atividade bacteriostática frente a oito bactérias e atividade bactericida frente a seis cepas. Ainda, inibiu a formação de biofilme, porém não houve destruição do mesmo. Estes resultados possibilitam que novos estudos sejam realizados para contribuir na sua utilização na indústria de alimentos, como um conservante de origem natural e, também, na área clínica.
- As nanoemulsões contendo extrato do cálice de *P. peruviana* não apresentaram citotoxicidade *in vitro* frente a linhagem celular não tumoral (3T3 fibroblastos) e foram citotóxicas frente a linhagem celular tumoral (MCF-7 câncer de mama), conferindo resultados promissores. Também, não foi evidenciada toxicidade *in vivo* nas condições avaliadas. Além disso, a liberação sob condições gastrointestinais simuladas demonstrou que as nanoemulsões contendo o extrato apresentaram melhor controle da liberação em relação ao extrato livre, protegendo a rutina frente à degradação, propiciando que novos estudos viabilizem a aplicabilidade na área alimentos.
- As nanoemulsões desenvolvidas têm potencial para preservar a capacidade antioxidante do extrato do cálice de *P. peruviana*, favorecendo a reutilização desse subproduto. Portanto, o uso da nanotecnologia associado com resíduos de plantas é de grande interesse para a ciência de alimentos e o desenvolvimento de novos produtos alimentares.

REFERÊNCIAS

ABDENNACER, B. et al. Determination of phytochemicals and antioxidant activity of methanol extracts obtained from the fruit and leaves of Tunisian Lycium intricatum Boiss. **Food Chemistry**, v. 174, p. 577-584, 2015.

ALMEIDA, A. S. et al. Aplicação de nanotecnologia em embalagens de alimentos. **Polímeros**, São Carlos , v. 25, n. spe, p. 89-97, 2015.

ARANCIBIA, C. et al. Comparing the effectiveness of natural and synthetic emulsifiers on oxidative and physical stability of avocado oil-based nanoemulsions. **Innovative Food Science & Emerging Technologies,** v. 44, p. 159-166, 2017.

BALASUNDRAM, N.; SUNDRAM, K.; SAMMAN, S. Phenolic compounds in plants and agriindustrial by-products: Antioxidant activity, occurrence, and potential uses. **Food Chemistry**, v. 99, n. 1, p. 191-203, 2006.

BALLESTEROS-VIVAS, D. et al. A multi-analytical platform based on pressurized-liquid extraction, in vitro assays and liquid chromatography/gas chromatography coupled to high resolution mass spectrometry for food by-products valorisation. Part 2: Characterization of bioactive compound. **Journal of Chromatography A,** v. 1584, p. 144-154, 2019a.

BALLESTEROS-VIVAS, D. et al. A multi-analytical platform based on pressurized-liquid extraction, in vitro assays and liquid chromatography/gas chromatography coupled to high resolution mass spectrometry for food by-products valorisation. Part 1: Withanolides-rich fractions from goldenberry (Physalis peruviana L.) calyces obtained after extraction optimization as case study. **Journal of chromatography. A**, v. 1584, p. 155-164, 11 jan. 2019b.

BATAGLION, G. A. et al. Determination of the phenolic composition from Brazilian tropical fruits by UHPLCMS/MS. **Food Chemistry**, v. 180, 280-287, 2015.

BAZANA, M. T.; CODEVILLA, C. F.; MENEZES, C. R. Nanoencapsulation of bioactive compounds: challenges and perspectives. **Current Opinion in Food Science**, v. 26, p. 47-56, 2019.

BETTAZZI. F.; PALCHETTI, I. Nanotoxicity assessment: A challenging application for cutting edge electroanalytical tools. **Analytica Chimica Acta**, v. 1072, p. 61-74, 2019.

BOMBARDELLI, E.; MORAZZONI, P. The flavonoids: new perspectives in biological activities and therapeutics. **Chimica Oggi**, v. 11, p. 25-28, 1993.

BOUCHEMAL, K. et al. Nano-emulsion formulation using spontaneous emulsification: solvent, oil and surfactant optimisation. **International Journal of Pharmaceutics,** v. 280, n. 1, p. 241-251, 2004.

BOYLE, S. P. et al. Bioavailability and efficiency of rutin as an antioxidant: A human supplementation study. **European Journal of Clinical Nutrition**, v. 54, p. 774-782, 2000.

BRENNER, S. The genetics of *Caenorhabditis elegans*. **Genetics**, v. 77, n. 1, p. 71-94, 1974.

BRIGHENTI, A. F. et al. Cultura da *Physalis* no planalto catarinense e a influência de sistemas de condução na qualidade dos frutos. In: CONGRESSO BRASILEIRO DE FRUTICULTURA, 20., 2008. **Anais...** Vitória: SBF/UFES, 2008. CD/ROOM.

BRIONES-LABARCA, V. et al. Extraction of β-Carotene, Vitamin C and Antioxidant Compounds from *Physalis peruviana* (Cape Gooseberry) Assisted by High Hydrostatic Pressure. **Food and Nutrition Sciences,** v. 4, n. 8, p. 109-118, 2013.

CAILLET, S. et al. Fenton reaction applied for screening natural antioxidants. **Food Chemistry**, v. 100, n. 2, p. 542-552, 2007.

CALABRÒ, M. L. et al. The rutin/b-cyclodextrin interactions in fully aqueous solution: Spectroscopic studies and biological assays. **Journal of Pharmaceutical and Biomedical Analysis**, v. 36, n. 5, p. 1019-1027, 2005.

