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PRODUCTION OF NANOPARTICLES BY ULTRASOUND-ASSISTED DOUBLE EMULSION CONTAINING ORANGE POMACE EXTRACTS OBTAINED FROM SEQUENTIAL EXTRACTION PROCESSES

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Dissertação submetida ao Programa de Pós-Graduação em Engenharia Química da Universidade Federal de Santa Catarina para a obtenção do título de Mestre em Engenharia Química.

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O presente trabalho em nível de mestrado foi avaliado e aprovado por banca examinadora composta pelos seguintes membros:

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To future me:

Being stuck isn't always a bad thing. Stillness can help see movement more clearly.

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RESUMO

Atualmente o Brasil é o maior produtor e exportador de suco de laranja do mundo e, consequentemente, a geração de bagaço da laranja (resíduo) é proporcionalmente alta (CITRUSBR, 2021). A obtenção de extratos a partir destes resíduos industriais é uma alternativa de crescente interesse para agregar valor aos resíduos gerados. Neste sentido, o sequenciamento de processos de extração é uma estratégia promissora, visto o maior fracionamento de bioativos a partir de uma mesma matriz. Uma vez que tais extratos possuem componentes que são sensíveis à degradação devido à oxidação, o encapsulamento é uma estratégia promissora para protegê-los de variações do ambiente, contribuindo para a estabilidade e durabilidade de sua atividade, mesmo no caso daqueles que possuem propriedades antioxidativas. Portanto, o objetivo deste trabalho foi obter nanopartículas impregnadas com extratos do bagaço da laranja obtidos por diferentes métodos de extração. Para tanto, foram realizadas extrações utilizando Soxhlet (SE) e métodos verdes: Extração supercrítica (SFE) e extração por líquido pressurizado (PLE) de forma sequencial com dois métodos SFE+SE e SFE+PLE. Após as extrações, foi realizada a encapsulação dos extratos obtidos por método de dupla emulsão, com auxílio de ultrassom, para obter partículas de tamanho nanométrico. A extração supercrítica com dióxido de carbono como primeira etapa extrativa apresentou rendimentos médios de 1,033%. As extrações sequenciais, que usaram Soxhlet e extração com líquido pressurizado como métodos, foram realizadas com etanol como solvente. Os resultados de rendimento para ambas extrações sequenciais foram de 31,13% para Soxhlet e 18,33% para extração com líquido pressurizado. Além disso, todos os extratos apresentaram atividade antioxidante, com destaque para os extratos sequenciais, com valores de EC_{50} de 1,78 $mg.mL^{-1}$ e 2,69 $mg.mL^{-1}$ para Soxhlet e PLE, respectivamente. Em geral, para a formulação da dupla emulsão são utilizados solventes orgânicos para seu preparo; porém, a água destilada foi utilizada como meio contínuo neste trabalho. As emulsões foram realizadas utilizando Pluronic F127 como surfactante hidrofílico e Lecitina e Manosileritritol lipídios-B como surfactantes lipofílicos. Todas as formulações resultaram em nanopartículas com tamanhos de 263 até 410 nm e emulsões monodispersas. O biossurfactante manosileritritol lipídios-B apresentou partículas menores, com tamanho de partículas entre 249 até 363,9 nm; porém com menor valor de potencial Zeta variando de -8,9 a -13,6 mV. Portanto, a aplicação de processos de extrações sequenciais foi considerada vantajosa visto que o rendimento de recuperação de extratos foi alto, resultando em extratos com boa atividade antioxidante e possibilitando o aumento do aproveitamento do resíduo industrial. O desenvolvimento de dupla emulsão com partículas de tamanho nanométrico mostrou-se viável com a incorporação dos extratos.

Palavras-chave: extração sequencial; bagaço da laranja; nanoencapsulação; dupla emulsão.

RESUMO EXPANDIDO

Introdução

O Brasil representa aproximadamente 50% da produção de suco de laranja do mundo (Neves et al., 2009). A produção de laranja brasileira no ciclo de 2020 representa 4,4% maior quando comparada ao ano de 2019 (Nogueira, 2020). A produção de suco de laranja produz resíduos que são frequentemente descartados em lixões. Transformar os resíduos industriais em diferentes produtos pode gerar benefícios financeiros para essas indústrias, provenientes da venda desses produtos e a diminuição dos resíduos gerados. Produtos secundários como óleos essenciais e outros extratos vegetais podem ser utilizados na indústria com diferentes funções. Por exemplo a vitamina C, encontrada em diversos extratos, pode ser utilizada como agente antienvelhecimento em cosméticos, ou ainda, o óleo de lavanda que pode ser utilizado devido à sua propriedade calmante em aromaterapia. Extratos vegetais podem ter propriedades antioxidantes e antimicrobianas, porém são sensíveis ao meio ao qual estão inseridos. Mudança de temperatura, incidência de luz, presença de oxigênio e cristalização são alguns dos fatores que colaboram na degradação desses extratos. Nesse contexto, a encapsulação de óleos essenciais e extratos vegetais pode ser usada para protegê-los do estresse do meio ao qual estão inseridos. Além da proteção dos extratos vegetais, a encapsulação também auxilia com outras dificuldades como a liberação do produto na localização correta ou ainda na extensão de tempo de liberação para aumentar o tempo de efeito do extrato.

Objetivos

O principal objetivo deste trabalho foi obter extratos a partir do bagaço da laranja que apresentassem atividade atividade antioxidante e encapsular esses extratos em nanopartículas usando métodos verdes. Para isso, os extratos de bagaço da laranja foram obtidos usando o sequenciamento de métodos, sendo extração supercrítica como primeiro etapa e Soxhlet e extração por líquido pressurizado como segundas etapas. A atividade antioxidante dos extratos foi avaliada pelos métodos de sequestro de radicais livres com DPPH e método de clareamento com β-caroteno. Ademais, para a produção das nanopartículas, o método de dupla emulsão sem uso de solvente orgânico assistida por ultrassom foi aplicado. Além disso, o efeito de dois surfactantes hidrofílicos (lecitina e manosileritritol lipídios-B) foi analisado.

Metodologia

As extrações e análises de atividades antioxidantes foram realizadas no Laboratório de Termodinâmica e Tecnologia Supercrítica/LATESC (Departamento de Engenharia Química e Engenharia de Alimentos, UFSC, SC/BR). A quantidade de água em amostras que foram usadas para extração deve ser reduzida (Pourmortazavi; Hajimirsadeghi, 2007). Para isso, após 8 horas de secagem a 50 °C, as amostras de bagaço da laranja apresentaram umidade de 3,78 g/ 100 g. Etanol PA e Hexano PA foram utilizados como solventes para as extrações realizadas em segunda etapa. A extração supercrítica (SFE) utilizou CO_2 como solvente supercrítico e ocorreu nas condições combinadas de 200 e 300 bar, com temperaturas de 40 e 50 °C. As extrações sequenciais, Soxhlet (SE) e líquido pressurizado (PLE), ocorreram após a extração supercrítica e utilizaram o resíduo da condição de extração selecionada de 200 bar/50 °C como material sólido. A extração com Soxhlet ocorreu seguindo a metodologia AOAC (2005) onde um invólucro contendo 5 g de material sólido foi colocado na câmara de extração e 150 mL de solvente foram adicionados ao balão de fundo redondo. O aparelho extrator foi colocado em manta de aquecimento para evaporação e refluxo do solvente durante 6 horas. A extração com líquido pressurizado utilizou 5 g de material sólido; sob vazão de 3 $mL.min^{-1}$, etanol PA foi usado como solvente, durante 30 minutos sob condições de 100 bar/60 °C. A análise antioxidante segundo o método de sequestro de radicais livres com uso de DPPH seguiu a metodologia de Mensor et.al. (2001) com adaptações de concentração e volume. O segundo

método para análise de atividade antioxidante foi o método de clareamento com β-caroteno seguindo a metodologia de Matthäus (2002). Formulações para produção das nanopartículas foram feitas no Laboratório de Controle de Processos e Polimerização/LCP (Departamento de Engenharia Química e Engenharia de Alimentos, UFSC, SC/BR). Lecitina, Pluronic F127 e Manosileritritol Lipídio B foram usados como surfactantes. Ácido esteárico puro foi usado como o lipídio para a formação das nanopartículas lipídicas sólidas. A primeira emulsão foi formada pela junção da fase aqueosa (W1) e fase orgânica (O) contendo água destilada, ácido esteárico, lecitina ou manosileritritol lipídio B e presença ou não de extrato SFE 200 bar/50 °C. W1 e O foram unidas e submetidas à agitação magnética e aquecimento em chapa magnética aquecedora a aproximadamente 70 °C durante cerca de 1 minuto ou menos e, então, submetidas à dispersão, com uso de sonda ultrassônica por 15 segundos. A segunda emulsão foi composta de W1/O e da segunda fase aquosa (W2), onde W2 foi feita com água destilada, Pluronic F127 e presença ou não de extratos SE ou PLE. Então, W1/O e W2 foram unidas e submetidas à dispersão com sonda ultrassônica por 1 minuto (em 4 rodadas de 15 segundos, sendo 10 segundos com a sonda e 5 s sem a sonda). Foram feitas análises de tamanho de partícula (Dp), polidispersividade (PDI) e potencial zeta (Z).

Resultados e Discussão

Considerando-se os extratos obtidos por extração supercrítica, todas as condições de extração apresentaram rendimentos significativamente similares aos resultados apresentados para as extrações feitas com hexano (2,57 %). Cruz et al. (2017) e Mazzutti et al. (2018) obtiveram, para semente de butiá e casca de semente de cacau, rendimentos similares quando diferentes condições de extração foram aplicadas. Apesar da baixa recuperação apresentada por SFE (com valores entre 0,93 % a 1,15 %), extratos com componentes apolares são obtidos, o que significa que bioativos diferentes são recuperados. Benelli et al. (2010) apresentaram rendimentos de extração supercrítica para bagaço de laranja similares aos obtidos neste trabalho, e a mudança de pressão causou uma leve melhora na recuperação de extratos. Porém, as extrações com líquido pressurizado e Soxhlet apresentaram desempenhos superiores para extratos sequenciais e não sequenciais. Os rendimentos de SE obtidos usando diferentes solventes mostraram que o solvente com polaridade maior apresentou maior recuperação tanto para extratos sequenciais (31,13 %) como não sequenciais (32,26 %). Resultados semelhantes ocorreram para o estudo de menta feito por Almeida et al. (2012). Os rendimentos obtidos para PLE foram significativamente similares (sequencial: 18,33 % e não sequencial: 15,74 %). Entre os extratos etanólicos, os sequenciais obtiveram maior eficiência de extração. Os maiores rendimentos obtidos para os extratos sequenciais podem ser atribuídos à etapa anterior onde foi aplicada maior pressão a qual pode ter causado ruptura das paredes celulares do material vegetal e, então o processo secundário pôde se beneficiar e obter maiores valores de rendimento. Comportamento similar foi observado por Dias et al. (2019) e Ferro et al. (2019) onde extratos sequenciais obtiveram maiores recuperações do que os não sequenciais. A variação entre os resultados de SE e PLE podem ser consequência das diferentes condições de extração percebidas entre os dois métodos, onde o primeiro ocorre à temperatura maior e o outro à pressão maior. O método de sequestro de radicais livres com DPPH é expresso em equivalente de concentração a 50% (EC_{50}), o que significa que quanto menor a concentração obtida, melhor a atividade antioxidante do composto. Entre os resultados obtidos para SFE, o melhor resultado foi apresentado para a condição 200 bar/50 °C com EC_{50} de 1,24 mg. mL^{-1}. Melhores resultados de EC_{50} foram apresentados tanto Soxhlet (1,39 mg. mL^{-1}) quanto para PLE (2,05 mg. mL^{-1}) para a matéria-prima inicial. Ainda que a boa atividade antioxidante seja apresentada pela matéria-prima inicial, o uso do resíduo de SFE ainda é vantajoso, pois leva a um melhor uso do material sólido. Mazzutti et al. (2018) apresentaram que extratos sequenciais eram melhores do que àqueles onde apenas SFE era aplicado. O método de clareamento com β-caroteno não

apresentou diferença significativa entre resultados obtidos para os extratos sequenciais, porém mostrou que há também atividade antioxidante demonstrada por esse método. Para a análise das nanopartículas lipídicas sólidas formadas, três parâmetros foram avaliados: tamanho de partícula (Dp), índice de polidispersividade (PDI) e potencial zeta (Z). As partículas obtidas apresentaram tamanhos variando de 263 a 410 nm. As partículas que continham em sua formulação manosileritritol lipídio B apresentaram partículas menores. As formulações que continham extratos tanto em W1 quanto em W2 apresentaram Dp menores tanto para lecitina quanto para MEL-B. Resultados similares foram apresentados por Peres et al. (2016) que elaboraram formulações similares usando lecitina. As formulações feitas neste trabalho obtiveram PDI variando entre 0,092 e 0,174, o que mostra que as formulações se apresentam como monodispersas. Das e Chaudhury (2011) declaram que valores menores do que 0,3 indicam monodispersividade. Mohanraj e Chen (2006) afirmam que valores maiores que 30 mV, em módulo, apresentam emulsões estáveis. O potencial zeta apresentou valores que confirmam uma baixa estabilidade das emulsões, com valores compreendidos entre 8,9 e 21,1 mV, em módulo. Especialmente para formulações contendo MEL-B os resultados apresentaram menor valor de potencial zeta e, apesar disto, MEL-B produz partículas menores.

Considerações Finais

Neste estudo, extratos foram obtidos a partir do bagaço da laranja utilizando-se de três diferentes métodos de extração: Soxhlet, extração supercrítica e extração com líquido pressurizado. A combinação dos métodos de extração foi feita com sucesso onde a extração supercrítica foi seguida por extração com líquido pressurizado (ou Soxhlet, para comparação). Os extratos obtidos com o bagaço da laranja foram utilizados em formulações para obtenção de nanopartículas lipídicas sólidas, sem uso de solvente orgânico. O método usado para nanoencapsulação foi o de dupla emulsão assistida por sonda ultrassônica para que extratos polar e apolar fossem incorporados na mesma partícula. As extrações apresentaram bons rendimentos, inclusive para os extratos sequenciais, e excelente atividade antioxidante. Portanto, a utilização de bagaço da laranja para recuperação de componentes é de grande valia e pode ser utilizada em pesquisas adicionais de antioxidantes naturalmente obtidos. As partículas obtidas apresentaram tamanhos dentro da escala nano, como consequência do uso da sonda ultrassônica, e emulsão monodispersa.

Palavras-chave: extração sequencial; bagaço da laranja; nanoencapsulação; dupla emulsão.

ABSTRACT

Currently, Brazil is the biggest producer and exporter of orange juice in the world, as a consequence, the production of orange pomace (residue) is proportionally high (CITRUSBR, 2021). The obtention of extracts from industrial residues is an ever-growing alternative of interest to aggregate value to generated residue. In this regard, the sequencing of extraction processes is a promising strategy, seeing the bigger fractioning of bioactive from the same matrix. Whereas these extracts have components that are sensitive to degradation due to oxidation, encapsulation is a promising alternative to protect them from environmental variability, contributing to their stability and durability, even in those with antioxidant properties. Therefore, the aim of this study was to obtain nanoparticles impregnated with orange pomace extracts obtained by different extraction methods. For that, extractions using Soxhlet (SE) and green methods: supercritical extraction (SFE) and pressurized liquid extraction (PLE) in sequential manner with two methods SFE+SE and SFE+PLE. Following the extractions, the encapsulation of the obtained extracts was done using the double emulsion method assisted by ultrasound to obtain nano sized particles. The supercritical extraction with carbon dioxide as the first extraction step presented an average global yield of 1.033%. The sequential extractions, that used Soxhlet and pressurized liquid extraction as methods, were performed using ethanol as solvent. Global yield results for both sequential extractions were 31.13% for Soxhlet and 18.33% for pressurized liquid extraction. Moreover, all extracts presented antioxidant activity, highlighting EC_{50} values of 1.78 mg. mL^{-1} and 2.69 mg. mL^{-1} for Soxhlet and PLE, respectively, obtained for sequential extracts. In general, for the preparation of double emulsion, organic solvents are generally used for this preparation; however, distilled water was used as continuous phase in this study. The emulsions were formulated using Pluronic F127 as the hydrophilic and lecithin and mannosylerythritol lipid-B as the lipophilic surfactants. All formulations presented nanoparticles with sizes from 263 to 410 nm and monodispersed emulsions. With particle sizes between 249 to 363.9 nm, the biosurfactant mannosylerythritol lipid-B presented smaller particles, but lower values of zeta potential varying between -8.9 to -13.6 mV. Hence, the application of sequential extraction processes was considered advantageous given that the yield for recuperation of extracts was high, resulting in extracts with good antioxidant activity and increasing the use of the industrial residue. The development of double emulsion with nano sized particles incorporating the obtained extracts proved viable.

Keywords: sequential extraction; orange pomace; nanoencapsulation; double emulsion.

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AA%	Percentual de atividade antioxidante
Abs	Absorbância
AM	Atividade antimicrobiana
AO	Atividade antioxidante
ASE	Extração de solvente acelerada
Dp	Tamanho de partícula
DPPH	1,1-diphenyl-2-picrylhydrazil
EC50	Concentração efetiva à 50%
GC	Cromatografia gasosa
HLB	Equilíbrio hidrófilo-lipófilo
HPLC	Cromatografia líquida de alta eficiência
LEC	Lecitina
MELs	Manosileritritol
MEL-B	Manosileritritol lipídio B
O	Fase orgânica
O/W/O	Óleo em água em óleo
P.A.	Para análise
PDI	Índice de polidispersividade
PGPR	Poliglicerol poliricilnoleato
PHSE	Extração de água quente pressurizada
PHBV	Poli (hidroxibutirato-co-hidroxi-valerato)
PLE	Extração com líquido pressurizado
PLGA	Poli (ácido lático-co-glicólico)
PLLA	Poli(ácido l-lático)
SE	Extração soxhlet
SFE	Extração supercrítica
SLNs	Nanopartículas lipídicas sólidas
SWE	Extração com água subcrítica
TPC	Fenólicos totais contidos
W	Watt (unidadede potência)
W1	Fase aquosa interna
W2	Fase aquosa externa

W/O/W	Água em óleo em água
WP	Whey protein
ZP	Potencial zeta

LISTA DE SÍMBOLOS

g	Gramas
$g.min^{-1}$	Gramas por minuto
L	Litro
h	Hora
$L.h^{-1}$	Litro por hora
min	Minuto
mg	Miligramas
$mg.mL^{-1}$	Miligramas por mililitros
mL	Mililitros
$mL.min^{-1}$	Mililitros poor miinuto
mM	Milimolar
Mm	Milímetros
mV	Milivolt
nm	Nanometro
s	Segundo
t	Tempo
t_{CER}	Tempo da taxa constante de extração
t_{FER}	Tempo da taxa de decaimento da extração
X_0	Rendimento global de extração
w_e	Massa de extrato em gramas
w_f	Massa final da amostra
w_i	Massa inicial da amostra
w_r	Massa da matéria prima inicial

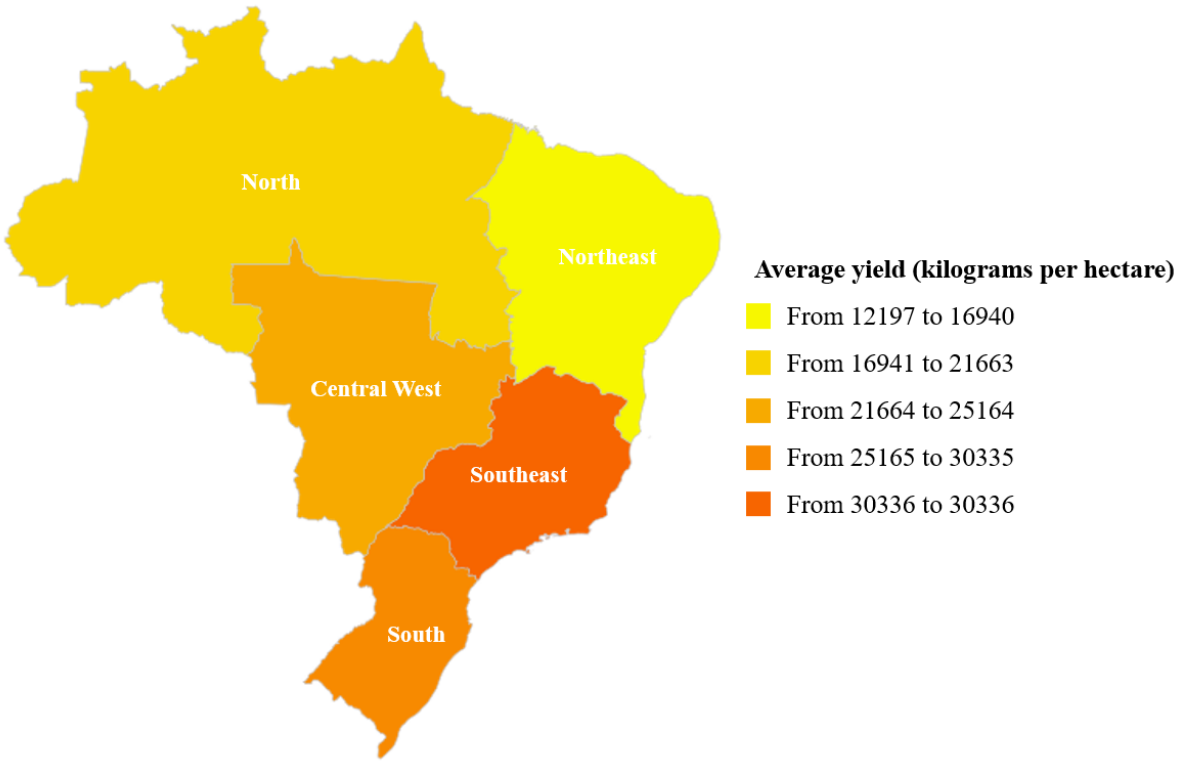
SUMÁRIO

CHAPTER I

1 INTRODUCTION

The Brazilian production of orange fruit has been increasing since 1960 (GRO INTELLIGENCE, 2015). According to the Brazilian Institute of Geography and Statistics (IBGE) and the United States Department of Agriculture (USDA), the 2020 cycle of Brazilian crop production will be 4.4% higher, when compared to 2019 (crop production of 17.6 tons) (NOGUEIRA, 2020). The orange fruit is composed of pectin, potassium, magnesium, beta-carotenes, vitamin C, among other nutrients. Ascorbic acid, or vitamin C, is well-known to assist the production of collagen, absorption of iron and improvement of immune system (BARRETO, 2018). Pectin can induce low cholesterol content (PECTIN, 2018); beta-carotene, a pigment related to the orange color, is a powerful antioxidant associated to the prevention of heart diseases (OLSEN, 2020).

Figure 1 Average yield of Orange Production in Brazil for April/2022.



Source: IBGE (2022).

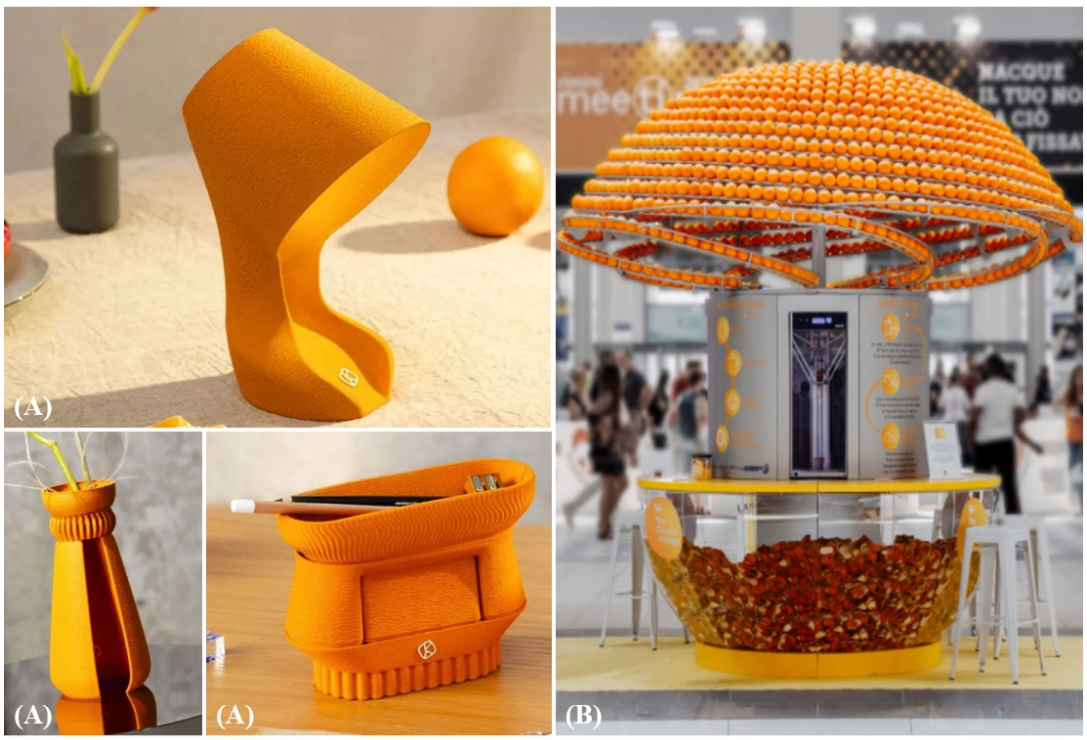
Since the orange production is substantial, and not all of it is consumed *in natura*, the processing of orange fruit into juice is a large industry. According to a report elaborated by

CitrusBR (National Association of Citrus Juice Exporters), Brazil represents approximately 50% out of the world orange juice production. In addition, Brazil exports 98% out of the total production (NEVES *et al*., 2009).

The production of orange juice generates residues that are often discarded in landfills. The use of residues from food industry, also called industrial waste, has increased due to researches that showed that such residues can be used to create other products, such as essential oils, d-limonene, terpenes and citric pulp flour from orange, lime, lemon, tangerine and grapefruit (NEVES *et al*., 2009).

Some practical examples that make use of residues are the Feel the Peel machine and the companies Green Spot Technologies and Krill Design. The squeezer "Feel the Peel" was designed to show the complete usage of the orange fruit. The installation produces orange juice and the peels enter the equipment to be processed and turned into cups that are 3D-printed promptly (CARLO RATTI ASSOCIATI, 2019). Moreover, Green Spot Technologies uses fermentation to transform by-products of orange, apple, beetroot, tomato and others to create nutritious flour rich in protein and dietary fiber while it is low on sugar (GREEN SPOT TECHNOLOGY, 2016). In addition, the Italian design company Krill Design creates home décor from orange peels (KRILL DESIGN, 2021).

Figure 2 (A) Krill Design products (B) Feel the Peel machine.



Source: (A) Krill Design (2021) (B) Carlo Ratti Associati (2019).

Thus, repurposing industrial residues into different products can be better for the industry due to the financial profit generated from their sales. Besides, a decrease on expenses to transport the residues to places where they can be discarded properly is an advantage.

Some of the products that can be produced from industrial residues are the essential oils and plant-based extracts. These products can have multiple functions, such as their usage in cosmetics to aid in formulations and functionality of products, or in aromatherapy where properties of different vegetable sources are used for different types of treatments. For instance, extracts with a high amount of vitamin C are used in antiaging cosmetics (LA ROCHE-POSAY, 2021). Even though essential oils can have multiple functions, such as antioxidant and antimicrobial, they are still sensible to the environment in which they are preserved. In other words, essential oils can be sensitive to the incidence of direct light, oxidation, crystallization, and others. Thereby, these products must be protected and properly preserved. A solution for their protection is to insert these essential oils in capsules that can protect them from light and oxidation due to a physical barrier between the oil and the surrounding environment.

In this context, the encapsulation of essential oils and plant extracts can be used to protect them from environmental stress. In addition to the protection of the essential oil, the encapsulation can also assist with other difficulties such as the release of the product in the correct location or even an increase of time release to extend their effect. However, many methods used make use of organic solvents as the continuous phase in the encapsulation process.

Overall, this work studied some alternatives applied to the areas of residue reuse, such as extraction and encapsulation. Thus, it provides a new perspective on the concept of green chemistry of orange juice industry chain, with application of combined extraction methods with low use of organic solvents. Secondarily, it describes the encapsulation of orange pomace extracts by double emulsion without the use of water instead of organic solvents in the formulation, which can increase the applicability of such products and their formation using greener methods.

1.1 OBJECTIVES

The main objective of this work is to evaluate the possibility of the use of green methods for the obtention of orange pomace extracts with antioxidant activity. The second main objective of this study is to encapsulate these extracts within nano sized particles, using green methods without the use of organic solvents.

To achieve this research aim, the specific objectives are described below:

- To obtain orange pomace extracts using supercritical fluid extraction, Soxhlet and pressurized liquid extraction;
- To obtain orange pomace extracts by combining extraction methods, with supercritical fluid extraction as first step; followed by Soxhlet or pressurized liquid extraction as second steps;
- To evaluate antioxidant activity of the obtained extracts by radical scavenging method using DPPH and β-carotene bleaching method;
- To produce nanoparticles by free-organic solvent double emulsion method assisted with ultrasound, encapsulating the obtained extracts;
- To evaluate the effect of two different hydrophilic surfactants in double emulsion nanoparticles.

1.2 RESEARCH STRUCTURE

This dissertation was divided into five chapters. In a simple manner of explanation, the theoretical part presents the necessary fundaments for the understanding and development of this research. The methodologies for the practical development along with results, discussions and conclusions were divided by topic. The themes are presented separately with its own chapter. In order to keep this dissertation more objective, all the writing and discussions were done in shorter sets of texts with a general explanation of the topic with advantages and disadvantages.

In Chapter I the research aim is presented, with contextualization and the reasons as to why the theme was chosen. Chapter II presents a literature review on the methods used to obtain extracts from orange bagasse and the method used to encapsulate these extracts. Chapter III describes the obtainment of extracts from orange bagasse with the use of three different extraction techniques and their integration, in addition to presenting the results for antioxidant analyses performed for these extracts. Chapter IV describes the adapted methodology of double emulsion applied to obtain nanoparticles without the use of toxic solvents; that is, with the use of distilled water as solvent. Chapter V presents the conclusion and suggestions for future researches.

CHAPTER II

2 LITERATURE REVIEW

In this chapter, a brief literature review presents the relevant subjects to this research. Three, highly known, methods of extraction are presented: Soxhlet, supercritical fluid and pressurized liquid extractions. Then, the double emulsion encapsulation method is detailed.

2.1 EXTRACTION METHODS OF BIOACTIVE COMPOUNDS

Multiple methods have been developed over the years. The facility of operation and maintenance of the equipment, non-necessity of specialized professionals and financial return, are among the reasons as to why some of them are used more often than others.

2.1.1 Soxhlet (SE)

The Soxhlet method is considered a standard method of extraction (LUQUE DE CASTRO; PRIEGO-CAPOTE, 2010). It can be used to validate the results obtained for global extraction yield, when compared to other methods (VIGANÓ *et al*., 2016), and the sequential approach.

The Soxhlet extractor was introduced in the late 19th century for the determination of milk fat (JENSEN, 2007). Since then, it has been used as a standard extraction method. This traditional method is important when evaluating new methods to verify if they are worth the time, cost or even if it can reproduce the same or better results.

Regarding the advantages of Soxhlet, the technical simplicity, the low operational cost and the simultaneous performance which can be maintained from multiple extractions. It is a consolidated extraction method. However, it is time, organic solvent and energy-consuming (LUQUE DE CASTRO; PRIEGO-CAPOTE, 2010; POURMORTAZAVI; HAJIMIRSADEGHI, 2007).

The Table 1 shows some examples of materials that were extracted using Soxhlet methodology.

Table 1 Examples of Soxhlet extraction applied to multiple solid materials and solvents.

Material	Solvent	Tests	Reference
Rosemary	Acetone, methanol	GC/HPLC	Bicchi et al. (2000)
Grape pomace	Ethanol, hexane, butanol, dichloromethane, ethylacetate	Yield, TPC[1], AO[2], Composition	De Campos et al. (2008)
Green coffee	Isopropanol and water	Yield, AO[2], Composition	Madhava Naidu et al. (2008)
Green coffee	Petroleum ether	Quantification of free diterpenes by HPLC	Tsukui et al. (2014)
Passion fruit seed	Hexane	Yield, AO[2], Tocopherol and tocotrienol composition	Barrales et al. (2015)
Chia seed cake	Hexane, ethylacetate, ethanol	Yield, TPC[1], AO[2], AM[3], Fatty acid composition	Guindani et al. (2016)
Juçara	Ethanol	TPC[1], AO[2], Monomeric anthocyanins content	Garcia-Mendoza et al. (2017)
Mangaba	Water, ethanol, hexane	Yield, TPC[1], AO[2]	Dias et al. (2018)
Pumpkin	Hexane	TPC[1], AO[2], Chromatography	Can-Cauich et al. (2019)
Piper diravicatum	n-hexane	Yield	De Oliveira et al. (2019)

[1]Total phenolics content, [2]Antioxidant activity, [3]Antimicrobial activity.

2.1.2 Supercritical fluid extraction

The supercritical fluid extraction (SFE) is a green modern extraction method that uses solvents at pressures and temperatures higher than the critical point of solvent. It is known to obtain select components depending on conditions and solvents used (KHAW *et al*., 2017).

The advantages of SFE include, no or low-organic solvent, selective extraction, preservation of components that are sensitive to high temperatures, among others. However, the high cost of the equipment, and specialist operators are significant disadvantages (KHAW *et al*., 2017).

Supercritical fluids present intermediate properties between liquid and gas, mainly the following interchangeable properties diffusion coefficient and viscosity. At supercritical conditions the diffusion coefficient is higher, which increases the efficiency of extraction, it also decreases the viscosity (compared to liquids), which enhances molecular diffusion that

accelerates extraction process (CAMEL, 2001). Supercritical fluids can be used for the extraction of bioactive compounds.

Carbon dioxide is the most common supercritical fluid used for this method due to its low critical point (approximately 73 atm and 31 °C), easy obtained, low cost, inert and not toxic (ASBAHANI *et al.*, 2015). Although, other gases also have interesting supercritical properties; such as nitrogen, methanol and water; however, due to high costs, high critical pressure and temperatures, risk of explosions and toxicity, for instance, they are rarely used. The Table 2 presents examples of SFE.

Table 2 Supercritical Fluid Extraction of bioactive compounds.

Material	Conditions	Assays	Reference
Rosemary	30, 40 °C 100,150, 200, 250, 300 bar	Yield, AO[2], Composition	Carvalho et al. (2005)
Spearmint	40,50,60°C 100,200,300 bar	Different extraction conditions on the recovery of flavonoid compounds	Bimakr et al. (2011)
Spent coffee grounds and coffee husks	40,50,60°C 100,200,300 bar	Yield, TPC[1], AO[2], Composition	Andrade et al. (2012)
Cordia verbenacea	30,40,50°C 100,150,200,300 bar	Yield, Cytotoxicity, Antitumor effects	Parisotto et al. (2012)
Grape pomace	50,60°C 150,200,250,300 bar	Yield, AM[3], Phenolic profile	Oliveira et al. (2013)
Red, green and brown Propolis	50°C 350 bar	TPC[1], AO[2], AM[3], Flavonoids, Arthepillin C, p-Coumaric	Machado et al. (2016)
Butia	40, 50, 60°C 100, 200, 300 bar	Yield, TPC[1], AO[2], AM[3], Composition	Cruz et al. (2017)
Quinoa	40, 50, 60°C 200, 300, 400 bar	Yield, AO[2], Composition, Tocophenol	Benito-Román et al. (2018)
Feijoa	40, 55°C 200, 300 bar	Yield, TPC[1], AO[2], AM[3], Composition	Santos et al. (2019)
Piper diravicatum	35,55°C 100,300,500 bar	Yield, AO[2], Composition, Inhibition of acetylcholinesterase	De Oliveira et al. (2019)

[1]Total phenolics content, [2]Antioxidant activity, [3]Antimicrobial activity.

2.1.3 Pressurized liquid extraction

At the Pittcon Conference, in 1995, a new method of extraction was introduced by Dionex Corporation that applied high pressure and high temperature (below critical point) -

Pressurized Liquid Extraction (PLE) (MUSTAFA; TURNER, 2011). This extraction method is related to the concept of green technology. PLE is also known as Accelerated Solvent Extraction (ASE), Pressurized Hot-Solvent Extraction (PHSE), and receives a specific name when the solvent used is water such as Subcritical Water Extraction (SWE) (CARABIAS-MARTÍNEZ *et al*., 2005; VAZQUEZ-ROIG; PICÓ, 2015). The Table 3 shows examples of PLE.

Table 3 Examples of Pressurized Liquid Extraction applied to multiple solid material sources.

Material	Solvent	Conditions	Tests	Reference
Blackberry residues	Water, acidified water, ethanol, mixture of ethanol and water	60, 80, 100°C 75 bar	Yield, TPC[1], AO[2], Monomeric anthocyanins	Machado et al. (2015)
Passion fruit rinds	ethanol	30, 45, 60°C 100 bar	Yield, TPC[1], AO[2], Phenolic composition	Viganó et al. (2016)
Juçara	Ethanol, water, mixture of ethanol + water	40, 60, 80°C 100 bar	TPC[1], AO[2], Anthocyanin's content	Garcia-Mendoza et al. (2017)
Buriti Shell	Mixture of ethanol + water	35, 50, 65, 71°C 100 bar	Yield, TPC[1], AO[2]	Rudke et al. (2019)
Feijoa	Ethanol, water, mixture of ethanol + water	40, 55, 80°C 100 bar	Yield, TPC[1], AO[2], AM[3], Composition	Santos et al. (2019)

[1]Total phenolics content, [2]Antioxidant activity, [3]Antimicrobial activity.

PLE presents advantages such as relative fast time of extraction, the reduced amount of solvent and the high recovery yield of extract. The high pressure increases the mass transfer rate by disrupting the solid matrix cell walls. Additionally, the high pressure combined with the high temperature can break intermolecular forces on the matrix that increases the efficiency of the process. In addition, the high temperature decreases the viscosity of the solvent, improving the diffusion rate (HERRERO *et al*., 2015; MUSTAFA; TURNER, 2011).

2.1.4 Sequential extraction

During the 90s, the use of green chemistry started to be developed. One of the principles states that there is a need to reduce the amount of waste and the impact that the chemical industry has introduced in the environment (LENARDÃO *et al*., 2003). In this context, the development of sequential extraction techniques contributes with this concept due to the reuse

of the same raw materials in order to exploit the most of it and also to avoid or reduce the use of non-green solvents. Table 4 presents some examples of sequential extraction being implemented to various source materials.

Table 4 Examples of Sequential Extraction applied to multiple solid material sources.