CASA, C. L. et al. Evidence for protective and antioxidante properties of rutin, a natural flavone, against ethanol induced gastric lesions. **Journal of Ethnopharmacology**, v. 71, p. 45-53, 2000.

CASTRO, A.; RODRIGUEZ, L.; VARGAS, E. Dry gooseberry (*Physalis peruviana* L) with pretreatment of osmotic dehydration. **Vitae - Revista de la Facultad de Química Farmacéutica**, v. 15, n. 2, p. 226-231, 2008.

CASTRO, J.; OCAMPO, Y.; FRANCO, L. Cape Gooseberry [*Physalis peruviana* L.] Calyces Ameliorate TNBS Acid-induced Colitis in Rats. **Journal of Crohn's and Colitis,** v. 9, n. 11, p. 1004-1015, 2015.

CEDEÑO, M.; MONTENEGRO, D. **Plan exportador, logístico y comercialización de uchuva al mercado de Estados Unidos para FRUTEXPO SCI Ltda**. Trabajo de grado (Ingeniería Industrial) – Facultad de Ingeniería, Pontificia Universidad Javeriana, Bogotá, 2004.

CHAARI, M. et al. Encapsulation of carotenoids extracted from halophilic *Archaea* in oil-in-water (O/W) micro- and nano-emulsions. **Colloids and Surfaces B: Biointerfaces,** v. 161, supplement C, p. 219-227, 2018.

CHUA, L. S. A review on plant-based rutin extraction methods and its pharmacological activities. **Journal of Ethnopharmacology**, v. 150, p. 805-817, 2013.

CODEVILLA, C. et al. Nanoestruturas contendo compostos bioativos extraídos de plantas. **Ciência e Natura**, v. 37, p. 142-151, 15 dez. 2015.

CONSELHO NACIONAL DO MEIO AMBIENTE. Resolução CONAMA nº 313, de 29 de outubro de 2002. Dispõe sobre o Inventário Nacional de Resíduos Sólidos Industriais. **Diário Oficial da União**, Brasília, DF, 22 nov. 2002. Seção 1, p. 85-91. Disponível em: <http://www.mma.gov.br/port/conama/legislacao/CONAMA_RES_CONS_2002_313.pdf>. Acesso em: 27 nov. 2017.

CONTRI, R.V. et al. Skin penetration and dermal tolerability of acrylic nanocapsules: Influence of the surface charge and a chitosan gel used as vehicle. **International Journal of Pharmaceutics**, v. 507, p. 12–20, 2016.

COSTA, N. M. B; ROSA, C. O. B. **Alimentos funcionais** - componentes bioativos e efeitos fisiológicos, Cap 1- Alimentos funcionais: Histórico, Legislação e Atributos. 2. ed. Rio de Janeiro: Editora Rubio, 2016.

CUEVA, M. B. R. et al. Antibacterial Effects of Uvilla (*Physalis peruviana* L.) extracts against Listeria spp. Isolated from Meat in Ecuador. **International Journal of Current Microbiology and Applied Sciences**, v. 6, n. 4, p. 1146-1153, 2017.

DAG, D.; KILERCIOGLU, M.; OZTOP, M. H. Physical and chemical characteristics of encapsulated goldenberry (*Physalis peruviana* L.) juice powder. **LWT - Food Science and Technology,** v. 83, Supplement C, p. 86-94, 2017.

DINAN, L.; SARKER, S.; SIK, V. 28-Hydroxywithanolide E from *Physalis peruviana*. **Photochemistry**, v. 44, p. 509-512, 1997.

DUDONNÉ, S. et al. Comparative Study of Antioxidant Properties and Total Phenolic Content of 30 Plant Extracts of Industrial Interest Using DPPH, ABTS, FRAP, SOD, and ORAC Assays. **Journal of Agricultural and Food Chemistry**, v. 5, n. 5, p. 1768-1774, 2009.

DUNCAN, T. V. Applications of nanotechnology in food packaging and food safety: Barrier materials, antimicrobials and sensors. **Journal of Colloid and Interface Science,** v. 363, n. 1, p. 1-24, 2011.

EMBABY, H. E.-S.; MOKHTAR, S. M. Chemical composition and nutritive value of lantana and sweet pepper seeds and nabak seed kernels. **Journal of Food Science**, v. 76, n. 5, p. C736-C741, 2011.

ERKAN, N.; AYRANCI, G.; AYRANCI, E. Antioxidant activities of Rosemary (*Rosmarinus officinalis* L.) extract, blackseed (*Nigelly sativa* L.) essential oil, carnosic acid, rosmarinic acid and sesamol. **Food Chemistry**, v. 110, p. 76-82, 2008.

ERTÜRK, Ö. et al. Antioxidant, antimicrobial activities and phenolic and chemical contents of Physalis peruviana L. from Trabzon, Turkey. **Indian Journal of Pharmaceutical Education and Research**, v. 51, n. 3, p. S213-S216, 2017.

ETZBACH, L. et al. Characterization of carotenoid profiles in goldenberry (*Physalis peruviana* L.) fruits at various ripening stages and in different plant tissues by HPLC-DAD-APCI-MSn. **Food Chemistry,** v. 245, Supplement C, p. 508-517, 2018.

EZHILARASI, P. N. et al. Nanoencapsulation Techniques for Food Bioactive Components: A Review. **Food and Bioprocess Technology,** v. 6, n. 3, p. 628-647, 2013.

FERRARI, G.; MARESCA, P.; CICCARONE, R. The application of high hydrostatic pressure for the stabilization on functional foods: Pomegranate juice. **Journal of Food Engineering**, v. 100, n. 2, p. 245-253, 2010.

FORSTER-CARNEIRO, T. et al. Biorefinery study of availability of agriculture residues and wastes for integrated biorefineries in Brazil. **Resources Conservation and Recycling**, v. 77, p. 78-88, 2013.

FRANCO, L. A. et al. Actividad antinflamatoria de extractos y fracciones obtenidas de cálices de *Physalis peruviana* L. **Biomédica,** v. 27, p. 110-115, 2007.

FRANCO, L. A. et al. Sucrose Esters from *Physalis peruviana* Calyces with Anti-Inflammatory Activity. **Planta Med,** v. 80, n. 17, p. 1605-1614, 2014.

FRANKEL, E. N.; MEYER, A. S. The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. **Journal of the Science of Food and Agriculture**, v. 80, p. 1925-1941, 2000.

FREY, C. et al. Comparative cytotoxicity of alkyl gallates on mouse tumor cell lines and isolated rat hepatocytes. **Comparative biochemistry and physiology. Part A, Molecular & integrative physiology**, v. 146, n. 4, p. 520–527, 2007.

FRÖHLICH, E. et al. Cytotoxity of nanoparticles is influenced by size, proliferation and embryonic origin of the cells used for testing. **Nanotoxicology**, v. 6, n. 4, p. 424-439, 2012.

GAUTAM, S. K.; DWIVEDI, D. H.; KUMAR, P. Preliminary studies on the bioactive phytochemicals in extract of Cape gooseberry (*Physalis peruviana* L.) fruits and their Products. **Journal of Pharmacognosy and Phytochemistry,** v. 3, n. 5, p. 93-95, 2015.

GIRONÉS-VILAPLANA, A. et al. Evaluation of Latin-American fruits rich in phytochemicals with biological effects. **Journal of Functional Foods,** v. 7, n. 1, p. 599-608, 2014.

GOOT, A. J. et al. Concepts for further sustainable production nof foods. **Journal of Food Engineering**, v. 168, p. 42-51, 2016.

GULLÓN, B. et al. Rutin: A review on extraction, identification and purification methods, biological activities and approaches to enhance its bioavailability. **Trends in Food Science and Technology**, v. 67, p. 220-235, 2017.

GUPTA, A. et al. Nanoemulsions: formation, properties and applications. **Soft Matter**, v. 12, n. 11, p. 2826-2841, 2016.

HAMAD, A. F. et al. The intertwine of nanotechnology with the food industry. **Saudi Journal of Biological Sciences**, v. 25, n. 1, p. 27-30, 2018.

HARBORNE, J. B.; WILLIAMS, C. A. Advances in flavonoid research since 1992. **Phytochemistry**, n. 5, p. 481-504, 2000.

HASSANIEN, M. F. R. *Physalis peruviana*: A Rich Source of Bioactive Phytochemicals for Functional Foods and Pharmaceuticals. **Food Reviews International,** v. 27, n. 3, p. 259-273, 2011.

HILLEGASS, J. M. et al. Assessing nanotoxicity in cells *in vitro*. **Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology,** v. 32, n. 3, p. 219-231, 2010.

HOU, D. X. Potential mechanism of cancer chemoprevention by anthocyanin. **Current Advancements in Molecular Medicines**, v. 3, p. 149-159, 2003.

HUANG, C. et al. The rapid discovery and identification of physalins in the calyx of *Physalis alkekengi* L.var.*franchetii* (Mast.) Makino using ultra-high performance liquid chromatography–quadrupole time of flight tandem mass spectrometry together with a novel three-step data mining strategy. **Journal of Chromatography A,** v. 1361, Supplement C, p. 139-152, 2014a.

HUANG, H. W. et al. Responses of microorganisms to high hydrostatic pressure processing. **Food Control**, v. 40, p. 250-259, 2014b.

HUANG, Q.; YU, H.; RU, Q. Bioavailability and Delivery of Nutraceuticals Using Nanotechnology. **Journal of Food Science,** v. 75, n. 1, p. R50-R57, 2010.

ISO/TC 229 **Nanotechnologies**. Technical committees. Disponível em: <https://www.iso.org/committee/381983.html>. Acesso em: 27 nov. 2019.

JI, L. et al. Physalins with anti-inflammatory activity are present in *Physalis alkekengi* var. *franchetii* and can function as Michael reaction acceptors. **Steroids,** v. 77, n. 5, p. 441-447, 2012.

JIANG, P. et al. Rutin and flavonoid contents in three buckwheat species *Fagopyrum esculentum, F. tataricum,* and *F. homotropicum* and their protective effects against lipid peroxidation. **Food Research International**, v. 40, p. 356-364, 2007.

KAMALAKKANNAN, N.; PRINCE, S. M. P. Rutin improves the antioxidant status in streptozotocin-induced diabetic rat tissues. **Molecular and Cellular Biochemistry**, v. 293, p. 211-219, 2006.

KAMAU, P. K. et al. Antimicrobial evaluation and phytochemical screening of aqueous and dichloromethane crude extracts of Kenyan *Physalis Peruviana* L (Cape Gooseberry). **IOSR. Journal of Dental and Medical Sciences,** v. 16, n. 5, p. 101-109, 2017.

KARUPPANAPANDIAN, T. et al. Reactive oxygen species in plants: their 34 generation, signal transduction, and scavenging mechanisms. **Australian Journal of Crop Science,** v. 5, n. 6, p. 709-725, 2011.

KHODDAMI, A.; WILKES, M.; ROBERTS, T. Techniques for Analysis of Plant Phenolic Compounds. **Molecules,** v. 18, n. 2, p. 2328-2375, 2013.