Material	Methods	Tests	Reference
Olives	SE + Microwave	Extraction efficiency improvement	Virot et al. (2007)
Passion fruit seed	SFE + Ultrasound	Yield, AO[2], Fatty acid composition, Tocopherol and Tocotrienol composition	Barrales et al. (2015)
Green propolis	SFE + PLE	Yield, TPC[1], AO[2], AM[3], Color, Composition	Monroy et al. (2017)
Artemisia	SFE + Maceration	Yield, TPC[1], AM[3], Flavonoids	Martinez-Correa et al. (2017)
Cocoa bean hulls	SFE + PLE	Yield, TPC[1], AO[2], Composition	Mazzutti et al. (2018)
Yellow passion fruit rinds	PLE + Ultrasound	TPC1, Flavonoid's profile, Pectin yield	De Souza et al. (2018)
Sida rhombifolia leaves	SFE + PLE	Yield, TPC[1], AO[2], Flavonoids, Composition	Ferro et al. (2019)

[1]Total phenolics content, [2]Antioxidant activity, [3]Antimicrobial activity.

The main advantage of having sequential extraction steps is that, even when the same extraction method is applied in a sequence, when the extraction conditions changes, the recovered material is also different. For instance, using two sequential PLEs, if the solvent or temperature changes from the first to the second extraction, the recovered extracts will have a different composition. That is, from the same sample, different components can be obtained. Therefore, the matrix was better explored, when compared to a single-step extraction (GARCIA-MENDOZA *et al*., 2017 and GIL-RAMIREZ *et al*., 2018).

2.2 ENCAPSULATION

This part of the literature review will approach the encapsulation process. More specifically the encapsulation of plant-based extracts and double emulsion method.

2.2.1 Encapsulation of essential oils

Essential oils are a volatile, natural, complex mixture of compounds characterized by a strong odor. They can present components that have antioxidant and/or antimicrobial activity. However, such volatile components are often sensitive to the variation of ambient condition such as elevated temperature, pH, moisture, presence of light and oxygen. In order to overcome the steadiness problems of essential oils, stabilization is important, by encapsulation process, for instance. Encapsulation protects sensitive oils against degradation and also can mask undesirable flavors and odors (MEZZOMO *et al.*, 2016; RODRÍGUEZ *et al*., 2016; TURASAN *et al*., 2015).

Encapsulation is a technique that preserves the core material against environmental factors due to the presence of a physical barrier between the core material and the surrounding mean. Encapsulation of bioactive compounds can improve the action of targeted compounds such as efficient approach to allow a controlled release of the core material, increase of the physical stability, protection from oxidation, decrease of volatility, enhancement of bioactivity, prevention of microbial contamination, reduction of toxicity, and enhancement of the solubility of hydrophobic compounds in an aqueous media that increases its range of application (ANDRADE *et al.,* 2017; DESAI *et al.*, 2019; RAVI KUMAR, 2000).

Another factor that may contribute to the improvement of encapsulation effects is the size of the particles. For instance, nanoparticles, due to their reduced size, can achieve different places than microparticles. Therefore, nanoparticles can be applied with a more specific function. Nanoparticles can promote a faster release, improve specificity and solubility, prolong the effect of the core material, improve intracellular penetration in biological tissues and skin retention and present higher degradability, because of its higher surface area. Nanoencapsulation of essential oils can also lower volatilization, which reduces instability and loss of material (FREIBERGER *et al*., 2015; RODRÍGUEZ *et al.,* 2016).

Some examples of encapsulation of essential oils using multiple wall materials and different methods are presented in Table 5.

Furthermore, the material used for the wall formation of the capsule can also be a contributing factor for the application of the capsules. For instance, some materials are not recommended when encapsulated material is applied to food or cosmetics because it can cause allergic reactions or become toxic with time. Depending on the function and the mean, the capsules will be inserted, which can change the choice of wall material. For instance, if the mean is aqueous, the material should be more hydrophilic to aid with the interaction and avoid phase separation. In addition to the wall properties, their behavior towards the mean in which it is inserted should be analyzed. The polarity, solubility and thickness can influence the choice

depending on how fast or slow the core material should be released (FERNANDES *et al*., 2014 and RODRÍGUEZ *et al*., 2016).

Table 5 Examples of different methods of encapsulation of essential oils.

Method	Essential oil	Wall Material	Reference
Spray drying	Green coffee	Maltodextrin, skim milk powder	Desai et al. (2019)
Freeze and Spray drying	Spent coffee grounds	Maltodextrin, Gum Arabic	Ballesteros et al. (2017)
Freeze drying	Rosemary	Maltodextrin, Whey Protein concentrate	Turasan et al. (2015)
Direct complexation	Tea tree	Amorphous beta-cyclodextrin	Shrestha et al. (2017)
Pressurized Antisolvent co-precipitation (PAS)	Propolis	Water soluble polyethylene glycol	Yang et al. (2014)
Supercritical Anti-Solvent (SAS)	Rosemary	Poloxamers (ethylene oxide and propylene oxide)	Visentin et al. (2012)
Ionic gelation	Beetroot	Sodium alginate	Ferreira (2018)
Emulsification/Ionic gelation	Peppermint and Green tea	Chitosan	Shetta et al. (2019)
Mini-emulsification/Solvent evaporation	Roasted coffee	Poly (l-lactic acid) (PLLA), poly (hydroxybutyrate-co-hydroxy valerate) (PHBV)	Freiberger et al. (2015)
Emulsification/Solvent Extraction	Pink pepper	Poly lactic acid	Andrade, K. S. et al. (2017)

Various materials are used as wall materials can be used separately or combined to improve their properties. Some of the most common examples are maltodextrin, gum arabic, poly (lactic-co-glycolic acid) (PLGA) and chitosan. Nevertheless, there are new materials being developed such as the one developed by Kaderides and Goula (2017) that utilized orange juice by-products to create a new wall material and applied this formulation to encapsulate pomegranate peel essential oil using spray drying method (KADERIDES; GOULA, 2019).

2.2.2 Double emulsion

Double emulsions are also known as emulsions of emulsions, layer by layer emulsions and multiple emulsions. The multiplicity of layers is used when the components within needs more protection from the surrounding environment or needs to be released in a longer period of time. Double emulsions can carry both hydrophilic and hydrophobic components depending on how it is layered (IQBAL *et al*., 2015). In can be an oil in water in oil (O/W/O) or the most

common one, water in oil in water (W/O/W), where the aqueous phase contains the hydrophilic component and the organic phase contains the hydrophobic component. The layers in double emulsions must communicate with each other so separation of phases does not occur. This stability is provided by surfactants that are able to link both phases within its layer.

This method is based on emulsification-solvent evaporation technique (DAS; CHAUDHURY, 2011; RODRÍGUEZ *et al.*, 2016). However, the solvent removal changes the name of the method. For emulsification-solvent evaporation, the particles are formed with the polymer precipitation during solvent evaporation. Although, there is a variation of the method called emulsion diffusion in which the emulsion gets diluted and then the polymer that will form the particles involves the droplets, and after that, the suspension goes through an evaporation process (KAKRAN; ANTIPINA, 2014).

Double emulsions differentiate from single emulsion due to the use of two different surfactants. One of them having high hydrophile-lipophile balance (HLB) value, hydrophilic surfactant, and the other having a low HLB, lipophilic surfactant. The lipophilic surfactant assists the stabilization of the aqueous dispersed phase of the emulsion, whereas the hydrophilic surfactant does the same for the organic dispersed phase whether they are the most inner or outer layer of the droplets.

Two destabilization phenomena can occur in an emulsion; they are called coalescence and Ostwald ripening and can cause the particles growth. Coalescence occurs due to the breakage of the droplet walls whereas Ostwald ripening occurs because of the transport of dispersed phase molecules from the smaller to the larger droplets through the continuous phase. Temperature rise, decreased viscosity and high shear of the continuous aqueous phase may favor droplet coalescence. Coalescence can cause phase separation, change in viscosity and decrease encapsulation efficiency. The rate of Ostwald ripening in emulsions depends on chemical structure of the nonpolar phase, the type and concentration of surfactant present, the movement of molecules across the interfacial phases and the presence of surfactant micelles in the continuous phase. Ostwald ripening can be influenced by solubilization, interfacial tension, and colloidal interactions (BAMBA *et al*., 2018; LEISTER; KARBSTEIN, 2020; SANTOS *et al*., 2017; WEISS *et al*., 1999).

Depending on the particle size, an emulsion can change its function or even its application. Also, depending on its particle size, the methods for the formation of particle change. For example, in cosmetics, there was an increase in the pursuit for emulsions that utilize the nano scale. For the achievement of this size, high energy processes are applied with the aid of different devices such as ultrasound, high pressure homogenizer and microfluidizer. These

devices work on the high shear produced by cavitation, collision and turbulence, which causes the droplets to form a more uniform dispersion with small dispersity of particle sizes (LAMBA *et al*., 2015). These high energy methods aid with a better control on particle size and their distribution. However, the smaller the particle, the more susceptible it is to instability that can lead to coalescence (gathering of particles). The emulsifier's properties to adsorb onto the created surfaces can cause overprocessing the emulsions and the high Brownian motion, increases the chances of collisions leading to coalescence at high microfluidization pressure (ALKANAWATI *et al*., 2018).

Time, ultrasonic frequency, surfactant concentration, oil concentration, pressure and temperature are some of the parameters that interfere in the particle sizes (MOHANRAJ; CHEN, 2006; RODRÍGUEZ *et al.*, 2016).

As for the application of double emulsions, this method can be used for the production of lipid nanoparticles loaded with hydrophilic drugs (DAS, 2011), it has also shown potential as drug delivery system for bioactive ingredients such as probiotics, vitamins, polyphenolic components and minerals (LEISTER; KARBSTEIN, 2020; MUDRIĆ *et al*., 2019). They can also be used in applications where controlled release is desired. These emulsions of emulsions also showed that they can improve the absorption of molecules that have low bioavailability, achieve a more accurate release depending on environment conditions such as pH and temperature at the right time. They can eliminate or at least minimize the unpleasant taste of bioactive compounds and provide products with healthier lipidic profile by reducing their lipid content (LAMBA *et al*., 2015; MUDRIĆ *et al*., 2019).

Some advantages of double emulsion are the additional protection against lipid oxidation and coalescence of particles, controlled release of active ingredients, particle size can be better controlled depending on preparation conditions (GONÇALVES *et al*.; VÉLEZ-ERAZO *et al*., 2018). On the other hand, the limited range of available lipophilic surfactants, the low thermodynamic stability, the high volumes of water that need to be eliminated, the leakage of components that are soluble in water, Ostwald ripening, and phase separation due to poor interface interaction are some of the disadvantages that make this a difficult method to work with (GONÇALVES *et al.*; LAMBA *et al*., 2015; MUDRIĆ *et al*., 2019). Some examples of studies with the method of double emulsion are shown in Table 6.

Table 6 Double emulsion.

Encapsulated substance	Surfactants/Wall materials	Study	Reference
Mango peel extract	Tween 20, Tween 80, lecithin, PGPR[1]	Encapsulation efficiency and stability	Velderrain-Rodríguez et al. (2019)
Blueberry pomace extract	Corn oil, PGPR[1], WP[2] isolate	Physical parameters for double emulsion preparation	Bamba et al. (2018)
-	Polyvinyl alcohol, Human serum albumin	Size, Zeta potential, stability, rheological behavior	Zambaux et al. (1998)
Trans-resveratrol and vitamin B12	PGPR[1], Miglyol, Sodium carboxymethyl cellulose	Encapsulation efficiency and yield, particle size and distribution	Matos et al. (2015)
Chia oil	WP[2] concentrate, Pectin	Kinetic stability, Zeta potential, mean droplet diameter, microstructure and rheological behavior	Vélez-Erazo et al. (2018)
Fragrance	PGPR[1], Tween 60, Tween 20, Tergitol 15-S-12		Stasse et al. (2020)
Bovine serum albumine	PLGA[3], poly (ε-caprolactone)	Size, polydispersity, percentage of encapsulation, release time	Agnihotri; Vavia (2003)
Aspartame	Sunflower oil, gelatin and Gum Arabic	Size, encapsulation yield, hygroscopy, solubility, morphology	Rocha-Selmi et al. (2013)

[1]PGPR = polyglycerol polyricinoleate, [2]WP = whey protein, [3]PLGA = poly (lactic-co-glycolic acid).

2.2.3 Nanoencapsulation

The main goal of encapsulation is to protect sensitive substances, isolating them from physical environmental stress. Nanoencapsulation is a technology that describes encapsulation on the nanometer scale using films, layers, or nano dispersions. Controlling particle size, controlling bioactive material release, fast drug release, reduce liquid volatility, mask unpleasant flavor, decrease of needed dosage to obtain the same biological efficiency and site-specific release of active agent such as pharmaceuticals are within the advantages of nanoencapsulation. Another advantage is the need for smaller amounts of emulsifier and increased stability which means less chance of flocculation and creaming. On the other hand, due to the small size of the particles, there is a greater risk of aggregation during transportation or even storage. However, the use of biodegradable copolymers can be used to aid decreasing coalescence (ASSIS *et al*., 2012; BAMBA *et al*., 2018; FREIRE, 2017; JAFARI, 2017 and

MOHANRAJ and CHEN, 2006). Table 7 presents some examples of nanoencapsulation to different applications.

Table 7 Examples of application of nanoencapsulation.

Nanoencapsulation of:	For:	Reference
Quercetin	Formation of biodegradable nanoparticles	Kumari et al. (2010)
Centella asiatica L. Urban	Enhancement of the skin-protective activities (cosmetics related)	Kwon et al. (2012)
Hydrophobic phytochemicals	Antioxidant and antimicrobial of Guabiroba fruit (*Campomanesia xanthocarpa* O. Berg)	Pereira et al. (2015)
Phenolic compounds	Protection against environmental stresses and low bioavailability	Faridi Esfanjani; Jafari (2016)
Passion fruit by-product extracts	Enhanced antimicrobial activity	Oliveira et al. (2017)
Thymus capitatus and *Origanum vulgare*	Food preservation	Granata et al. (2018)

2.2.4 Biosurfactants applied in emulsion formulation

Biosurfactants can be synthesized by various microorganisms such as bacteria, fungi and yeasts. Biosurfactants are amphipathic surface-active compounds that decrease interfacial and superficial tension and stabilize solutions by forming emulsions (SANTOS *et al.*, 2016). Due to microbial origin, low toxicity and biodegradability, these types of surfactants can be considered superior to synthetic surfactants and they have gained more and more attention (GUDIÑA *et al*., 2013).

Some of the general properties of biosurfactants are lower toxicity, antimicrobial activities, structural diversity, probiotic nature, higher biodegradability, higher selectivity and specific activity at extreme conditions: higher foaming ability. Due to its amphiphilic structure with polar and non-polar cores, biosurfactants are able to be used in different types of industry such as cosmetics, pharmacological and food industry. They are used as potential antimicrobial agents and to remove soil contaminants such as toxic pollutants (ARUTCHELVI *et al*., 2008; GUDIÑA *et al*., 2013).

Mannosylerythritol lipid (MELs) are biosurfactant produced by *Pseudozyma* sp. as a major component. Due to being a biosurfactant with environmental compatibility, structural diversity, versatile biochemical function and mild production conditions, they recently regained attention even though they have been known for over five decades (ARUTCHELVI *et al*., 2008).

Among MELs properties, one can cite easy biodegradability, environmental compatibility, non-toxicity, low critical micelle concentrations, antiaging (active agent), excellent surface and interfacial tension, which can be associated with nanoparticles to enhance cytotoxicity and solubility as well as be used as coating agent, lower surface tension of water. MELs have the ability to form thermodynamically stable particles, can be used for drug delivery systems and count with a self-assembling property which is a reversible and spontaneous molecular organization into an ordered structure (ARUTCHELVI *et al*., 2008; COELHO *et al*., 2020; KITAMOTO *et al*., 2002; WORAKITKANCHANAKUL *et al*., 2009).

Similar to modern extraction methods, the application of biosurfactant is related to the concept of green chemistry, including its production using alternative culture medium (ANDRADE *et al*., 2017).

2.3 STATE OF ART

Although orange bagasse is a massive residue, the most of data available in the literature are related to orange peel. However, several authors have studied other parts of orange pomace that is not only the peel. Table 8 presents examples of researches in the different fields that used orange pomace as main material.

As presented in the previous table, the use of orange pomace has been widely reported in the literature, for different areas. The study on drying isotherm of orange pomace showed that the increase of temperature decreases drying time and raises the drying rate. Researches on extraction of distinct bioactive were done with Supercritical, Soxhlet, Ultrasound, hydrodistillation and microwave methods using different solvents to evaluate antimicrobial, antibiofilm and antioxidant properties of the orange pomace. In addition, encapsulation with different methods (such as spray drying, double emulsion and co-precipitation), different wall materials, particle size and applications (such as a functional drink, reductant agent and cover) were done.

However, there are a lot to be explored about the use of orange pomace for obtaining active extracts. Extraction methods were already applied for orange pomace, but used separately, not as sequential process as in the present work, Also, the double emulsion method, used before with the aid of spray dry, is used along with ultrasound to produce nanoparticles in this research.

Table 8 Examples of researches from literature reporting the use of extracts obtained from different parts of orange pomace.

Orange part	Investigation	Reference
Peel	Encapsulation by co-precipitation	Beristain et al. (1996)
Peel	Operation conditions for Supercritical extraction	Mira, B; Blasco, M; Subirats (1996)
Not specified	Encapsulating in a spray dried double emulsion O/W/O	Edris; Bergnståhl (2001)
Peel	Encapsulation was applied to eliminate inhibition in fermentation of orange waste to ethanol	Pourbafrani et al. (2007)
Entire pomace	Determination of kinetics and isotherms for drying	Fiorentin et al. (2010)
Entire pomace	Extraction using Supercritical fluid, Soxhlet, ultrasound and hydrodistillation, with evaluation of antioxidant and antimicrobial activities	Benelli et al. (2010)
Not specified	Microencapsulation by complex coacervation	Jun-xia et al. (2011)
Entire pomace	Physical and chemical characterization of orange pomace flour	Clemente et al. (2012)
Peel and leaves	Phenolic contents and antioxidant activity of multiple varieties of orange	Lagha-Benamrouche; Madani (2013)
Peel	Extraction using ultrasonic and Soxhlet methods	Xhaxhiu et al. (2013)
Not specified	Effect of ionic strength, pH, freeze–thaw and light on the physicochemical stability of primary and secondary emulsions	Zhao et al. (2014)
Peel	Extraction of essential oil, pectin and polyphenols using microwave and ultrasound in a bio-refinery form	Boukroufa et al. (2015)
Peel	Spray drying the extracts, analyses of the moisture sorption isotherms	Edrisi Sormoli; Langrish (2016)
Peel	Encapsulated extract mixed with green tea to form a functional drink	Rasouli Ghahroudi et al. (2017)
Entire pomace	Pomace was used to obtain a high dietary fiber powder to be used as carrier material	Kaderides; Goula (2017)
Not specified	Encapsulating vitamin E, analyses of the influence of heating temperature	Raikos (2017)
Peel	Extraction using microwave assisted and hot water, Antimicrobial and antibiofilm analyses	Caputo et al. (2018)
Peel	Silver nanoparticles were produced using the orange peel oil as a reductant agent and cover	Veisi et al. (2019)

CHAPTER III

3 OBTAINMENT OF ANTIOXIDANT EXTRACTS FROM ORANGE POMACE USING SEQUENTIAL EXTRACTION METHODS

In this chapter the first part of this work is presented; the obtainment of orange pomace extracts using sequential extraction methods. The extracts obtained were evaluated according to the results of global yield and antioxidant activity. These extracts were selected regarding those parameters and used in the second phase of this project that is described in section Chapter IV.

3.1 MATERIAL AND METHODS

The following topics will present the materials and the methods utilized in the operation conditions for the process of orange bagasse extracts obtention, as well as the evaluation of antioxidant activity. The extraction experiments were conducted at the Laboratory of Thermodynamics and Supercritical Technology/LATESC (Chemical Engineering and Food Engineering Department, UFSC, SC/BR).