KIM, K. H. et al. Optimal recovery of high-purity rutin crystals from the whole plant of *Fagopyrum esculentum* Moench (buckwheat) by extraction, fractionation, and recrystallization. **Bioresource Technology**, v. 96, p. 1709-1712, 2005.

KUMAR, V. et al. Photoinduced green synthesis of silver nanoparticles using aqueous extract of *Physalis angulata* and its antibacterial and antioxidant activity. **Journal of Environmental Chemical Engineering,** v. 5, n. 1, p. 744-756, 2017.

KUSPKA, M.; JÉLEN, H. H. In-tube extraction for the determination of the main volatile compounds in *Physalis peruviana* L. **Journal of Separation Science**, v. 40, n. 2, p. 532-541, 2016.

LICODIEDOFF, S. **Caracterização físico-química e compostos bioativos em *Physalis peruviana* e derivados**. 2012. 119 p. Tese (Doutorado em Tecnologia de Alimentos) – Universidade Federal do Paraná, Curitiba, PR, 2012.

LIU, Y. et al. Protective effect of rutin against acute gastric mucosal lesions induced by ischemia-reperfusion. **Pharmaceutical Biology,** v. 51, n. 7, p. 914-919, 2013.

MARTÍNEZ, W. et al. *In vitro* studies on the relationship between the anti-inflammatory activity of *Physalis peruviana* extracts and the phagocytic process. **Immunopharmacology and Immunotoxicology,** v. 32, n. 1, p. 63-73, 2010.

MASON, T. G. et al. Nanoemulsions: formation, structure, and physical properties. **Journal of Physics: Condensed Matter,** v. 18, n. 41, p. R635-R666, 2006.

MAZORRA, M. Aspectos anatómicos de la formación y crecimiento del fruto de uchuva *Physalis peruviana* (Solanaceae). **Acta Biológica Colombiana**, v. 11, n. 1, p. 69-81, 2006.

MCCLEMENTS, D. J. Nanoemulsions versus microemulsions: terminology, differences, and similarities. **Soft Matter,** v. 8, p. 1719-1729, 2012.

MCCLEMENTS, D. J.; BAI, L.; CHUNG, C. Recent Advances in the Utilization of Natural Emulsifiers to Form and Stabilize Emulsions. **Annual Review of Food Science and Technology,** v. 8, n. 1, p. 205-236, 2017.

MEDINA, S. et al. Potential of Physalis peruviana calyces as a low-cost valuable resource of phytoprostanes and phenolic compounds. **Journal of the Science of Food and Agriculture**, v. 99, n. 5, p. 2194-2204, 2019.

MEDINA-MEDRANO, J. R. et al. Phenolic constituents and antioxidant properties of five wild species of *Physalis* (Solanaceae). **Botanical Studies,** v. 56, n. 1, p. 24, 2015.

MEHRNIA, M.-A. et al. Crocin loaded nano-emulsions: Factors affecting emulsion properties in spontaneous emulsification. **International Journal of Biological Macromolecules,** v. 84, Supplement C, p. 261-267, 2016.

MENDES, L. P. et al. Biodegradable nanoparticles designed for drug delivery: The number of nanoparticles impacts on cytotoxicity. **Toxicology in Vitro**, v. 29, n. 6, p. 1268–1274, 2015.

MENDES, L. P. et al. Multicompartimental nanoparticles for Co-encapsulation and multimodal drug delivery to tumor cells and neovasculature. **Pharmaceutical Research**, v. 31, n. 5, p. 1106–1119, 2014.

MISSAOUI, W. N.; ARNOLD, R. D.; CUMMINGS, B. S. Toxicological status of nanoparticles: What we know and what we don't know. **Chemico-Biological Interactions,** v. 295, p. 1-12, 2018.

MOKHTAR, S. M.; SWAILAM, H. M.; EMBABY, H. E. S. Physicochemical properties, nutritional value and techno-functional properties of goldenberry (*Physalis peruviana*) waste powder concise title: Composition of goldenberry juice waste. **Food Chemistry**, v. 248, p. 1-7, 2018.

MUNIZ, J. et al. Sistemas de condução para o cultivo de *Physalis* no planalto catarinense. **Revista Brasileira de Fruticultura,** v. 33, n. 3, p. 830-838, 2011.

NEO, Y. P. et al. Encapsulation of food grade antioxidant in natural biopolymer by electrospinning technique: A physicochemical study based on zein–gallic acid system. **Food Chemistry,** v. 136, n. 2, p. 1013-1021, 2013.

OLIVARES-TENORIO, M.-L. et al. Health-promoting compounds in cape gooseberry (*Physalis peruviana* L.): Review from a supply chain perspective. **Trends in Food Science & Technology**, v. 57, p. 83-92, 2016.

OLIVEIRA, I. R. W.Z; FERNANDES, S. C.; VIEIRA, I. C. Development of a biosensor based on gilo peroxidase immobilized on chitosan chemically crosslinked with epichlorohydrin for determination of rutin. **Journal of Pharmaceutical and Biomedical Analysis**, v. 41, n. 2, p. 366-372, 2006.

PATIL, B. S. et al. Bioactive Compounds: Historical Perspectives, Opportunities, and Challenges. **Journal of Agricultural and Food Chemistry,** v. 57, n. 18, p. 8142-8160, 2009.

PATRA, J. K. et al. Photo-mediated Biosynthesis of Silver Nanoparticles Using the Non-edible Accrescent Fruiting Calyx of *Physalis peruviana* L. Fruits and Investigation of its Radical Scavenging Potential and Cytotoxicity Activities. **Journal of Photochemistry and Photobiology B: Biology**, v. 188, p. 116-125, 2018.

PRADHAN, N. et al. Facets of Nanotechnology as Seen in Food Processing, Packaging, and Preservation Industry. **BioMed Research International,** v. 2015, p. 17, 2015.

PRIOR, R. L. Absorption and metabolism of anthocyanins: Potential health effects. In: MESKIN, M. et al. (Ed.). **Phytochemicals:** Mechanisms of action. Boca Raton, FL: CRC Press, 2004. p. 1-19.

PUENTE, L. A. et al. *Physalis peruviana* Linnaeus, the multiple properties of a highly functional fruit: A review. **Food Research International,** v. 44, n. 7, p. 1733-1740, 2011.

QU, J. et al. Zinc accumulation and synthesis of ZnO nanoparticles using *Physalis alkekengi* L. **Environmental Pollution,** v. 159, n. 7, p. 1783-1788, 2011.

RAMADAN, M. F. Bioactive phytochemicals, nutritional value, and functional properties of cape gooseberry (*Physalis peruviana*): An overview. **Food Research International**, v. 44, n. 7, p. 1830-1836, 2011.

RAMADAN, M. F.; MOERSEL, J. T. Impact of enzymatic treatment on chemical composition, physicochemical properties and radical scavenging activity of goldenberry (*Physalis peruviana* L.) juice. **Journal of the Science of Food and Agriculture,** v. 87, n. 3, p. 452-460, 2007.

RAMADAN, M. F.; MÖRSEL, J. T. Oil Goldenberry (*Physalis peruviana* L.). **Journal of Agricultural and Food Chemistry,** v. 51, n. 4, p. 969-974, 2003.

RAO, J.; MCCLEMENTS, D. J. Food-grade microemulsions and nanoemulsions: Role of oil phase composition on formation and stability. **Food Hydrocolloids,** v. 29, n. 2, p. 326-334, 2012.

RASHID, M.; SABIR, S. Biosynthesis of Self-Dispersed Silver Colloidal Particles Using the Aqueous Extract of *P. peruviana* for Sensing dl -Alanine. **ISRN Nanotechnology**, p. 1–7, 2014.

REZAEI, A.; FATHI, M.; JAFARI, S. M. Nanoencapsulation of hydrophobic and low-soluble food bioactive compounds within different nanocarriers. **Food Hydrocolloids**, v. 88, p. 146-162, 2019.

ROCKETT, F. C. et al. **Relatório final de atividades do projeto Biodiversidade para Alimentação e Nutrição (BFN) da Região Sul.** Porto Alegre, RS: Universidade Federal do Rio Grande do Sul, 2018.

RODRIGUES, F. A. et al. Caracterização do ponto de colheita de *Physalis peruviana* L. na região de Lavras, MG. **Bioscience Journal**, Uberlândia, v. 28, n. 6, p. 862-867, 2012.

ROP, O. et al. Bioactive content and antioxidant capacity of Cape gooseberry fruit. **Central European Journal of Biology,** v. 7, n. 4, p. 672-679, 2012.

RUFATO, A. R. et al. A cultura da *Physalis.* In: KRETZSCHMAR, A. A.; RUFATO, L.; PELIZZA, T. R. (Org.). **Pequenas frutas.** Florianópolis: UDESC, 2013. p. 172-238 (Série Fruticultura, 2).

SALEM, M. A.; EZZAT, S. M. Nanoemulsions in food industry. In: MILANI, J. M. (Ed.). **Some new aspects of colloidal systems in foods.** [S.l.]: IntechOpen, 2018. cap. 3, p. 31–51,https://doi. org/10.5772/intechopen.79447. Disponível em: <https://www.intechopen. com/books/some-new-aspects-of-colloidal-systems-in-foods/nanoemulsions-in-foodindustry>. Acesso em: 29 set. 2019.

SCOTT, N.; CHEN, H. Nanoscale science and engineering for agriculture and food systems. **Industrial Biotechnology**, v. 9, n. 1, p. 17-18, 2013.

SEVERO, J. et al. Atividade antioxidante e fitoquímicos em frutos de *Physalis* (*Physalis peruviana*, L.) durante o amadurecimento e o armazenamento. **Revista Brasileira de Agrociência,** Pelotas, v. 16, n. 1-4, p. 77-82, 2010.

SHIN, G. H.; KIM, J. T.; PARK, H. J. Recent developments in nanoformulations of lipophilic functional foods. **Trends in Food Science and Technology**, v. 46, n. 1, p. 144-157, 2015.

SHISHIR, M. R. I. et al. Advances in micro and nano-encapsulation of bioactive compounds using biopolymer and lipid-based transporters. **Trends in Food Science and Technology**, v. 78, p. 34-60, 2018.

SILVA, H. D.; CERQUEIRA, M. Â.; VICENTE, A. A. Nanoemulsions for Food Applications: Development and Characterization. **Food and Bioprocess Technology,** v. 5, n. 3, p. 854-867, 2012.

SILVA, S. S. **Desenvolvimento de formulação nanoestruturada contendo extrato de *Physalis peruviana***. 2018. 82p. Dissertação (Mestrado em Ciência e Tecnologia dos Alimentos) – Universidade Federal de Santa Maria, Santa Maria, RS, 2018.

SILVA, S. S. et al. Physicochemical characterization and evaluation of *in vitro* and *in vivo* toxicity of goldenberry extract nanoemulsion. **Ciência Rural**, v. 49, n. 8, p. 1–7, 2019.

SINGH, B. et al. Phenolic composition and antioxidant potential of grain legume seeds: A review. **Food Research International,** v. 101, Supplement C, p. 1-16, 2017.

SOLANS, C. et al. Nano-emulsions. **Current Opinion in Colloid & Interface Science,** v. 10, n. 3, p. 102-110, 2005.