3.1.1 Obtention and pre-treatment of the raw material

After orange juice is prepared in the industry, the residue is called orange pomace. The pomace used for this project was kindly donated by the juice industry SUQ, located in São José, SC/Brazil, from Valentia cultivar. The raw material is part of a single lot of the plantation of origin and was collected in the period of one production day. After pickup, the raw material was stored in a freezer and portions of the material were separated for the homogenization.

Given that the raw material is composed by peel, seeds and fruit membranes that come in different sizes and consistency the homogenization step was necessary so the drying process was performed with higher efficiency and homogeneity. The raw material can be seen in Figure 3.

The homogenized samples of the orange bagasse presented initial humidity of approximately 69%, measured in the Humidity Analyzer Smart Turbo, at the laboratory PROFI, UFSC. The samples were then submitted to drying process in air circulation oven (DeLeo, Porto Alegre/RS, Brazil) for 8 hours at 50°C.

After drying, the samples were grounded in knife mill (De Leo, Porto Alegre/RS, Brasil). Then, to obtain a medium particle size from the milling process, the samples were sieved in vertical sieve (Bertel Indústria Metalúrgica Ltda., Caieiras/SP, Brasil) and a medium particle diameter of 0.256 mm was obtained. All mesh sizes were used for the extraction processes for better fill of the extraction column and simulation of a real industry process. Lastly, the samples were placed in polyethylene bags and stored in domestic freezer at -18°C until experiments started.

Figure 3 Raw material: (A) as received, (B) after size homogenization, (C) after drying.

Source: the author.

3.1.2 Determination of moisture content

The determination of moisture content and volatile substances for the orange bagasse was performed accordingly to the method 925.09 of AOAC (2005), in which the procedure is based upon the loss of moisture and volatile substances when the sample is submitted to the temperature of 105°C.

The procedure was conducted firstly by the preparation of the aluminum capsules that must be placed in oven without circulation at 105°C for 1 hour (DeLeo, Porto Alegre/RS, Brazil), then cooled in desiccator until it reaches room temperature and weighted in analytical scale (AY220, SHIMADZU do Brazil Ltda., São Paulo/SP, Brazil). Then, 5 g of raw material, weighted in the aluminum capsules in the analytical scale were taken to the oven at 105°C for 24 hours, cooled in the desiccator until room temperature and weighted again. The samples were heated again for 3 more hours, cooled and weighted to see if it any change would occur to their weight, or if it would continue constant. For the calculus of humidity and volatiles, Equation 1 was used, where w_i is the initial sample weight (g) and w_f is the final sample weight (g).

$$Moisture\ content\ (\%) = \left(\frac{w_i - w_f}{w_i}\right).100 \quad (1)$$

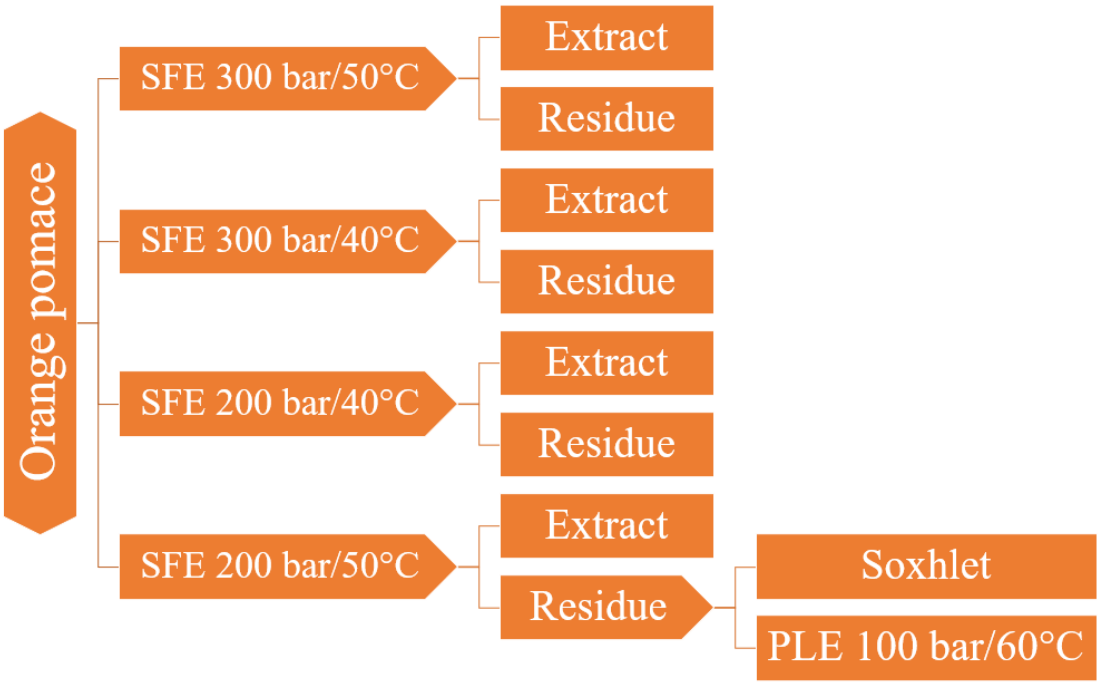
The determination of moisture content and volatiles substances of the orange bagasse was performed in triplicate and the results were expressed as mean value ± standard deviation.

3.1.3 Extraction with sequential extraction processes

In this work, the sequential extraction methods occurred in two manners: Integration of Supercritical fluid extraction with Pressurized liquid extraction and Integration of Supercritical fluid extraction with Soxhlet extraction. The second integration was performed to use Soxhlet as a standard method of comparison with results obtained from Pressurized liquid extraction.

For the integration process to happen, the residue from the SFE extractions had to be selected before the second step. Hence, the analysis of global yield and antioxidant activity of the extracts obtained after the first extraction were performed. Only the solid residue of the selected extract was used for the integration of the processes. The flowchart in Figure 4 shows the extraction steps executed. Thus, the second step was performed with the extraction residue of the previous extraction. The parameters to select the best extract were high global yield and antioxidant activity results.

Figure 4 Flowchart of the sequential extraction process.



Source: the author.

3.1.3.1 Supercritical extraction

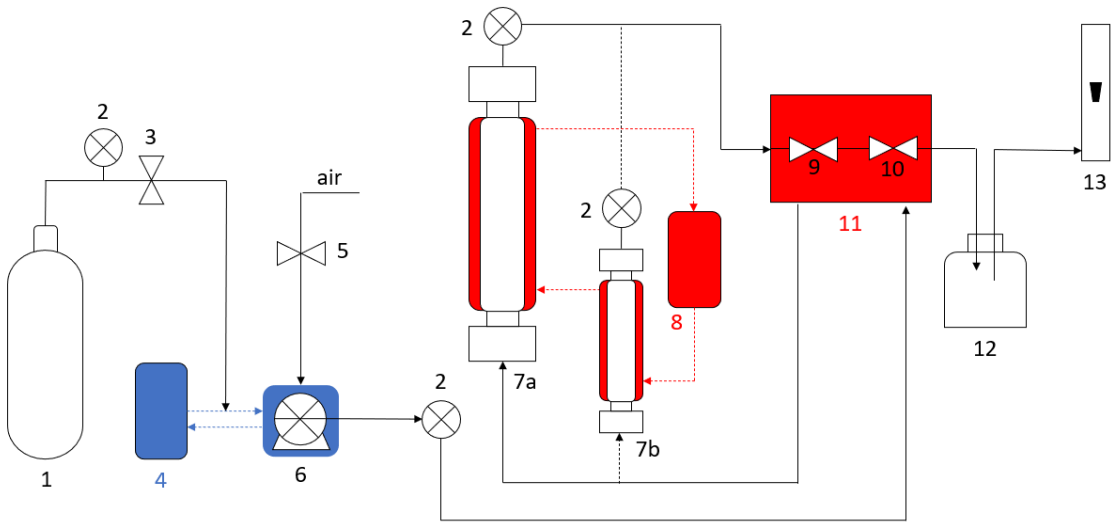
The supercritical extraction (SFE) experiments were performed using an extraction unit that can operate up to a maximum pressure of 300 bar and 111 to 1000 $L.h^{-1}$ of solvent flow interval.

The supercritical extraction unit was employed to obtain extracts of orange bagasse, with the use of pure carbon dioxide. Kinetic tests were performed in order to stablish extraction conditions such as raw material weight for bed formation and extraction time.

The extraction unit used for this part consists in an apparatus that contains two extraction jacketed vessels, one of 138 mL (internal diameter of 20 mm and height of 440 mm) and another of 622 mL (internal diameter of 45 mm and height of 391.1 mm) made of AISI316 stainless steel. A schematic diagram of the extraction unit presenting details of side equipment can be seen in Figure 5.

Two methods were used for this extraction. The static method is characterized by keeping the supercritical solvent in contact with the solid matrix, during a pre-determined period of time, for greater saturation of the solid matrix. For this reason, the supercritical solvent is able to penetrate deeper into the solid matrix and supports the increase on global extraction yield (PESSOA *et al*., 2015).The dynamic method of supercritical extraction was employed in the kinetics experiments for the determination of global yield with the aid of the static method. The dynamic method is characterized by the continuous passage of the solvent through the solid matrix (FERREIRA *et al*., 1999).

Figure 5 Scheme of the unit extraction SFE device.



Notes: 1: CO_2 cylinder; 2: Manometer; 3: CO_2 pressure regulator/reducer; 4: Cooling bath; 5: Compressed air regulator; 6: CO_2 pump; 7a: Jacketed extraction vessel; 7b: Jacketed extraction vessel; 8: Heating; 9: Regulator needle valve; 10: Block needle valve; 11: Heating bath for valves; 12: SFE glass flask; 13: Rotameter. Source: the author.

The preliminary test made with the use of Soxhlet extractor and hexane as solvent presented a global yield of 2.38 %. Given that the aim of the SFE is not only the evaluation of global yield of the extracts but also to obtain a bigger quantity of mass for the sequential stages of this work the vessel of 622 mL was chosen to perform all supercritical extractions. Therefore, for each extraction performed in triplicate, approximately 230 g of raw material was added to the extraction column along with cotton and glass beads to make the fixed bed compacted, so it could not create preferential paths for the passage of the supercritical solvent.

The influence of pressure and temperature on the global extraction yield verification was performed. The variables analyzed were pressure, in two levels of 200 and 300 bar, and temperature, also in two levels of 40 and 50 °C. The levels were applied accordingly to Table 9. The solid residues of all extraction conditions were collected and stored in freezer. However, the residue of only one condition was used as source material for the next extraction step.

Table 9 Assay plan for the supercritical extraction process

Variables	Levels	
T (°C)	40	50
P (bar)	200	200
	300	300

3.1.3.1.1 SFE kinetic experiments – extraction curves and kinetic parameters

The kinetic experiments for the obtention of extraction curves were performed using approximately 230 g of raw material. The material was weighted in analytical scale (BG2000, GEHAKA, São Paulo/SP, Brazil), with decigram of precision. The weight of the raw material was selected to obtain a compacted bed in which the height is equal to twice the size of the diameter of the extraction cell, so that in this way the axial dispersion can be contemned. The flasks used for the kinetic experiment were weighted in analytical scale, with a tenth of milligram precision. Time gaps were determined previously for practical collection of extracts. After collection, the flasks with extracts were weighted again, so extract weight could be determined with the passing of time. Extraction curves were built by crossing data of accumulated extract weight and time.

The kinetics experiments were performed with the lowest extraction condition and used pure CO_2 with solvent flow of 17 ± 2 g.min^{-1}, pressure of 200 bar and temperature of 40°C. The experimental values of the extract weight versus extraction time obtained for this extraction curve is presented in Table A, Appendix I.

The kinetic curve was built by collecting extracts from pre-determined time gaps. Each time extraction interval was obtained from the extraction curves data. The time gaps t_{CER} and t_{FER} represent the end of constant rate and decreasing rate of extraction, respectively shown in Figure 7.

3.1.3.2 Soxhlet extraction (SE)

Soxhlet extraction was performed according to the method 920.39C of AOAC (2005) using solvents Ethanol P.A. and Hexane P.A. The methodology used 5 g of solid material wrapped in filter paper and inserted in the extraction chamber. Then, 150 mL of solvent was added to a round flask and placed in a heating device so the solvent could reach boiling point and start the extraction with solvent reflux. The extraction process lasted 6 hours.

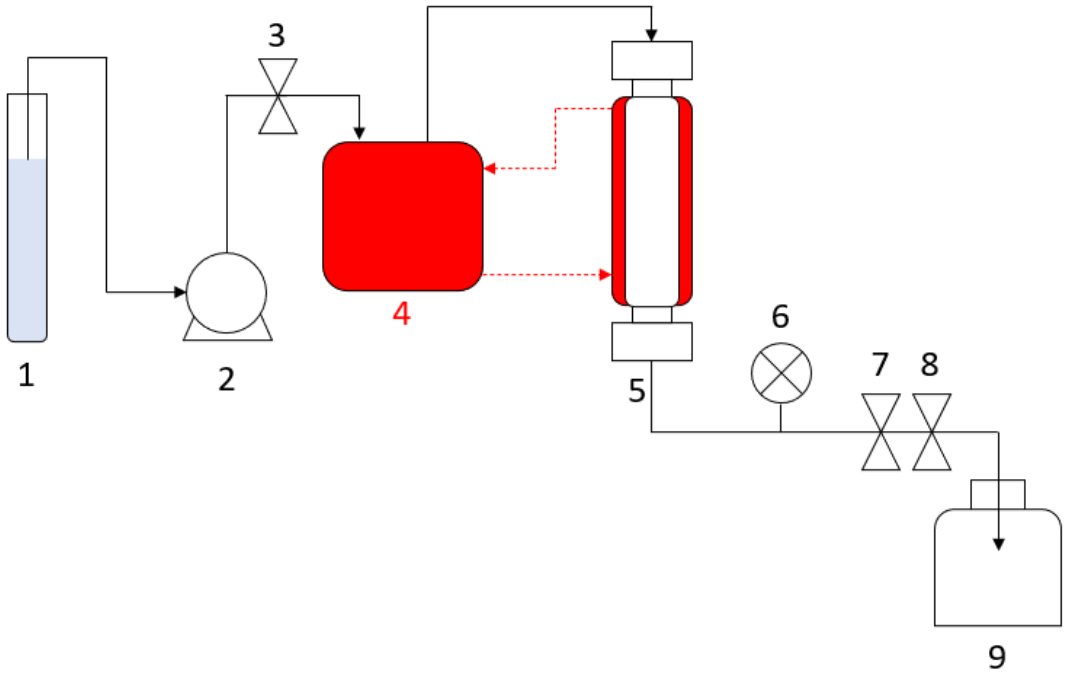
For previous determination of what to expect from Supercritical fluid extraction a preliminary test with raw material and hexane P.A. was performed. The extraction with ethanol and hexane was performed to compare results with the sequential extracts. For the sequential extractions, the solid material used was the residue from the supercritical fluid extractions with ethanol and hexane as solvents; due to the lack of references, these extractions were necessary for comparison of results.

The extraction was performed in triplicate and the extracts obtained after solvent evaporation were placed in dark flasks and stored in domestic freezer, at -18 °C. Extraction yield was calculated in the same way described previously with Equation 2.

3.1.3.3 Pressurized liquid extraction (PLE)

The PLE experiments were performed in a customized equipment, as presented by Mazzutti et al. (2018);the apparatus diagram is shown in Figure 6.

Figure 6 Scheme of the customized PLE unit extraction.



Notes: 1: Solvent reservoir; 2: Pump; 3: Block needle valve; 4: Heating bath; 5: Jacketed extraction vessel; 6: Manometer; 7,8: Regulator needle valve thick and fine adjustment; 9: PLE collector flask. Source: the author.

The extraction was performed by adding approximately 5 g of sample, raw material or residue from the previous extraction step, into the extraction cell. The volume of the extraction cell was completed with glass beads and cotton layers, to have a compacted bed without leaving space to create preferential paths for the solvent. The extractions were performed at 60 °C and 100 bar, in duplicate. The experimental procedure was based in the instructions given by Santos *et al*. (2019). Hexane P.A. and Ethanol P.A. were used as solvents. The extracts obtained were submitted to evaporation of the solvents and were stored in amber flasks in freezer until analyses resumed.

3.1.3.3.1 PLE kinetic experiments – extraction curves and kinetic parameters

The kinetic experiments for the obtention of the extraction curves were performed using approximately 5 g of residue material collected from the previous SFE step. The material was weighted in analytical scale, with a tenth of milligram precision. The weight of the raw material was selected to define a compacted bed. The flasks used for the kinetic experiment were weighted in the same analytical scale. Time gaps were determined previously for practical collection of extracts, considering the volume of solvent used for each time gap. After evaporation of the solvent, the flasks with extracts were weighted in analytical scale. Extraction curves were obtained as a function of accumulated extract weight by time, and are presented in Figure 10.

The kinetics experiments were performed using solvent flow of 3 ± 0.2 $mL.min^{-1}$, pressure of 100 bar and temperature of 60 °C (conditions based on the work of Barrales *et al*. (2018) and Rudke *et al.* (2019)) and ethanol as solvent. The experimental values of the extract weight versus extraction time obtained for this extraction curve is presented in Table B, Appendix I.

Each time extraction interval was calculated from the extraction curves data. The time gaps t_{CER} and t_{FER} represent the end of constant rate and decreasing rate of extraction, respectively.

3.1.4 Determination of global extraction yield (X_0)

The global extraction yield of oil (X_0) is the quantity of extracted oil present in the solid matrix (orange pomace) in a determined extraction pressure and temperature. The experiments, in this research, were performed for each sample in duplicate, with bed height and raw material weight of approximately 300 mm and 230 g, respectively. The top and bottom end of the extraction cell were coated with cotton to avoid solid particles breaking into the extraction line. The rest of the extraction cell was filled with glass beads, in the entrance of the extraction cell, before the raw material.

The global extraction yield was calculated through the ratio between extract weight (w_e) obtained and raw material weight (w_r) used to form the fixed bed, as shown in Equation 2. This same equation was used for the calculus of global extraction yields of the sequential extraction steps.

$$X_0(\%) = \frac{w_e}{w_r} \quad (2)$$

The flasks containing the extracts were weighted in analytical scale and stored in domestic freezer at -18 °C.

3.1.5 Antioxidant activity

3.1.5.1 Free radical scavenging activity – DPPH

The free radical scavenging of the orange bagasse extracts was evaluated using the 1,1-diphenyl-2-picrylhydrazil (DPPH) method, as described by Mensor *et al.* (2001) and adapted here to use a smaller amount of reagents, solvent and extracts. The determination of antioxidant activity trough the DPPH method is about the reaction of an ethanolic solution of DPPH in a 0.3 mM of concentration with increasing concentrations of the orange bagasse extracts during 30 minutes, in room temperature and in the dark. After reaction time is complete, the absorbances are read in spectrophotometer (FEMTO, 800 XI, São Paulo, SP) at 517 nm.

The higher the antioxidant activity of the sample, the more stable the DPPH radical becomes, causing discoloration of the solution containing the reagent and the extract therefore reducing their absorbance. Thus, the percentage of sample inhibition of the tested samples can be calculated converting the absorbance in percentual of antioxidant activity (AA%), accordingly to Equation 4: Where Abs_{sample} is the absorbance value of the sample, Abs_{blank} is the is the absorbance value of the blank sample and $Abs_{control}$ is the absorbance value of the control sample.

$$AA(\%) = 100 - \left[\frac{(Abs_{sample} - Abs_{blank})}{Abs_{control}}\right] \quad (4)$$

For the experiments performed for the orange bagasse, before the definitive concentrations in which all extracts were evaluated, a concentration test was executed. Using previously determined concentrations (0.625; 1.25; 2.5; 5 and 10 mg. mL^{-1}) for the extracts, it was possible to achieve the real concentrations used to build the curves with concentrations of 0.75; 1; 1.5; 2; 3 mg. mL^{-1} and find the EC_{50} values. The absorbance values were measured at 517 nm in spectrophotometer after 30 min at room temperature and converted into percentage of antioxidant activity (AA%).