SOLANS, C.; SOLÉ, I. Nano-emulsions: Formation by low-energy methods. **Current Opinion in Colloid & Interface Science,** v. 17, n. 5, p. 246-254, 2012.

SONJA, D.; JASNA, Č.-B.; GORDANA, Ć. By-products of fruits processing as a source of phytochemicals. **Chemical Industry & Chemical Engineering Quarterly**, v. 15, n. 4, p. 191-202, 2009.

SUDHA, M. L.; BASKARAN, V.; LEELAVATHI, K. Apple pomace as a source of dietary fiber and polyphenols and its effect on the rheological characteristics and cake making. **Food Chemistry**, v. 104, n. 2, p. 686-692, 2007.

SUZUKI, T. et al. Physiological roles of rutin in the buckwheat plant. **Japan Agricultural Research Quarterly**, v. 49, p. 37-43, 2015.

TALUKDAR, D. Total flavonoids, phenolics, tannins and antioxidant activity in seeds of lentil and grass pea. **International Journal of Phytomedicine,** v. 4, n. 4, p. 537-542, 2013.

TAPIA, M.; FRIES, A. **Guía de campo de los cultivos andinos**. Lima: FAO y ANPE, 2007.

TOMASSINI, T. C. B. et al. Gênero *Physalis* - uma revisão sobre vitaesteróides. **Química Nova**, v. 23, n. 1, p. 47–57, 2000.

TORO, R. M. et al. Phytochemical analysis, antioxidant and anti-inflammatory activity of calyces from *Physalis peruviana*. **Natural Product Communications,** v. 9, n. 11, p. 1573-1575, 2014.

TORRES-LEÓN, C. et al. Mango seed: Functional and nutritional properties. **Trends in Food Science and Technology**, v. 55, p. 109-117, 2016.

TSAO, R. Chemistry and biochemistry of dietary polyphenols. **Nutrients**, v. 2, n. 12, p. 1231-1246, 2010.

WALKER, R.; DECKER, E.; MCCLEMENTS, D. J. Development of food-grade nanoemulsions and emulsions for delivery of omega-3 fatty acids: Opportunities and obstacles in the food industry. **Food & Function**, v. 6, n. 1, p. 41-54, 2015.

WEBSTER, R. P.; GAWDE, M. D.; BHATTACHARYA, R. K. Protective effect of rutin, a flavonol glycoside, on the carcinogen-induced DNA damage and repair enzymes in rats. **Cancer Letters**, v. 109, p. 185-191, 1996.

WU, S. J. et al. Antioxidant Activities of *Physalis peruviana*. **Biological and Pharmaceutical Bulletin,** v. 28, n. 6, p. 963-966, 2005.

WU, T. et al. *Caenorhabditis elegans* as a complete model organism for biosafety assessments of nanoparticles. **Chemosphere**, v. 221, p. 708-726, 2019.

XU, B. et al. High-performance liquid chromatography (HPLC) determination of five active ingredients in the calyces of *Physalis Alkekengi* L. var. *franchetii* (mast.) Mskino. **Journal of Medicinal Plants Research**, v. 6, n. 12, p. 2438-2442, 2012.

YANG, J.; GUO, J.; YUAN, J. *In vitro* antioxidant properties of rutin. **LWT–Food Science and Technology**, v. 41, p. 1060-1066, 2008.

YEN, C. Y. et al. 4beta-Hydroxywithanolide E from Physalis peruviana (golden berry) inhibits growth of human lung cancer cells through DNA damage, apoptosis and G2/M arrest. **BMC Cancer**, v. 10, p. 46, p. 1-8, 2010.

YILDIRIM, S. T.; OZTOP, M. H.; SOYER, Y. Cinnamon oil nanoemulsions by spontaneous emulsification: Formulation, characterization and antimicrobial activity. **LWT - Food Science and Technology,** v. 84, Supplement C, p. 122-128, 2017.

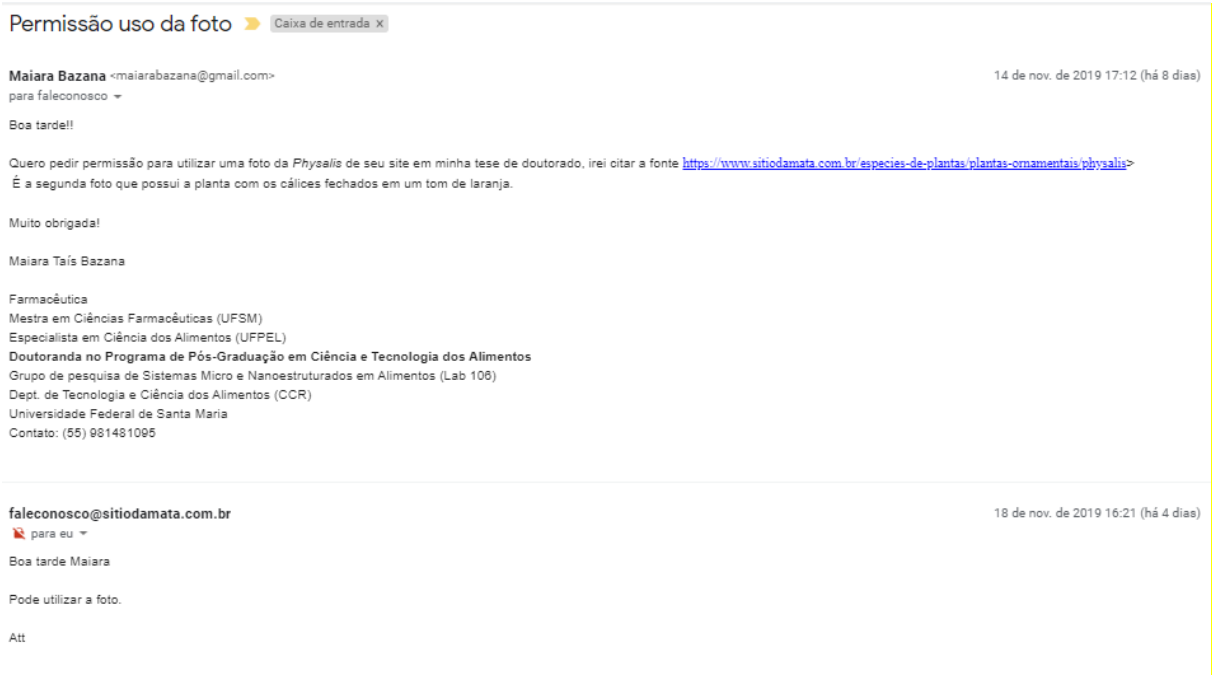
ZARIF, L. Nanocochleate cylinders for oral & parenteral delivery of drugs. **Journal of Liposome Research**, v. 13, n. 1, p. 109-110, 2003.

ZHANG, Y. et al. Oxidative stability of sunflower oil supplemented with carnosic acid compared with synthetic antioxidants during accelerated storage. **Food Chemistry**, v. 118, p. 656-662, 2010.

ZHAO, J. et al. Advanced development in analysis of phytochemicals from medicine and food dual purposes plants used in China. **Journal of Chromatography A,** v. 1218, n. 42, p. 7453-7475, 2011.

ANEXO A – AUTORIZAÇÕES DE USO DAS IMAGENS

Autorização da Figura 1 do referencial teórico



Permissão uso da foto Caixa de entrada ×

Maiara Bazana <maiarabazana@gmail.com> 14 de nov. de 2019 17:12 (há 8 dias)
para faleconosco

Boa tarde!!

Quero pedir permissão para utilizar uma foto da *Physalis* de seu site em minha tese de doutorado, irei citar a fonte https://www.sitiodamata.com.br/especies-de-plantas/plantas-ornamentais/physalis>
É a segunda foto que possui a planta com os cálices fechados em um tom de laranja.

Muito obrigada!

Maiara Taís Bazana

Farmacêutica
Mestra em Ciências Farmacêuticas (UFSM)
Especialista em Ciência dos Alimentos (UFPEL)
Doutoranda no Programa de Pós-Graduação em Ciência e Tecnologia dos Alimentos
Grupo de pesquisa de Sistemas Micro e Nanoestruturados em Alimentos (Lab 106)
Dept. de Tecnologia e Ciência dos Alimentos (CCR)
Universidade Federal de Santa Maria
Contato: (55) 981481095

faleconosco@sitiodamata.com.br 18 de nov. de 2019 16:21 (há 4 dias)
para eu

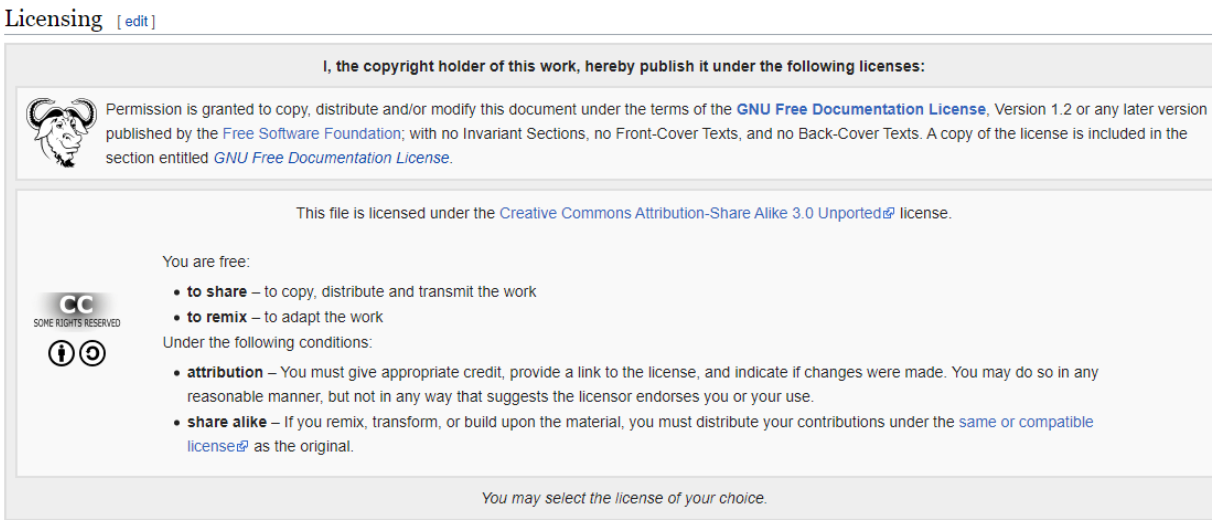
Boa tarde Maiara

Pode utilizar a foto.

Att

A Figura 1B do referencial teórico foi encontrada em imagens marcadas para reutilização, sem direitos autorais. Segue o link:

Figura 1B: https://commons.wikimedia.org/wiki/File:Physalis_Nahaufnahme.JPG



Licensing [edit]

I, the copyright holder of this work, hereby publish it under the following licenses:

Permission is granted to copy, distribute and/or modify this document under the terms of the **GNU Free Documentation License**, Version 1.2 or any later version published by the Free Software Foundation; with no Invariant Sections, no Front-Cover Texts, and no Back-Cover Texts. A copy of the license is included in the section entitled *GNU Free Documentation License*.