This activity was also presented as the effective concentration at 50% (EC_{50}). EC_{50} means the concentration of the solution required to give 50% decrease in the absorbance of the sample solution, compared to a blank, and expressed in mg. mL^{-1} (CRUZ *et al*., 2017). The EC_{50} values were calculated from the linear regression of the AA% curves obtained for all extract concentrations. The AA% and EC_{50} for all extracts were obtained considering the average of triplicate assays.

3.1.5.2 Discoloration method of β-carotene/linoleic acid

The determination of antioxidant activity using the discoloration method of β-carotene/linoleic acid is based on spectrophotometric measurements of the coloration loss (oxidation) of β-carotene induced by the oxidative degradation of linoleic acid. The methodology used for this work was described by Matthäus (2002). For this method, the system formed by β-carotene and linoleic acid is induced to fast discoloration when in the absence of an antioxidant compound. The discoloration rate of the β-carotene solution is determined by the difference in the initial (time = 0 minutes) and final (time = 120 minutes) absorbance at 470 nm.

The methodology consists in the preparation of two base emulsions, one with the β-carotene and another without, called blank solution. The extracts are also prepared with dilution using 5 mg of extract, diluted in 3 mL of solvent. In this case, the solvent used was ethanol. After emulsions and extract solutions were added together, the initial absorbance was measured in spectrophotometer at 470 nm. Then, in water bath at 40°C for 120 minutes, the samples were placed and the absorbance was measured again in the same wavelength. The results of antioxidant activity (AA%) were calculated with Equation 5 and presented as average ± standard deviation. Where $Abs_{t:120}$ is the absorbance value of the sample at 120 minutes, $Abs_{t:0}$ is the absorbance value of the sample at initial time, $Abs_{control:120}$ is the absorbance value of the control sample at 120 minutes and $Abs_{control:0}$ is the absorbance value of the control sample at initial time.

$$AA\% = 1 - \left\{\left[\frac{(Abs_{t:120} - Abs_{t:0})}{(Abs_{control:120} - Abs_{control:0})}\right].100\right\} \quad (5)$$

3.2 RESULTS AND DISCUSSION

3.2.1 Moisture content

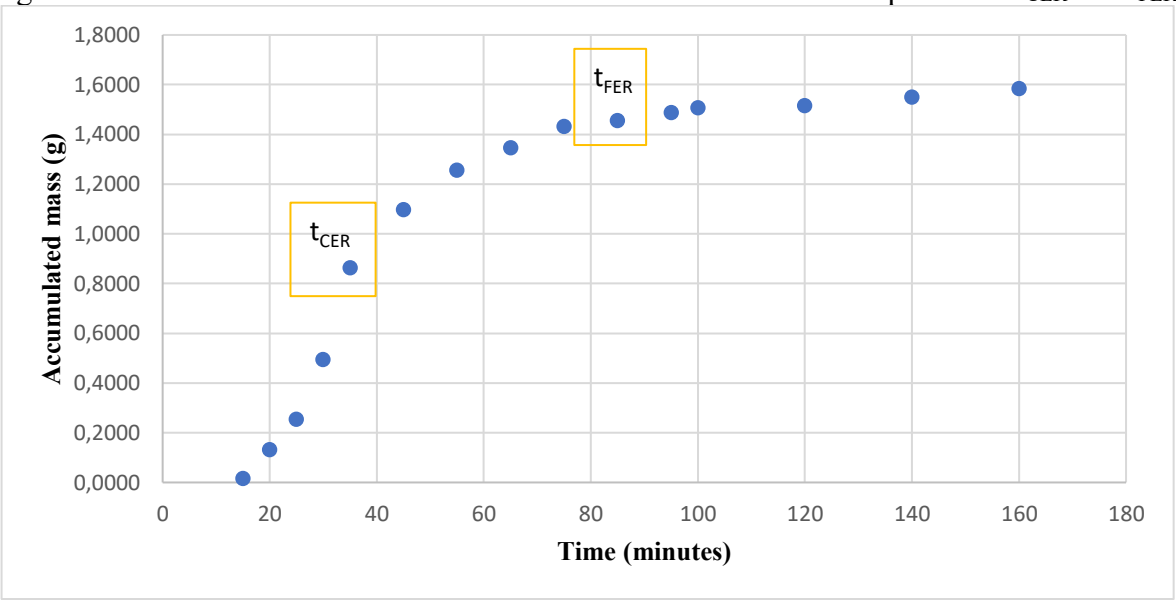
The moisture content determined for the orange pomace is 3.78 ± 0.19 g/100 g, after drying process. According to Pourmortazavi; Hajimirsadeghi (2007), the water present in the samples can affect the extraction yields, which means that, by reducing the amount of water in the samples, the higher the yields values will be. Thus, to avoid undesired interactions between the solid matrix, the solvent used for the extractions and the water; the water content must be reduced. The samples went through a drying process described previously to achieve this reduction.

3.2.2 Preliminary tests

3.2.2.1 SFE kinetic experiments

Preliminary test for determination of the extraction parameters was performed. The test, presented in Figure 7, was done to determine the extraction time in which the orange bagasse would stay in the SFE cell. It was performed at the lowest extraction condition determined before with 200 bar and 40 °C. It is necessary to have a predetermined period of time for assays that evaluate the yield. This period was found based on the extraction curves obtained with the kinetic tests.

Figure 7 Kinetics of SFE for determination of extraction time with emphasis on t_{CER} and t_{FER}.



Source: the author.

The kinetic graphic is divided in three steps. The first stage goes from time zero up to t_{CER}, at 35 minutes, named constant extraction rate (CER) where the particles are completely involved with a layer of solute. The process is characterized by mass transfer with convection between the surface of the solid matrix and the solvent. In this case, according to the yield ratio (ratio between the partial and complete extraction yields), approximately 55 % of extract has been recovered up to t_{CER}. The second stage occurs between t_{CER} and t_{FER}, with the decrease of the extraction rate due to the decrease of the solute in the surface of the particles and so it begins the mass transfer through diffusion. The final stage occurs after t_{FER}, here at 85 minutes of extraction, with falling extraction rate (FER), and it is characterized by the almost nonexistent extraction rate, in which the curve decreases significantly and presents the approximate value of extractable content, in this case up to 92 % of the extract had been obtained at t_{FER} (FERREIRA *et al*., 1999). After t_{FER}, 35 more minutes of extended extraction were applied to increase the percentage of recovery.

After evaluation of the curve of the SFE kinetic, the evaluation of the extraction yield ratio and to make sure that the extract was obtained at its maximum amount, the extraction time for SFE process was set to 120 minutes for all extraction conditions.

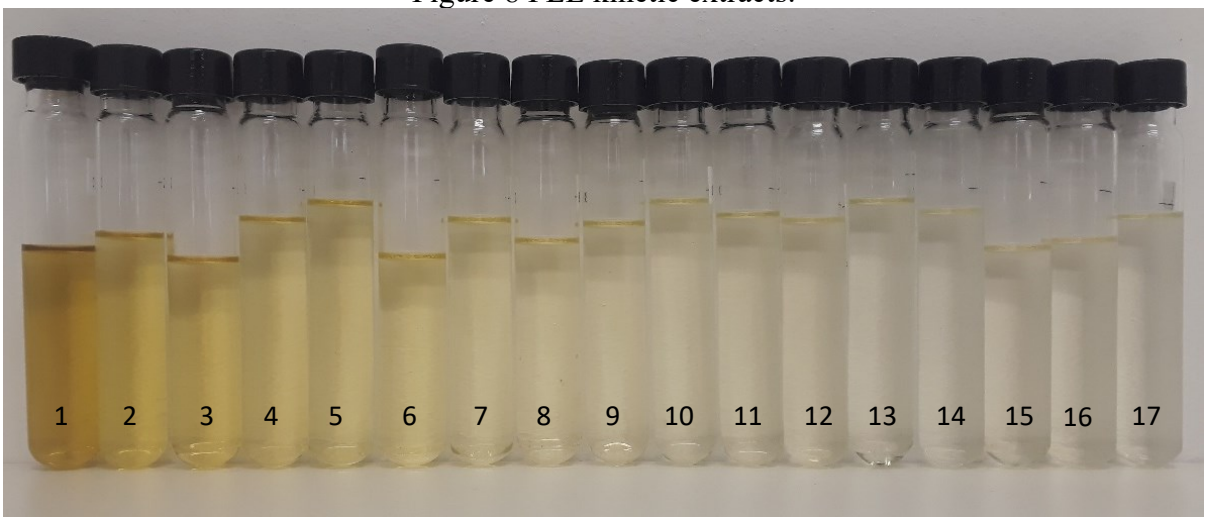
3.2.2.2 PLE kinetic experiments

The behavior presented by PLE extraction was different from the extracts obtained with SFE. For SFE, there is a clearer line of separation according to the data gathered. However, for

PLE, not only is the data in accumulated mass and the yield ratio are evaluated but also the coloration of the extracts throughout a certain period of time. In Figure 8, the discoloration of the extracts can be seen. As seen in the picture, flask number 5 represents when t_{CER} is achieved and flask number 8 represents t_{FER}

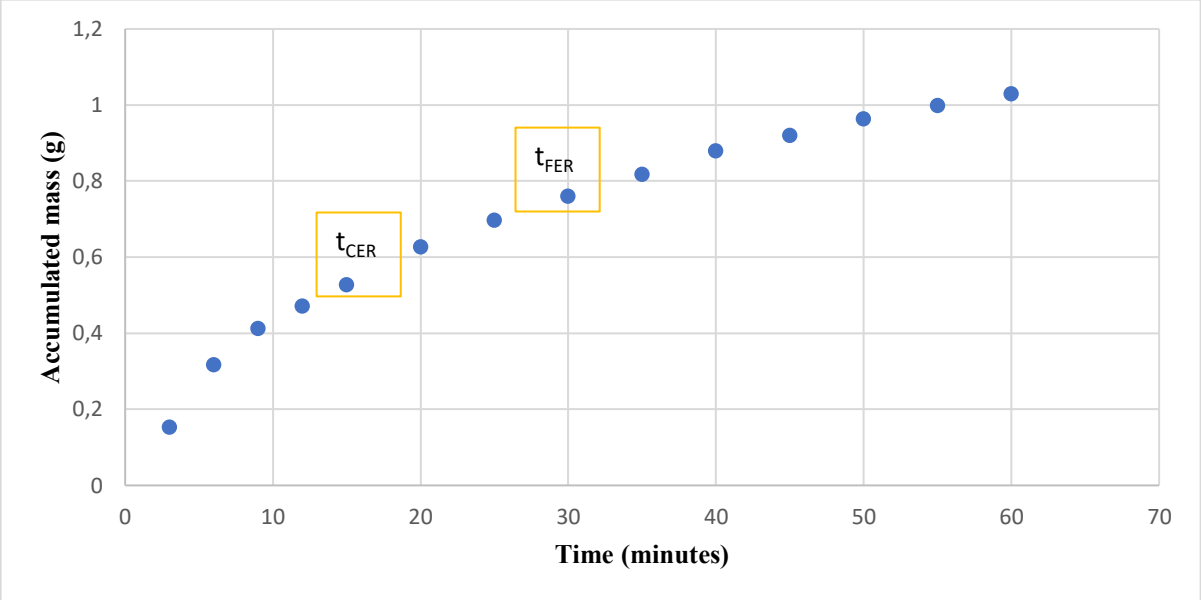
The PLE kinetic and values for t_{CER} and t_{FER} are shown in Figure 9. It can be observed that t_{CER} is achieved within 15 minutes and t_{FER} is achieved at 30 minutes. Both times were obtained with evaluation of yield ratio and coloration where t_{CER} was achieved when approximately 50 % of extract had already been obtained and t_{FER} was set when the extract has been obtained was just about 74 %. After evaluation of the curve, the coloration and the yield ratio, the extraction time for PLE process was set to 30 minutes. The time of extraction for the following process was set at t_{FER} due to the amount of extract recovered and also due to the amount of solvent that had already been used. The amount of extra solvent used to achieve a higher recovery rate was not advantageous.

Figure 8 PLE kinetic extracts.



Source: the author.

Figure 9 Kinetics of PLE for determination of extraction time with emphasis on t_{CER} and t_{FER}.



Source: the author.

3.2.3 Global extraction yield (X_0)

Results obtained for global extraction yields by the different extraction methods are presented in Table 10 along with extraction conditions. Preliminary tests performed using Soxhlet-hexane were done to obtain comparison to the SFE yields. It has been observed that the yield obtained with SFE is often lower than the yield obtained with Soxhlet-hexane. Accordingly, as Soxhlet-hexane presented extraction yields close to 2%, results for SFE showed that orange pomace would obtain yields at maximum of 2%.

Considering the extracts obtained for Supercritical fluid extraction, all conditions presented numerically similar results within the same method and with extraction performed with Soxhlet hexane. Similar behavior was observed by Cruz et al. (2017) and Mazzutti et al. (2018), in which the SFE extracts obtained from butia seeds and cocoa bean hulls using different extraction conditions presented similar yields regardless of extraction pressure and temperature. Benelli et al. (2010), who also obtained extracts from orange pomace, presented an analogous behavior with results for SFE yields presenting a small improvement on yield when pressure went from 200 to 300 bar.

On the other hand, PLE and SE showed a superior recovery of extracts for both sequential and non-sequential methods using ethanol as solvent. The high recovery numbers can be explained by the extraction conditions and the use of polar solvent that can extract more

polar substances. Despite the lower yield presented by SFE method, extracts with non-polar components are obtained, which means that different bioactive constituents are recovered.

Table 10 Global extraction yields for different extraction methods.

Method	P(bar)/T(°C); solvent	Sample	Extraction Yield (X_0)[1]
SE	Hexane	Orange pomace	2.57 ± 0.19^{C}
	Ethanol		32.26 ± 5.87^{A}
PLE	100/60; Hexane		1.17 ± 0.48^{C}
	100/60; Ethanol		15.74 ± 0.84^{B}
SFE	300/50; CO_2	Orange pomace	1.087 ± 0.151^{C}
	300/40; CO_2		1.147 ± 0.157^{C}
	200/50; CO_2		0.930 ± 0.253^{C}
	200/40; CO_2		0.968 ± 0.056^{C}
SE	Hexane	SFE Residue: 200/50	0.66 ± 0.25^{C}
	Ethanol		31.129 ± 0.532^{A}
PLE	100/60; Ethanol		18.330 ± 0.283^{B}

[1]Identical letters in the same column indicate that there is no significant difference ($p<0,05$).

Moreover, the SE yields obtained using different solvents also showed that the solvent with higher polarity presented higher recovery. Similar results occurred with Benelli et al. (2010), Mezzomo et al. (2010), Almeida et al. (2012) and Andrade, K. S. et al. (2017). In addition, the yield from SE for ethanolic sequential extraction demonstrated a slightly lower recovery than the yield obtained for the raw material extraction. This can be explained by the existence of a previous process where the material has already had a recovery and it is expected a lower recovery.

Furthermore, PLE extraction obtained high yields for ethanolic extractions. The same cannot be said for the extraction with hexane. This shows that the use of orange pomace, whether it is in original raw material or as residue for a second step extraction, presents more affinity with the polar solvent. Between ethanolic extracts, the sequential presented higher yield. This higher recovery could be achieved due to the previous step that applied higher pressure and may have caused cell wall ruptures. Dias et al. (2019) stated that the high yield presented by the sequential process was explained by the turbulence and shear forces that could have caused the cell wall breakage from the first step due to the use of ultrasound as integration method. Also, the high recovery was explained due to the previous process removing nonpolar components such as resins and waxes and the implementation of high pressure causing structural modifications in the solid matrix. In addition, Ferro et al. (2019) presented the same behavior in which PLE sequential extract obtained higher recovery than SFE.

The variation between SE and PLE results can be a consequence of the different extraction conditions. SE stays in room pressure and has higher temperature, at boiling point, and a longer period of time. PLE is performed using higher and controlled pressure with lower temperature and shorter period of time, of only 30 minutes. Given that the higher the temperature, the lower the surface tension and solvent viscosity (MAZZUTTI *et al*., 2018). The possibility of SE presenting higher yield is almost certain and, in this case, a reality.

3.2.4 Antioxidant activity

Antioxidant activity was evaluated for SFE and ethanolic extracts. Results obtained for β-carotene and DPPH assays are presented in Table 11. Free radical scavenging using DPPH is expressed in EC_{50}, which means that the lower the concentration obtained, the better is the antioxidant activity, considering that a smaller amount of extract will be necessary to achieve the 50% absorbance decay. Within results for SFE extracts, condition 200 bar/50 °C presented the best result with 1.24 ± 0.01 mg. mL^{-1} followed by 1.48 ± 0.05 mg. mL^{-1} on the condition 200 bar/40 °C. Both results are significantly different and yet present great antioxidant activity for this method. Results obtained for butia and tamarind seeds presented EC_{50} values which shows they have a lower antioxidant activity than the orange pomace (CRUZ *et al*., 2017; REIS *et al*., 2016). In addition, results obtained here are similar to the ones obtained in the same conditions as Benelli et al. (2010), which presented a study in orange pomace as well.

Within the same extraction method, Soxhlet and PLE both sequential and non-sequential methods presented good results; however, raw material with 1,39 mg. mL^{-1} for SE and for PLE 2,05 mg. mL^{-1} showed better results. The raw material extracts presenting better results can be explained by the presence of components that aid this property and that weren't extracted before due to the lack of a previous extraction step.

Soxhlet presented better value of EC_{50} for raw material. This is probably because the extraction time allows the recovery of more antioxidant components. Benelli et al. (2010) obtained similar results where low pressure extracts presented better EC_{50} than SFE. Although the raw material presents good antioxidant activity, the use of the residue (sequential method) is still more advantageous, as it leads to a better use of the raw material and a reduction of solid waste. Mazzutti et al. (2018) presented that sequential process extracts were better than SFE because of the smaller concentration of extracts needed to achieve the 50% decay of the method.

Table 11 Antioxidant Activity for orange pomace extracts.

Extraction Method	P(bar)/T(°C); solvent	EC_{50}[1] (mg. mL^{-1})	β-carotene (AA%)[1]
SFE	300/50; CO_2	1.79 ± 0.02^{C}	46.47 ± 2.97^{AB}
	300/40; CO_2	1.70 ± 0.06^{C}	36.16 ± 4.52^{B}
	200/50; CO_2	1.24 ± 0.01^{F}	48.31 ± 1.23^{A}
	200/40; CO_2	1.48 ± 0.05^{D}	10.17 ± 3.39^{C}
SE[2]	Ethanol	1.78 ± 0.02^{C}	40.46 ± 4.90^{AB}
PLE[2]	100/60; Ethanol	2.69 ± 0.02^{A}	45.07 ± 5.34^{AB}
SE	Ethanol	1.39 ± 0.01^{E}	-
PLE	100/60; Ethanol	2.05 ± 0.03^{B}	-

[1]Identical letters in the same column indicate that there is no significant difference ($p<0,05$); [2]Sequential extracts.