This file is licensed under the Creative Commons Attribution-Share Alike 3.0 Unported license.

You are free:
- **to share** – to copy, distribute and transmit the work
- **to remix** – to adapt the work

Under the following conditions:
- **attribution** – You must give appropriate credit, provide a link to the license, and indicate if changes were made. You may do so in any reasonable manner, but not in any way that suggests the licensor endorses you or your use.
- **share alike** – If you remix, transform, or build upon the material, you must distribute your contributions under the same or compatible license as the original.

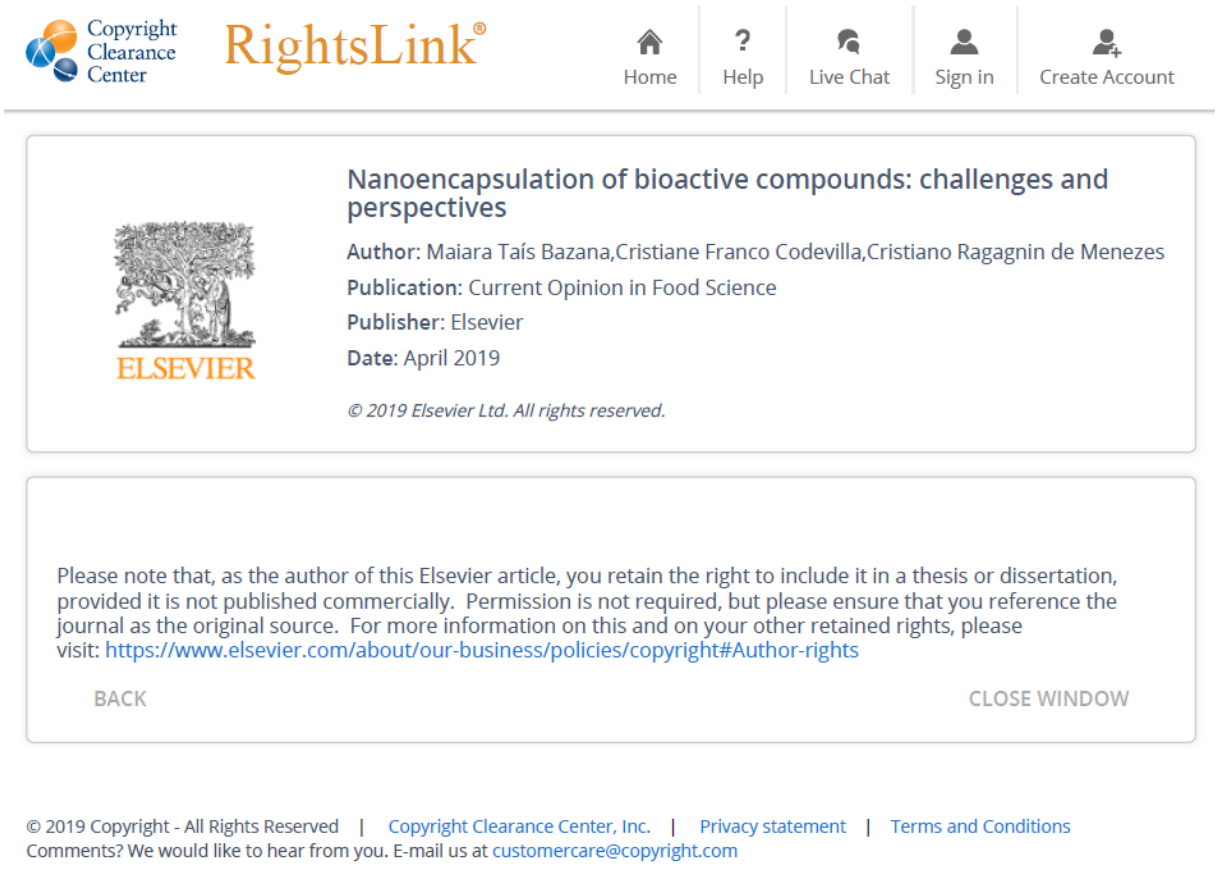
You may select the license of your choice.

As Figuras 2 e 3 do referencial teórico foram encontradas em imagens marcadas para reutilização, sem direitos autorais. Segue os links abaixo:

Figura 2: https://es.wikipedia.org/wiki/Archivo:Physalin_AB.png

Figura 3: https://de.wikipedia.org/wiki/Datei:Rutin_structure.svg

ANEXO B – AUTORIZAÇÃO DO ARTIGO 1



Copyright Clearance Center RightsLink®

Home | Help | Live Chat | Sign in | Create Account

ELSEVIER

Nanoencapsulation of bioactive compounds: challenges and perspectives

Author: Maiara Taís Bazana,Cristiane Franco Codevilla,Cristiano Ragagnin de Menezes

Publication: Current Opinion in Food Science

Publisher: Elsevier

Date: April 2019

© 2019 Elsevier Ltd. All rights reserved.

Please note that, as the author of this Elsevier article, you retain the right to include it in a thesis or dissertation, provided it is not published commercially. Permission is not required, but please ensure that you reference the journal as the original source. For more information on this and on your other retained rights, please visit: https://www.elsevier.com/about/our-business/policies/copyright#Author-rights

BACK CLOSE WINDOW

© 2019 Copyright - All Rights Reserved | Copyright Clearance Center, Inc. | Privacy statement | Terms and Conditions
Comments? We would like to hear from you. E-mail us at customercare@copyright.com

ANEXO C – AUTORIZAÇÃO DO ARTIGO 2