On the other hand, results shown for β-carotene are presented in percentage, which means that the higher the percentage, the higher the antioxidant activity demonstrated by the method. Lagha-Benamrouche; Madani (2013) presented a study in which extracts of different orange cultivars were obtained by maceration and presented up to 80 % of antioxidant activity for β-carotene method. All extracts presented some percentage of antioxidant activity pointed out by this method. However, SFE extract under condition 200/40 obtained the lowest result with 10.17 %. There is the possibility that the change in extraction conditions caused the insufficient recovery of antioxidant components when pressure was changed from 300 to 200 bar. All in all, even though this method showed good antioxidant activity, it did not present significant difference between extracts. In addition, sequential processes had similar results for the bleaching method.

Since extraction yields presented no significant difference between extracts, antioxidant activity became the decision factor to decide the residue from which SFE condition should be chosen to proceed with the integration of methods. For this reason, condition 200 bar/50 °C was chosen to be the first step in the sequential extraction process and as the inner component of first emulsion on the encapsulation process.

3.3 CONCLUSION

The results obtained in this study showed that repurposing the orange pomace, usually a solid waste, can be profitable due to the obtention of extracts with antioxidant activity and good recovery yield. Extracts with antioxidant activity were obtained from orange pomace, using different extraction methods. Also, the integration of the extraction methods was successful, especially when the extraction yield is considered. The sequential method, using supercritical fluid extraction (SFE) followed by pressurized liquid extraction (PLE), showed that residues from extraction can be as valuable as raw material. Furthermore, SFE and PLE are considered green methods and should be used more often.

The following extraction methods of PLE and SE obtained greater yields for polar solvent ethanol both for sequential and for the non-sequential methods. These results were superior than those obtained for SFE. However, SFE is a more selective method, hence the lower recovery percentage.

All extracts obtained from orange pomace presented good antioxidant activities, with some extracts being better than others. Sequential extracts and SFE extracts presented similar results for both methods of evaluation.

Considering the great amount of orange pomace that the juice industry produces, it is surprising that there is not a higher number of studies that use the entirety of the bagasse. Hopefully, this study will bring in more attention to this material given that it presents good recovery percentage and antioxidant potential. These results could be used in the generation of new products with high added value.

CHAPTER IV

4 NANOENCAPSULATION OF ORANGE POMACE EXTRACTS VIA DOUBLE EMULSION TECHNIQUE

This chapter shows the second part of this research. The extracts obtained in the previous chapter were selected and used in the formulation in order to obtain emulsions in the nano size spectrum.

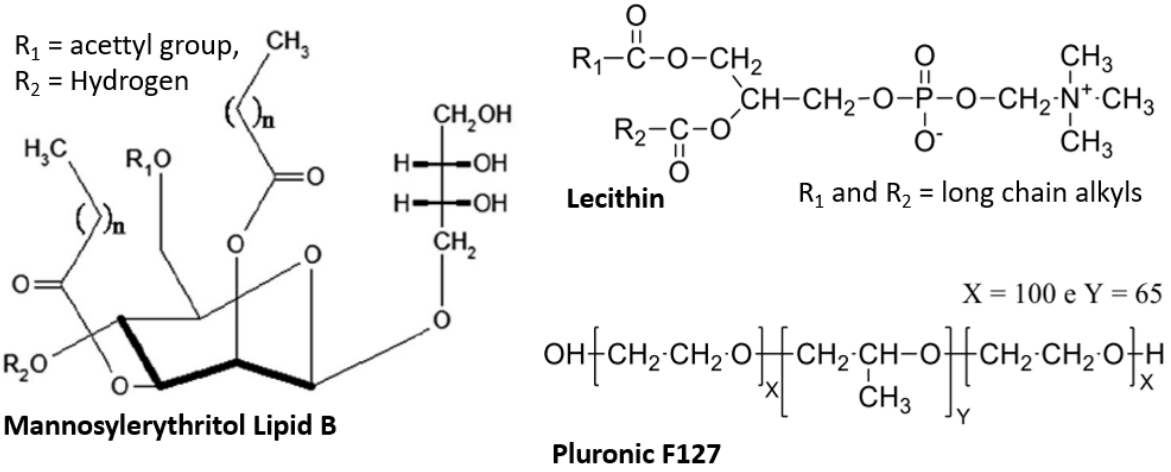
4.1 MATERIALS AND METHOD

The following topics will present the materials and the methods utilized in the formulation of the double emulsions for the creation of the solid lipid nanoparticles. The nanoencapsulation experiments were conducted at the Laboratory of Processes Control and Polymerization/LCP (Chemical Engineering and Food Engineering Department, UFSC, SC/BR).

4.1.1 Materials

Pure stearic acid (Vetec) was used as the lipid in the preparation of the solid lipid nanoparticles. Lecithin (Alfa Aesar), Pluronic F127 (Sigma-Aldrich) and Mannosylerythritol Lipid B (Toyobo/Japan, purity > 95 %) were used as surfactants (Figure 10). All reagents were used as received. Distilled water was used in all experiments.

Figure 10 Molecular structure of the surfactants utilized.



Source: Adapted from Andrade, C. J. De et al. (2017) and Peres (2016).

4.1.2 Double emulsion method

For the formation of the double emulsion, several steps were necessary. Several tests were performed to evaluate the formulations and parameters to obtain the emulsions and consequently produce solid lipid nanoparticles.

4.1.2.1 Preliminary tests

The parameters that needed to be determined in the preliminary tests were: time of sonication for the formation of the second emulsion in the ultrasound probe, amplitude of the ultrasound probe and concentration of the hydrophilic surfactant. The data analyzed was particle size, particle size distribution and zeta potential. The experimental procedure for these tests was the same as presented in section 4.1.2.2 Formulations. For the preliminary tests, the production of solid lipid nanoparticles was performed with a blank formulation, that is, no orange pomace extracts were used during tests. They were performed using lecithin given that it is a standard lipophilic surfactant.

Table 12 Experimental conditions used in the preliminary tests.

Test	Sonication Time (s)	Amplitude (%)	Concentration of Pluronic (%)
T1	45	45	10
T2	45	45	5
T3	60	45	10
T4	60	45	5
T5	60	50	10
T6	60	50	5

Hydrophilic surfactant Pluronic F127 was tested in concentrations of 5 and 10% (in relation to the total mass of W2 phase) given that a higher concentration did not solubilize. Time of sonication tested for the emulsification of the second emulsion was 45 s and 60 s, given that a longer period of time only lowered the zeta potential of the formulations and provided bigger particle sizes. The amplitude of the ultrasound probe (Eco-sonics, Ultronique, 500 W) was also tested 45% and 50%, given that amplitude higher than 50% resulted on lower zeta potential values for the formulations and lower than 45% resulted in bigger particles.

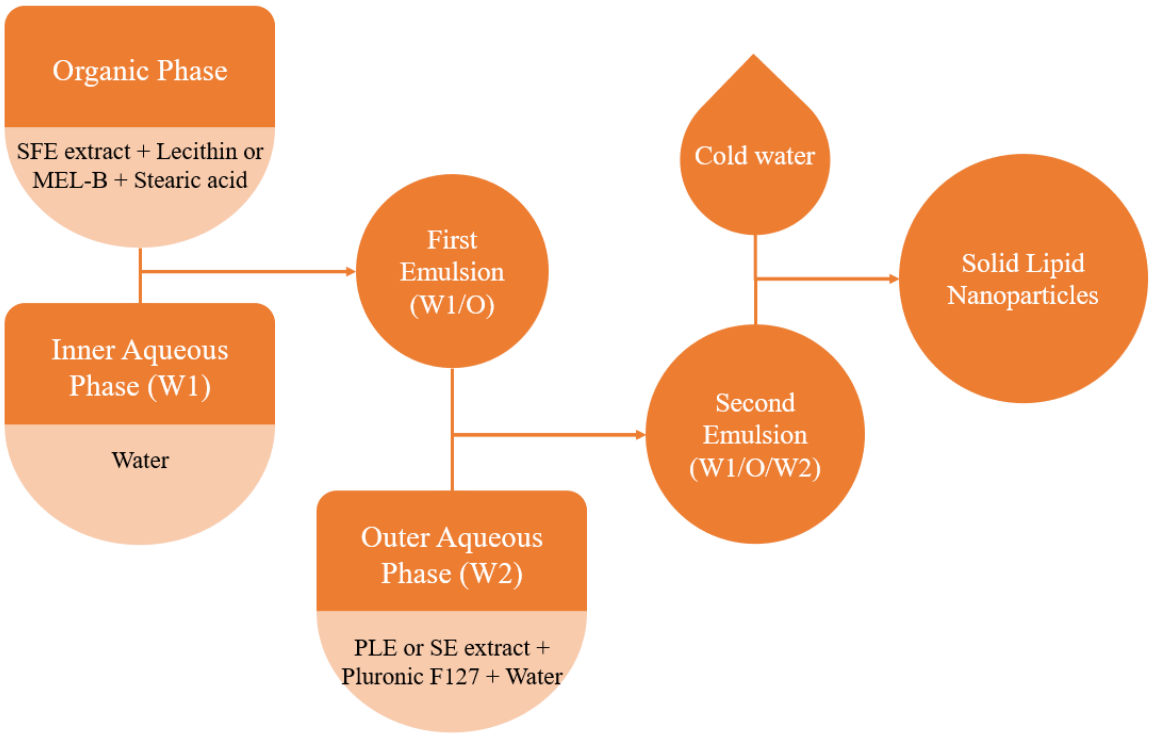
In the end of the tests, the selected conditions were: time was 60 s with amplitude of 50% and Pluronic concentration of 10%.

4.1.2.2 Formulations

The first emulsion was formed with the first aqueous phase (W1) that contained distilled water and an organic phase (O) containing stearic acid (0.3 g when in absence of SFE extract and 0.27 g when in the presence of SFE extract), 0.003 g of lecithin or Mannosylerythritol Lipid B, distilled water (0.1 g when in absence of secondary extract PLE or SE and 0.09 g when in the presence of secondary extracts) and presence or not of SFE 200 bar/50 °C extract (0.03 g). Both the aqueous phase (W1) and the organic phase (O) were submitted to heat and agitation in a heated plate (at approximately 70 °C) with magnetic agitation until homogeneity and the melting of the stearic acid for nearly 1 minute or less. Then, W1 and O were joined together and submitted to sonication in ultrasound probe for 15 s with 50% amplitude at room temperature (approximately 25 °C).

To obtain of the second emulsion, a second aqueous phase (W2) that contained distilled water (2.7 g), Pluronic F127 (0.3 g) and presence or not of SE or PLE extracts (0.01 g), was prepared. Pluronic F127 was used in 10% mass portion of the W2 phase. The ratio within the components of W2 do not change. Then the second aqueous phase (W2) was joined into the first emulsion and agitated in ultrasound probe for 60 s, (10 s on and 5 s off), with 50% of amplitude. After the second emulsion was made, cold distilled water (45 g) was added to the mixture to solidify and form the solid lipid nanoparticles. The experimental procedure is schematically shown in the diagram in Figure 11. Formulations for the formation of the double emulsions are presented in Table 13.

Figure 11 Diagram of the double emulsion.



Source: the author.

Table 13 Formulations for the preparation of the solid lipid nanoparticles using double emulsion without organic solvent.

Form. Number	Formulation Code	Lipid surf. (1%)[1]	SFE[2]	SE[3]	PLE[3]
F1	LEC-0	Lecithin	0	-	-
F2	LEC-PLE	Lecithin	0	-	10
F3	LEC-SFEPLE	Lecithin	10	-	10
F4	MEL-0	MEL-B	0	-	-
F5	MEL-PLE	MEL-B	0	-	10
F6	MEL-SFEPLE	MEL-B	10	-	10
F7	LEC-SE	Lecithin	0	10	-
F8	LEC-SFESE	Lecithin	10	10	-
F9	MEL-SE	MEL-B	0	10	-
F10	MEL-SFESE	MEL-B	10	10	-

[1]Percentage of lipid surfactant regarding the total amount of Stearic acid; [2]Weight percentage of SFE extract regarding the total amount of Stearic acid; [3]Weight Percentage of sequential SE or PLE extract regarding the total mass of W1.

Formulation codes signal the changing components of the formulation. That is, LEC-0 means formulation made with lecithin and no extract, LEC-PLE means formulation made with lecithin and PLE extract, LEC-SFEPLE means formulation made with lecithin and SFE and PLE extracts, LEC-SE means formulation made with lecithin and SE extract, LEC-SFESE means formulation made with lecithin and SFE and SE extracts, MEL-0 means formulation made with MEL-B and no extract, MEL-PLE means formulation made with MEL-B and PLE

extract, MEL-SFEPLE means formulation made with MEL-B and SFE and PLE extracts, MEL-SE means formulation made with MEL-B and SE extracts, and last MEL-SFESE means formulation made with MEL-B and SFE and SE extracts. After formation of the solid lipid nanoparticles and characterization, the nanoparticles were freeze-dried to avoid coalescence.

4.1.3 Particle characterization

Measurements for zeta potential (Z), particle size (Dp) and particle size distribution (PDI) were performed at LINDEN/UFSC using the equipment MALVERN's Zetasizer Nanosizer (0.3 nm – 10μm; MPT-2 Autotitrator, 173°) at 25 °C, without dilution. The formulations were inserted in a flask and placed in the equipment camera for reading.

4.2 RESULTS AND DISCUSSION

Solid lipid particles (SLNs) with the orange pomace extracts were obtained using the double emulsion technique. Characterization of particle size, size distribution and zeta potential of the SLNs is presented in the following topics.

Particle size (Dp) and particle size distribution (PDI) were analyzed and showed results with nano sized particles, with MEL-B particles among the formulations with smaller sizes. Peres (2016) did a similar formulation using lecithin and Pluronic F127 for double emulsion formulation and obtained particles size and distribution similar to the ones presented in this work. Zeta Potential (ZP), on the other hand, presented values that confirm a lower stability of the particles, especially for formulations containing MEL-B.

4.2.1 Effect on the particle size

The results of particle size for the SLNs are shown in Table 14. Comparison in pairs were done, to compare the effect of different variables on particle size. Considering the absence of extracts, formulations F1 and F4 can be analyzed according to their different surfactants. The MEL-B formulation presented particle sizes with more than 30 % reduction of size.

In regard to the presence of only external extracts (PLE or SE), the lecithin pair F2-F7 and the MEL-B pair F5-F9 can be observed. For these pairs, the change between extracts did not result in a notable difference in the particles sizes. Furthermore, examining the different surfactants within these formulations, other pairings must be done, the PLE pair F2-F5 and the

SE pair F7-F9. The formulations containing MEL-B (F5 and F9) presented 10 % of reduction in the size of the particles.

Table 14 Particle size results for different formulations.

Entry	Formulation Code	Dp (nm)[1]
F1	LEC-0	410.2 ± 1.3^{A}
F2	LEC-PLE	315.8 ± 1.6^{D}
F3	LEC-SFEPLE	308.9 ± 7.3^{D}
F4	MEL-0	259.7 ± 0.4^{FG}
F5	MEL-PLE	272.9 ± 5.1^{EF}
F6	MEL-SFEPLE	249.0 ± 0.9^{G}
F7	LEC-SE	331.0 ± 4.2^{C}
F8	LEC-SFESE	282.7 ± 2.8^{E}
F9	MEL-SE	262.7 ± 7.9^{FG}
F10	MEL-SFESE	363.9 ± 9.8^{B}

[1]Means that do not share a letter in the same column are significantly different.

Observing results obtained for the formulations containing or not SFE extract, other groups are formed. Within the lecithin formulations, F2-F3 containing PLE and F7-F8 containing SE extracts can be evaluated. Even though the first pair does not present significant difference, it is observed that the presence of SFE resulted in a smaller particle size. A size reduction of more than 10 % can be observed in the second pair, that presents significant difference in values. Examining MEL-B formulations, the presence of SFE extract did not assist with the conclusion that the presence of inner extract can aid in the decrease of particle size. Formulation containing PLE presented smaller particle size in the presence of SFE; however, SE formulation presented smaller particle size when in the absence of the inner extract.

The effect of two different lipophilic surfactants was also analyzed. Lecithin, a standard surfactant, and Mannosylerythritol-Lipid B, which is still a novelty and; in this case, was used to substitute lecithin. The average particle size (329.7 nm for lecithin and 281.6 nm for MEL-B) presented that MEL-B resulted in a particle reduction of almost 15 %, which shows that, in general, MEL-B presented smaller particles. In addition, comparisons between formulations that contained the same extracts were done and showed that formulations containing MEL-B, generally, presented smaller particle sizes. Kim et al. (2014) formulated emulsions using different variation of MEL and obtained nano sized particles with sizes varying from 130 to 200 nm depending on the concentration of MEL used.

The smaller particles sizes may be due to the surfactant's properties. Taking into account that lecithin is a powder and MEL-B is a thick, honey-like liquid; it can be considered that MEL-B blended and formed a more homogenic emulsion than lecithin. Another compelling

factor is the molecular structure of both surfactants: MEL-B presents in its structure (Figure 10) more functional groups that assist in the increase of its polarity. In addition, MEL-B presents excellent surface tension-lowering ability and low critical micelle concentrations (CMC) (KITAMOTO *et al*., 2002). Also, the self-assembling property of MEL-B probably allows for components in the formulations to form smaller vesicles upon the high shear caused by the ultrasound probe (YU *et al*., 2015).

4.2.2 Effect on the dispersity of the particles

The reduced value of dispersity means that the majority of the particles in the emulsion have the same size or they have approximate sizes. A number of researchers in the emulsion field accept dispersity values lower than 0.3 as an optimum value to demonstrate that the emulsion is monodispersed (DAS; CHAUDHURY, 2011). The results for the dispersity of the particles (Table 15) can also be evaluated in pairs.

Table 15 Polydispersity results for different formulations.

Entry	Formulation Code	PDI[1]
F1	LEC-0	0.144 ± 0.030^{AB}
F2	LEC-PLE	0.120 ± 0.034^{AB}
F3	LEC-SFEPLE	0.174 ± 0.016^{A}
F4	MEL-0	0.122 ± 0.021^{AB}
F5	MEL-PLE	0.132 ± 0.032^{AB}
F6	MEL-SFEPLE	0.160 ± 0.004^{AB}
F7	LEC-SE	0.161 ± 0.035^{AB}
F8	LEC-SFESE	0.163 ± 0.010^{AB}
F9	MEL-SE	0.092 ± 0.028^{B}
F10	MEL-SFESE	0.171 ± 0.032^{A}

[1]Means that do not share a letter in the same column are significantly different.

Polydispersity values did not show significant difference between most of the formulations. However, if the statistical analysis is set aside, within the formulations that do not present extracts, MEL-B presented a more homogenic emulsion when compared to the similar formulations made with lecithin. In addition, when analyzing formulations with inner and outer extracts, the presence of SFE extract exhibited emulsions with less uniform particle sizes. Therefore, disregarding the statistical analyses, we can deduce that the presence of extracts in the inner layer of the particles provides a bigger range of particle sizes in this study.

The change in surfactant does not seem to present a notable difference such as seen in F2-F5 and F3-F6, where F2 (lecithin) and F6 (MEL-B) showed that each formulation,

regardless of surfactant, obtained a smaller PDI. Peres et al. (2016) and Schubert; Müller-Goymann (2005) produced formulations with lecithin and obtained particle size distributions similar to the ones presented in this study. Thus, the effect of surfactant on the dispersity shows the surfactant do not present major effects in this study.

4.2.3 Effects on the stability of the particles:

The stability of the emulsions can be analyzed with zeta potential values that quantify the charge property on the surface of the nanoparticles. The higher the value, in modulus, the more stable the emulsion. Emulsions are considered stable when zeta potential values are higher than 30mV or lower than -30 mV. High zeta potential values indicate that there is a smaller chance of coalescence happening (DAS; CHAUDHURY, 2011; MOHANRAJ; CHEN, 2006). In advance, the formulations presented in this work did not present zeta potential values to be considered stable (Table 16), however it does not mean that the emulsions formulated are unstable.

Table 16 Zeta potential of the nanoparticles.

Entry	Formulation Code	ZP (mV)[1]
F1	LEC-0	-21.2 ± 0.7^{F}
F2	LEC-PLE	-17.2 ± 0.4^{D}
F3	LEC-SFEPLE	-16.9 ± 1.0^{D}
F4	MEL-0	-13.6 ± 0.1^{C}
F5	MEL-PLE	-9.3 ± 0.3^{A}
F6	MEL-SFEPLE	-11.9 ± 0.8^{BC}
F7	LEC-SE	-18.1 ± 0.6^{DE}
F8	LEC-SFESE	-19.2 ± 0.4^{E}
F9	MEL-SE	-8.9 ± 0.3^{A}
F10	MEL-SFESE	-11.8 ± 1.0^{B}

[1]Means that do not share a letter in the same column are significantly different.

In regard to the complete absence of extracts (F1 and F4), the lecithin formulation presented higher stability. Considering the presence of extracts, whether inner or outer extracts decreased the zeta value of the formulations when compared to the ones without extracts within formulations with the same surfactants.

In addition, examining the presence of the inner extract, in general, it presented a better stability than the ones without SFE extracts. On the other hand, when analyzing the pairings whose only difference is the surfactant, the emulsions with more stability were the formulations

containing lecithin. Schubert; Müller-Goymann (2005) produced solid lipid nanoparticles with lecithin and obtained similar zeta potential results for lecithin formulations.

Low HLB values, suggest higher free energy and lower amounts of hydrophilic antioxidants at the O/W2 interfacial region, which results in less stable emulsions. Given that lecithin and MEL-B present low HLB values of 8 and 8.8, respectively (VELDERRAIN-RODRÍGUEZ *et al*., 2019 and ANDRADE, C. J. *et al*., 2017), it was expected that formulations obtained here showed lower stability.

MEL-B presented, in general, a smaller stability than lecithin, even though is considered to be thermodynamically stable (ARUTCHELVI et al., 2008). Even though MEL-B has shown, in this study, to obtain a lower value of zeta potential, Kim et al. (2014) formulations using one type of MEL presented zeta potential with values of -60 mV or less.

The behavior presented here can be explained due to the molecular structure of the lecithin and MEL-B. In Figure 10, observing the molecular structure of lecithin, the functional groups that are present are less electronegative and, for that, there are a lack of electrons on the surrounding structure. That is, lecithin can be considered more stable than MEL-B.

4.3 CONCLUSION

For this part of the study, the goals that were defined before were achieved with the production of solid lipid nanoparticles impregnated with orange pomace extracts using double emulsion method assisted with ultrasound without the use of organic solvent as the continuous phase.

The double emulsion method, even though it is a laborious method, can be considered for further research when solid lipid particles are required. This study has also shown that it is possible to obtain nanoparticles with double emulsion assisted with ultrasound. Another point is the possibility of working with double emulsions with the use of water as the continuous mean solvent. In addition, this method allows nanoencapsulation without the use of other solvents that are not considered green.

In regards to the presence of the extracts, the inner non-polar extract can aid with the obtention of smaller particle sizes whereas the polar extracts in the outer phase do not collaborate much for this purpose. Moreover, the presence of extracts in the particles led to a decrease in the stability of the emulsions. The presence of SE and PLE in the formulations decrease even more the stability of the particles.

In addition, comparing the surfactants lecithin and MEL-B; the use of MEL-B resulted in smaller particles. Also, using the biosurfactant MEL-B, is interesting for processes of in which the production of these nanoparticles is desired to be green. Even though MEL-B is thermodynamically stable, in this case, all formulations that presented this surfactant presented smaller stability. In regards to the dispersity of the nano particles, they all presented values that indicate monodispersed emulsions, with low polydispersity indexes.

CHAPTER V

5 CONCLUSION

In this study, extracts from the orange pomace were obtained with the use of three different methods: supercritical fluid extraction, Soxhlet extraction and pressurized liquid extraction. A sequential extraction process was successfully performed, with supercritical fluid extraction followed by pressurized liquid extraction (or Soxhlet extraction, for comparison). Extraction yields ranged from 0.93 % to 1.147 % in the first step of the sequential process. For the second step of the sequential process global yields of 31.13 % and 18.33 % were obtained for Soxhlet and pressurized liquid extraction, respectively. Even though the SFE extracts obtained a smaller recovery, they present components with high value due to the selectivity of the process.

The extracts obtained presented good recovery yields even for the sequential extracts. They also presented antioxidant activity. EC_{50} values of 1.78 mg. mL^{-1} and 2.69 mg. mL^{-1} were presented for DPPH assay for sequential Soxhlet and pressurized liquid extraction, respectively. Also, values of EC_{50} ranging from 1.24 mg. mL^{-1} to 1.79 mg. mL^{-1} were obtained for SFE extracts. Extracts were also analyzed according to β-carotene bleaching method and SFE extracts presented antioxidant activity varying from 10.17 % to 46.47 %. For the same method Soxhlet (40.46 %) and PLE (45.07 %) also presented high antioxidant activities. Hence, the utilization of orange pomace is of great value due to the recovery of components that can be used in further research of naturally obtained antioxidants.

The extracts obtained from orange pomace were used in formulations for the obtention of solid lipid nanoparticles, without the use of organic solvents. The method for nanoencapsulation was double emulsion assisted with ultrasound, in order to allow the incorporation of both polar and non-polar extracts.

The solid lipid nanoparticles presented sizes within the range of 249.0 nm to 410.2 nm, as a result of the use of the ultrasound probe, and monodispersed values (with PDI varying from 0.092 to 0.174). However, formulations did not present high values of zeta potential (ranging from 8.9 mV to 21.2 mV, in module). Therefore, these formulations, once the zeta potential is increased by the use of other components that may aid in the surface charge of the particles, can be used in the industry of food and cosmetics given that they do not use toxic or any other problematic ingredient within their formulations.

5.1 SUGESTIONS FOR FUTURE WORKS

a) Use of different solvents for the extraction of the sequential extracts;
b) Characterization of the raw material, the solid residue from SFE and the extracts;
c) Evaluation of antimicrobial activity of the extracts;
d) Evaluation of other extraction conditions for pressurized liquid extraction, such as a change in the extraction temperature;
e) Use of different surfactants in the formulation of the nano emulsions;
f) Use of a different encapsulating agent, such as bee wax to replace the stearic acid;
g) Use of a stabilizer to increase the shelf life of the nano emulsions so they can be used for product formulations;
h) Study of the degradation of the particles due to hydrolysis;
i) Evaluation of the release of the extracts for the mean *in vitro*;
j) Evaluation of the encapsulation efficiency of the extracts into the SLNs;
k) Characterization of the nanoparticles, such as electronic microscopy;
l) Evaluation of antioxidant and antimicrobial activity of the formulations.

REFERÊNCIAS

AGNIHOTRI, S. M.; VAVIA, P. R. Influences of process parameters on nanoparticle preparation performed by a double emulsion pressure homogenization technique. **Journal of Surface Science and Technology**, v. 19, n. 3–4, p. 183–187, 2003.

ALKANAWATI, M. S.; WURM, F. R.; THÉRIEN-AUBIN, H.; LANDFESTER, K. Large-Scale Preparation of Polymer Nanocarriers by High-Pressure Microfluidization. **Macromolecular Materials and Engineering**, v. 303, n. 1, p. 1–7, 2018.

ALMEIDA, P. P.; MEZZOMO, N.; FERREIRA, S. R. S. Extraction of Mentha spicata L. Volatile Compounds: Evaluation of Process Parameters and Extract Composition. **Food and Bioprocess Technology**, v. 5, n. 2, p. 548–559, 2012.

ANDRADE, C. J.; ANDRADE, L. M.; ROCCO, S. A.; et al. A novel approach for the production and purification of mannosylerythritol lipids (MEL) by Pseudozyma tsukubaensis using cassava wastewater as substrate. **Separation and Purification Technology**, v. 180, p. 157–167, 2017.

ANDRADE, K. S.; GONALVEZ, R. T.; MARASCHIN, M.; et al. Supercritical fluid extraction from spent coffee grounds and coffee husks: Antioxidant activity and effect of operational variables on extract composition. **Talanta**, v. 88, p. 544–552, 2012.

ANDRADE, K. S.; PONCELET, D.; FERREIRA, S. R. S. Sustainable extraction and encapsulation of pink pepper oil. **Journal of Food Engineering**, v. 204, p. 38–45, 2017.

AOAC. Official Methods of Analysis of the AOAC International, 18th ed. **Official methods of analysis of AOAC International. Volume I, agricultural chemicals, contaminants, drugs/edited by William Horwitz. Gaithersburg (Maryland): AOAC International, 1997.**, 2005.

ARUTCHELVI, J. I.; BHADURI, S.; UPPARA, P. V.; DOBLE, M. Mannosylerythritol lipids: A review. **Journal of Industrial Microbiology and Biotechnology**, v. 35, n. 12, p. 1559–1570, 2008.

ASBAHANI, A. EL; MILADI, K.; BADRI, W.; et al. Essential oils: From extraction to encapsulation. **International Journal of Pharmaceutics**, v. 483, n. 1–2, p. 220–243, 2015. Elsevier B.V. Disponível em: <http://dx.doi.org/10.1016/j.ijpharm.2014.12.069>. .

ASSIS, L.; ZAVAREZE, E. DA R.; PRENTICE-HERNÁNDEZ, C.; SOUZA-SOARES, L. DE A. Revisão : Características de nanopartículas e potenciais aplicações em alimentos. **Brazilian Journal of Food Technolog**, v. 15, n. 2, p. 99–109, 2012.

BALLESTEROS, L. F.; RAMIREZ, M. J.; ORREGO, C. E.; TEIXEIRA, J. A.; MUSSATTO, S. I. Encapsulation of antioxidant phenolic compounds extracted from spent coffee grounds by freeze-drying and spray-drying using different coating materials. **Food Chemistry**, v. 237, p. 623–631, 2017. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.foodchem.2017.05.142>. .

BAMBA, B. S. B.; SHI, J.; TRANCHANT, C. C.; et al. Coencapsulation of polyphenols and anthocyanins from blueberry pomace by double emulsion stabilized by whey proteins: Effect of Homogenization parameters. **Molecules**, v. 23, n. 10, 2018.

BARRALES, F. M.; REZENDE, C. A.; MARTÍNEZ, J. Supercritical CO 2 extraction of passion fruit (Passiflora edulis sp .) seed oil assisted by ultrasound. **The Journal of Supercritical Fluids**, v. 104, p. 183–192, 2015. Elsevier B.V. Disponível em: <http://dx.doi.org/10.1016/j.supflu.2015.06.006>. .

BARRALES, F. M.; SILVEIRA, P.; BARBOSA, P. DE P. M.; et al. Recovery of phenolic compounds from citrus by-products using pressurized liquids — An application to orange peel. **Food and Bioproducts Processing**, v. 112, p. 9–21, 2018. Institution of Chemical Engineers. Disponível em: <https://doi.org/10.1016/j.fbp.2018.08.006>. .

BENELLI, P.; RIEHL, C. A. S.; SMÂNIA, A.; SMÂNIA, E. F. A.; FERREIRA, S. R. S. Bioactive extracts of orange (Citrus sinensis L. Osbeck) pomace obtained by SFE and low pressure techniques: Mathematical modeling and extract composition. **Journal of Supercritical Fluids**, v. 55, n. 1, p. 132–141, 2010.
BENITO-ROMÁN, O.; RODRÍGUEZ-PERRINO, M.; SANZ, M. T.; MELGOSA, R.; BELTRÁN, S. Supercritical carbon dioxide extraction of quinoa oil: Study of the influence of process parameters on the extraction yield and oil quality. **Journal of Supercritical Fluids**, v. 139, n. March, p. 62–71, 2018. Elsevier. Disponível em: <https://doi.org/10.1016/j.supflu.2018.05.009>. .
BERISTAIN, C. I.; VAZQUEZ, A.; GARCIA, H. S.; VERNON-CARTER, E. J. Encapsulation of orange peel oil by co-crystallization. **LWT - Food Science and Technology**, v. 29, n. 7, p. 645–647, 1996.
BICCHI, C.; BINELLO, A.; RUBIOLO, P. Determination of phenolic diterpene antioxidants in Rosemary (Rosmarinus officinalis L.) with different methods of extraction and analysis. **Phytochemical Analysis**, v. 11, n. 4, p. 236–242, 2000.
BIMAKR, M.; RAHMAN, R. A.; TAIP, F. S.; et al. Comparison of different extraction methods for the extraction of major bioactive flavonoid compounds from spearmint (Mentha spicata L.) leaves. **Food and Bioproducts Processing**, v. 89, n. 1, p. 67–72, 2011. Institution of Chemical Engineers. Disponível em: <http://dx.doi.org/10.1016/j.fbp.2010.03.002>. .
BOUKROUFA, M.; BOUTEKEDJIRET, C.; PETIGNY, L.; RAKOTOMANOMANA, N. Bio-refinery of orange peels waste : A new concept based on integrated green and solvent free extraction processes using ultrasound and microwave techniques to obtain essential oil , polyphenols and pectin. **Ultrasonics - Sonochemistry**, v. 24, p. 72–79, 2015. Elsevier B.V. Disponível em: <http://dx.doi.org/10.1016/j.ultsonch.2014.11.015>. .
CAMEL, V. Recent extraction techniques for solid matrices - Supercritical fluid extraction, pressurized fluid extraction and microwave-assisted extraction: Their potential and pitfalls. **Analyst**, v. 126, n. 7, p. 1182–1193, 2001.
DE CAMPOS, L. M. A. S.; LEIMANN, F. V.; PEDROSA, R. C.; FERREIRA, S. R. S. Free radical scavenging of grape pomace extracts from Cabernet sauvingnon (Vitis vinifera). **Bioresource Technology**, v. 99, n. 17, p. 8413–8420, 2008.
CAN-CAUICH, C. A.; SAURI-DUCH, E.; MOO-HUCHIN, V. M.; BETANCUR-ANCONA, D.; CUEVAS-GLORY, L. F. Effect of extraction method and specie on the content of bioactive compounds and antioxidant activity of pumpkin oil from Yucatan, Mexico. **Food Chemistry**, v. 285, n. September 2018, p. 186–193, 2019. Elsevier. Disponível em: <https://doi.org/10.1016/j.foodchem.2019.01.153>. .
CAPUTO, L.; QUINTIERI, L.; CAVALLUZZI, M.; LENTINI, G.; HABTEMARIAM, S. Antimicrobial and Antibiofilm Activities of Citrus Water-Extracts Obtained by Microwave-Assisted and Conventional Methods. **Biomedicines**, v. 6, n. 2, p. 70, 2018.
CARABIAS-MARTÍNEZ, R.; RODRÍGUEZ-GONZALO, E.; REVILLA-RUIZ, P.; HERNÁNDEZ-MÉNDEZ, J. Pressurized liquid extraction in the analysis of food and biological samples. **Journal of Chromatography A**, v. 1089, p. 1–17, 2005.
CARVALHO, R. N.; MOURA, L. S.; ROSA, P. T. V.; MEIRELES, M. A. A. Supercritical fluid extraction from rosemary (Rosmarinus officinalis): Kinetic data, extract's global yield, composition, and antioxidant activity. **Journal of Supercritical Fluids**, v. 35, n. 3, p. 197–204, 2005.
CLEMENTE, E.; FLORES, A. C.; ROSA, C. I. L. F.; OLIVEIRA, D. M. Características da farinha de resíduos do processamento de laranja. **Revista Ciencias Exatas e Naturais**, v. 14, n. 2, p. 257–269, 2012.
COELHO, A. L. S.; FEUSER, P. E.; CARCIOFI, B. A. M.; DE ANDRADE, C. J.; DE OLIVEIRA, D. Mannosylerythritol lipids: antimicrobial and biomedical properties. **Applied**

Microbiology and Biotechnology, v. 104, n. 6, p. 2297–2318, 2020. Applied Microbiology and Biotechnology.
CRUZ, P. N.; PEREIRA, T. C. S.; GUINDANI, C.; et al. Antioxidant and antibacterial potential of butia (Butia catarinensis) seed extracts obtained by supercritical fluid extraction. **Journal of Supercritical Fluids**, v. 119, p. 229–237, 2017. Elsevier B.V. Disponível em: <http://dx.doi.org/10.1016/j.supflu.2016.09.022>. .
DAS, S.; CHAUDHURY, A. Recent advances in lipid nanoparticle formulations with solid matrix for oral drug delivery. **AAPS PharmSciTech**, v. 12, n. 1, p. 62–76, 2011.
DESAI, N. M.; HAWARE, D. J.; BASAVARAJ, K.; MURTHY, P. S. Microencapsulation of antioxidant phenolic compounds from green coffee. **Preparative Biochemistry and Biotechnology**, v. 49, n. 4, p. 400–406, 2019. Taylor & Francis. Disponível em: <https://doi.org/10.1080/10826068.2019.1575858>. .
DIAS, J. L.; MAZZUTTI, S.; DE SOUZA, J. A. L.; et al. Extraction of umbu (Spondias tuberosa) seed oil using CO 2 , ultrasound and conventional methods: Evaluations of composition profiles and antioxidant activities. **Journal of Supercritical Fluids**, v. 145, n. November 2018, p. 10–18, 2019. Elsevier. Disponível em: <https://doi.org/10.1016/j.supflu.2018.11.011>. .
DIAS, M. J.; ÁVILA, C. R. DE; MEZZOMO, N.; LANZA, M. Evaluation of bioactive extracts of mangaba (Hancornia speciosa) using low and high pressure processes. **The Journal of Supercritical Fluids**, v. 135, p. 198–210, 2018. Elsevier. Disponível em: <https://doi.org/10.1016/j.supflu.2018.01.016>. .
EDRIS, A.; BERGNSTÅHL, B. Encapsulation of orange oil in a spray dried double emulsion. **Nahrung/Food**, v. 3803, n. April 2001, p. 133–137, 2001.
EDRISI SORMOLI, M.; LANGRISH, T. A. G. Spray drying bioactive orange-peel extracts produced by Soxhlet extraction: Use of WPI, antioxidant activity and moisture sorption isotherms. **LWT - Food Science and Technology**, v. 72, p. 1–8, 2016. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.lwt.2016.04.033>. .
FARIDI ESFANJANI, A.; JAFARI, S. M. Biopolymer nano-particles and natural nano-carriers for nano-encapsulation of phenolic compounds. **Colloids and Surfaces B: Biointerfaces**, v. 146, p. 532–543, 2016. Elsevier B.V. Disponível em: <http://dx.doi.org/10.1016/j.colsurfb.2016.06.053>. .
FERNANDES, R. V. D. B.; BORGES, S. V.; BOTREL, D. A. Gum arabic/starch/maltodextrin/inulin as wall materials on the microencapsulation of rosemary essential oil. **Carbohydrate Polymers**, v. 101, n. 1, p. 524–532, 2014. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.carbpol.2013.09.083>. .
FERREIRA, L. P. C. Microencapsulação de extrato de beterraba pelo processo de gelificação iônica. , p. 1–60, 2018.
FERREIRA, S. R. S.; NIKOLOV, Z. L.; DORAISWAMY, L. K.; MEIRELES, M. A. A.; PETENATE, A. J. Supercritical fluid extraction of black pepper (Piper nigrun L.) essential oil. **Journal of Supercritical Fluids**, v. 14, n. 3, p. 235–245, 1999.
FERRO, D. M.; MAZZUTTI, S.; VITALI, L.; OLIVEIRA MÜLLER, C. M.; FERREIRA, S. R. S. Integrated extraction approach to increase the recovery of antioxidant compounds from Sida rhombifolia leaves. **Journal of Supercritical Fluids**, v. 149, p. 10–19, 2019. Elsevier B.V. Disponível em: <https://doi.org/10.1016/j.supflu.2019.03.013>. .
FIORENTIN, L. D.; MENON, B. T.; ALVES, J. A.; et al. Determinação da cinética e das isotermas de secagem do bagaço da laranja. **Acta Scientiarum - Technology**, v. 32, n. 2, p. 147–152, 2010.
FREIBERGER, E. B.; KAUFMANN, K. C.; BONA, E.; et al. Encapsulation of roasted coffee oil in biocompatible nanoparticles. **LWT - Food Science and Technology**, v. 64, n. 1, p. 381–389, 2015.

FREIRE, N. F. **Desenvolvimento De Nanopartículas Poliméricas Contendo Óleo Essencial De Citronela (Cymbopogon Winterianus)**, 2017.
GARCIA-MENDOZA, M. DEL P.; ESPINOSA-PARDO, F. A.; BASEGGIO, A. M.; et al. Extraction of phenolic compounds and anthocyanins from juçara (Euterpe edulis Mart.) residues using pressurized liquids and supercritical fluids. **Journal of Supercritical Fluids**, v. 119, p. 9–16, 2017. Elsevier B.V. Disponível em: <http://dx.doi.org/10.1016/j.supflu.2016.08.014>. .
GIL-RAMIREZ, A.; SALAS-VEIZAGA, D. M.; GREY, C.; et al. Integrated process for sequential extraction of saponins, xylan and cellulose from quinoa stalks (Chenopodium quinoa Willd.). **Industrial Crops and Products**, v. 121, n. May, p. 54–65, 2018. Elsevier. Disponível em: <https://doi.org/10.1016/j.indcrop.2018.04.074>. .
GONÇALVES, O. H.; LEIMANN, F. V; STEINMACHER, F. R.; et al. Biodegradable Polymer Nanoparticles. **Biodegradable Polymer Nanoparticles**.
GRANATA, G.; STRACQUADANIO, S.; LEONARDI, M.; et al. Essential oils encapsulated in polymer-based nanocapsules as potential candidates for application in food preservation. **Food chemistry**, v. 269, n. March, p. 286–292, 2018. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/30100436>. .
GUDIÑA, E. J.; RANGARAJAN, V.; SEN, R.; RODRIGUES, L. R. Potential therapeutic applications of biosurfactants. **Trends in Pharmacological Sciences**, v. 34, n. 12, p. 667–675, 2013.
GUINDANI, C.; PODESTÁ, R.; BLOCK, J. M.; et al. Valorization of chia (Salvia hispanica) seed cake by means of supercritical fluid extraction. **Journal of Supercritical Fluids**, v. 112, p. 67–75, 2016. Elsevier B.V. Disponível em: <http://dx.doi.org/10.1016/j.supflu.2016.02.010>. .
HERRERO, M.; SÁNCHEZ-CAMARGO, A. DEL P.; CIFUENTES, A.; IBÁÑEZ, E. Plants, seaweeds, microalgae and food by-products as natural sources of functional ingredients obtained using pressurized liquid extraction and supercritical fluid extraction. **TrAC - Trends in Analytical Chemistry**, v. 71, p. 26–38, 2015. Disponível em: <http://dx.doi.org/10.1016/j.trac.2015.01.018>. .
IQBAL, M.; ZAFAR, N.; FESSI, H.; ELAISSARI, A. Double emulsion solvent evaporation techniques used for drug encapsulation. **International Journal of Pharmaceutics**, v. 496, n. 2, p. 173–190, 2015. Elsevier B.V. Disponível em: <http://dx.doi.org/10.1016/j.ijpharm.2015.10.057>. .
JAFARI, S. M. **An overview of nanoencapsulation techniques and their classification**. Elsevier Inc., 2017.
JENSEN, W. B. The origin of the Soxhlet extractor. **Journal of Chemical Education**, v. 84, n. 12, p. 1913–1914, 2007.
JUN-XIA, X.; HAI-YAN, Y.; JIAN, Y. Microencapsulation of sweet orange oil by complex coacervation with soybean protein isolate/gum Arabic. **Food Chemistry**, v. 125, n. 4, p. 1267–1272, 2011. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.foodchem.2010.10.063>. .
KADERIDES, K.; GOULA, A. M. Development and characterization of a new encapsulating agent from orange juice by-products. **Food Research International**, v. 100, n. June, p. 612–622, 2017. Elsevier. Disponível em: <http://dx.doi.org/10.1016/j.foodres.2017.07.057>. .
KADERIDES, K.; GOULA, A. M. Encapsulation of pomegranate peel extract with a new carrier material from orange juice by-products. **Journal of Food Engineering**, v. 253, n. February, p. 1–13, 2019. Elsevier. Disponível em: <https://doi.org/10.1016/j.jfoodeng.2019.02.019>. .
KAKRAN, M.; ANTIPINA, M. N. Emulsion-based techniques for encapsulation in biomedicine, food and personal care. **Current Opinion in Pharmacology**, v. 18, p. 47–55,

2014. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.coph.2014.09.003>. .
KHAW, K. Y.; PARAT, M. O.; SHAW, P. N.; FALCONER, J. R. Solvent supercritical fluid technologies to extract bioactive compounds from natural sources: A review. **Molecules**, v. 22, n. 7, 2017. MDPI AG.
KIM, M. K.; JEONG, E. S.; KIM, K. N.; PARK, S. H.; KIM, J. W. Nanoemulsification of pseudo-ceramide by molecular association with mannosylerythritol lipid. **Colloids and Surfaces B: Biointerfaces**, v. 116, p. 597–602, 2014. Elsevier B.V. Disponível em: <http://dx.doi.org/10.1016/j.colsurfb.2013.10.022>. .
KITAMOTO, D.; ISODA, H.; NAKAHARA, T. Functions and Potential applications of glycolipid biosurfactants. **J. Biosci. Bioeng.**, v. 94, n. 3, p. 187–201, 2002.
KUMARI, A.; YADAV, S. K.; PAKADE, Y. B.; SINGH, B.; YADAV, S. C. Development of biodegradable nanoparticles for delivery of quercetin. **Colloids and Surfaces B: Biointerfaces**, v. 80, n. 2, p. 184–192, 2010. Elsevier B.V. Disponível em: <http://dx.doi.org/10.1016/j.colsurfb.2010.06.002>. .
KWON, M. C.; CHOI, W. Y.; SEO, Y. C.; et al. Enhancement of the Skin-Protective Activities of Centella asiatica L. Urban by a Nano-encapsulation Process. **Journal of Biotechnology**, v. 157, n. 1, p. 100–106, 2012. Elsevier B.V. Disponível em: <http://dx.doi.org/10.1016/j.jbiotec.2011.08.025>. .
LAGHA-BENAMROUCHE, S.; MADANI, K. Phenolic contents and antioxidant activity of orange varieties (Citrus sinensis L. and Citrus aurantium L.) cultivated in Algeria: Peels and leaves. **Industrial Crops and Products**, v. 50, p. 723–730, 2013.
LAMBA, H.; SATHISH, K.; SABIKHI, L. Double Emulsions: Emerging Delivery System for Plant Bioactives. **Food and Bioprocess Technology**, v. 8, n. 4, p. 709–728, 2015.
LEISTER, N.; KARBSTEIN, H. P. Evaluating the stability of double emulsions— A review of the measurement techniques for the systematic investigation of instability mechanisms. **Colloids and Interfaces**, v. 4, n. 1, p. 1–18, 2020.
LENARDÃO, E. J.; FREITAG, R. A.; DABDOUB, M. J.; FERREIRA BATISTA, A. C.; DA CRUZ SILVEIRA, C. “Green chemistry” - Os 12 princípios da química verde e sua inserção nas atividades de ensino e pesquisa. **Quimica Nova**, v. 26, n. 1, p. 123–129, 2003.
LUQUE DE CASTRO, M. D.; PRIEGO-CAPOTE, F. Soxhlet extraction: Past and present panacea. **Journal of Chromatography A**, v. 1217, n. 16, p. 2383–2389, 2010. Elsevier B.V. Disponível em: <http://dx.doi.org/10.1016/j.chroma.2009.11.027>. .
MACHADO, A. P. D. F.; PASQUEL-REÁTEGUI, J. L.; BARBERO, G. F.; MARTÍNEZ, J. Pressurized liquid extraction of bioactive compounds from blackberry (Rubus fruticosus L.) residues: A comparison with conventional methods. **Food Research International**, v. 77, p. 675–683, 2015. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.foodres.2014.12.042>. .
MACHADO, B. A. S.; SILVA, R. P. D.; BARRETO, G. D. A.; et al. Chemical composition and biological activity of extracts obtained by supercritical extraction and ethanolic extraction of brown, green and red propolis derived from different geographic regions in Brazil. **PLoS ONE**, v. 11, n. 1, p. 1–27, 2016.
MADHAVA NAIDU, M.; SULOCHANAMMA, G.; SAMPATHU, S. R.; SRINIVAS, P. Studies on extraction and antioxidant potential of green coffee. **Food Chemistry**, v. 107, n. 1, p. 377–384, 2008.
MARTINEZ-CORREA, H. A.; BITENCOURT, R. G.; KAYANO, A. C. A. V.; et al. Integrated extraction process to obtain bioactive extracts of Artemisia annua L. leaves using supercritical CO2, ethanol and water. **Industrial Crops and Products**, v. 95, p. 535–542, 2017. Elsevier B.V. Disponível em: <http://dx.doi.org/10.1016/j.indcrop.2016.11.007>. .
MATOS, M.; GUTIÉRREZ, G.; IGLESIAS, O.; COCA, J.; PAZOS, C. Enhancing encapsulation efficiency of food-grade double emulsions containing resveratrol or vitamin

B12 by membrane emulsification. **Journal of Food Engineering**, v. 166, p. 212–220, 2015. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.jfoodeng.2015.06.002>. .
MATTHÄUS, B. Antioxidant activity of extracts obtained from residues of different oilseeds. **Journal of Agricultural and Food Chemistry**, v. 50, n. 12, p. 3444–3452, 2002.
MAZZUTTI, S.; RODRIGUES, L. G. G.; MEZZOMO, N.; VENTURI, V.; FERREIRA, S. R. S. Integrated green-based processes using supercritical CO2 and pressurized ethanol applied to recover antioxidant compouds from cocoa (Theobroma cacao) bean hulls. **Journal of Supercritical Fluids**, v. 135, n. December 2017, p. 52–59, 2018. Elsevier. Disponível em: <https://doi.org/10.1016/j.supflu.2017.12.039>. .
MENSOR, LUCIANA L; MENEZES, FÁBIO S; LEITÃO, GILDA G; REIS, ALEZANDRE S; SANTOS, TEREZA C; COUBE, CINTIA S; LEITÃO, S. G. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. **Phytotherapy Research**, v. 130, n. August, p. 127–130, 2001.
MEZZOMO, N.; MILEO, B. R.; FRIEDRICH, M. T.; MARTÍNEZ, J.; FERREIRA, S. R. S. Supercritical fluid extraction of peach (Prunus persica) almond oil: Process yield and extract composition. **Bioresource Technology**, v. 101, n. 14, p. 5622–5632, 2010. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.biortech.2010.02.020>. .
MEZZOMO, N.; OLIVEIRA, D. A.; COMIM, S. R. R.; FERREIRA, S. R. S. Encapsulation of extract from winery industry residue using the supercritical anti-solvent technique. **Brazilian Journal of Chemical Engineering**, v. 33, n. 3, p. 589–598, 2016. Assoc. Brasiliera de Eng. Quimica / Braz. Soc. Chem. Eng.
MIRA, B; BLASCO, M; SUBIRATS, S. Supercritical CO2 extraction of essential oils from Orange peel. **The Journal of Supercritical Fluids**, v. 9, n. 4, p. 238–243, 1996.
MOHANRAJ, V. J.; CHEN, Y. Nanoparticles - A review. **Tropical Journal of Pharmaceutical Research**, v. 5, n. 1, p. 561–573, 2006.
MONROY, Y. M.; RODRIGUES, R. A. F.; RODRIGUES, M. V. N.; et al. Brazilian green propolis extracts obtained by conventional processes and by processes at high pressure with supercritical carbon dioxide, ethanol and water. **Journal of Supercritical Fluids**, v. 130, n. August, p. 189–197, 2017. Elsevier. Disponível em: <http://dx.doi.org/10.1016/j.supflu.2017.08.006>. .
MUDRIĆ, J.; ŠAVIKIN, K.; IBRIĆ, S.; ĐURIŠ, J. Double emulsions (W/O/W emulsions): Encapsulation of plant bioactives. **Lekovite sirovine**, , n. 39, p. 76–83, 2019.
MUSTAFA, A.; TURNER, C. Pressurized liquid extraction as a green approach in food and herbal plants extraction: A review. **Analytica Chimica Acta**, v. 703, n. 1, p. 8–18, 2011. Elsevier B.V. Disponível em: <http://dx.doi.org/10.1016/j.aca.2011.07.018>. .
NEVES, M. F.; TROMBIN, V. G.; MILAN, P.; et al. **O retrato da citricultura brasileira**. Ribeirão Preto, 2009.
NOGUEIRA, B. P. **CONAB Análise Mensal - Laranja**. Brasília, 2020.
OLIVEIRA, D. A.; ANGONESE, M.; FERREIRA, S. R. S.; GOMES, C. L. Nanoencapsulation of passion fruit by-products extracts for enhanced antimicrobial activity. **Food and Bioproducts Processing**, v. 104, p. 137–146, 2017. Institution of Chemical Engineers. Disponível em: <http://dx.doi.org/10.1016/j.fbp.2017.05.009>. .
OLIVEIRA, D. A.; SALVADOR, A. A.; SMÂNIA, A.; et al. Antimicrobial activity and composition profile of grape (Vitis vinifera) pomace extracts obtained by supercritical fluids. **Journal of Biotechnology**, v. 164, n. 3, p. 423–432, 2013. Elsevier B.V. Disponível em: <http://dx.doi.org/10.1016/j.jbiotec.2012.09.014>. .
DE OLIVEIRA, M. S.; DA CRUZ, J. N.; GOMES SILVA, S.; et al. Phytochemical profile, antioxidant activity, inhibition of acetylcholinesterase and interaction mechanism of the major components of the Piper divaricatum essential oil obtained by supercritical CO 2. **Journal of Supercritical Fluids**, v. 145, n. December 2018, p. 74–84, 2019. Elsevier. Disponível em:

<https://doi.org/10.1016/j.supflu.2018.12.003>. .
PARISOTTO, E. B.; MICHIELIN, E. M. Z.; BISCARO, F.; et al. The antitumor activity of extracts from Cordia verbenacea D.C. obtained by supercritical fluid extraction. **Journal of Supercritical Fluids**, v. 61, p. 101–107, 2012. Elsevier B.V. Disponível em: <http://dx.doi.org/10.1016/j.supflu.2011.08.016>. .
PEREIRA, M. C.; HILL, L. E.; ZAMBIAZI, R. C.; et al. Nanoencapsulation of hydrophobic phytochemicals using poly (dl-lactide-co-glycolide) (PLGA) for antioxidant and antimicrobial delivery applications: Guabiroba fruit (Campomanesia xanthocarpa O. Berg) study. **LWT - Food Science and Technology**, v. 63, n. 1, p. 100–107, 2015. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.lwt.2015.03.062>. .
PERES, L. B. **SÍNTESE DE POLÍMEROS A PARTIR DE AMINOÁCIDOS E O DESENVOLVIMENTO DE SISTEMAS NANOPARTICULADOS PARA ENCAPSULAÇÃO DE FÁRMACOS**, 2016. Federal University of Santa Catarina.
PERES, LUANA BECKER; PERES, LAIZE BECKER; DE ARAÚJO, P. H. H.; SAYER, C. Solid lipid nanoparticles for encapsulation of hydrophilic drugs by an organic solvent free double emulsion technique. **Colloids and Surfaces B: Biointerfaces**, v. 140, p. 317–323, 2016. Elsevier B.V. Disponível em: <http://dx.doi.org/10.1016/j.colsurfb.2015.12.033>. .
PESSOA, A. S.; PODESTÁ, R.; BLOCK, J. M.; et al. Extraction of pequi (Caryocar coriaceum) pulp oil using subcritical propane: Determination of process yield and fatty acid profile. **Journal of Supercritical Fluids**, v. 101, p. 95–103, 2015. Elsevier B.V. Disponível em: <http://dx.doi.org/10.1016/j.supflu.2015.03.006>. .
POURBAFRANI, M.; TALEBNIA, F.; NIKLASSON, C.; TAHERZADEH, M. J. Protective effect of encapsulation in fermentation of limonene-contained media and orange peel hydrolyzate. **International Journal of Molecular Sciences**, v. 8, n. 8, p. 777–787, 2007.
POURMORTAZAVI, S. M.; HAJIMIRSADEGHI, S. S. Supercritical fluid extraction in plant essential and volatile oil analysis. **Journal of Chromatography A**, v. 1163, n. 1–2, p. 2–24, 2007.
RAIKOS, V. Encapsulation of vitamin E in edible orange oil-in-water emulsion beverages: Influence of heating temperature on physicochemical stability during chilled storage. **Food Hydrocolloids**, v. 72, p. 155–162, 2017. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.foodhyd.2017.05.027>. .
RASOULI GHAHROUDI, F.; MIZANI, M.; REZAEI, K.; BAMENI MOGHADAM, M. Mixed extracts of green tea and orange peel encapsulated and impregnated on black tea bag paper to be used as a functional drink. **International Journal of Food Science and Technology**, v. 52, n. 7, p. 1534–1542, 2017.
RAVI KUMAR, M. N. Nano and microparticles as controlled drug delivery devices. **Journal of pharmacy & pharmaceutical sciences**, v. 3, n. 2, p. 234–258, 2000.
REIS, P. M. C. L.; DARIVA, C.; VIEIRA, G. A. B.; HENSE, H. Extraction and evaluation of antioxidant potential of the extracts obtained from tamarind seeds (Tamarindus indica), sweet variety. , v. 173, p. 116–123, 2016.
ROCHA-SELMI, G. A.; BOZZA, F. T.; THOMAZINI, M.; BOLINI, H. M. A.; FÁVARO-TRINDADE, C. S. Microencapsulation of aspartame by double emulsion followed by complex coacervation to provide protection and prolong sweetness. **Food Chemistry**, v. 139, n. 1–4, p. 72–78, 2013. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.foodchem.2013.01.114>. .
RODRÍGUEZ, J.; MARTÍN, M. J.; RUIZ, M. A.; CLARES, B. Current encapsulation strategies for bioactive oils: From alimentary to pharmaceutical perspectives. **Food Research International**, v. 83, p. 41–59, 2016. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.foodres.2016.01.032>. .
RUDKE, A. R.; MAZZUTTI, S.; ANDRADE, K. S.; VITALI, L.; FERREIRA, S. R. S.

Optimization of green PLE method applied for the recovery of antioxidant compounds from buriti (Mauritia flexuosa L.) shell. **Food Chemistry**, v. 298, n. March, p. 125061, 2019. Elsevier. Disponível em: <https://doi.org/10.1016/j.foodchem.2019.125061>. .
SANTOS, A. P. P. DOS; SILVA, M. D. D. S.; COSTA, E. V. L. Biossurfactantes: Uma alternativa para o mercado industrial. **Fronteiras: Journal of Social, Technological and Environmental Science**, v. 5, n. 1, p. 88, 2016.
SANTOS, J.; CALERO, N.; TRUJILLO-CAYADO, L. A.; GARCIA, M. C.; MUÑOZ, J. Assessing differences between Ostwald ripening and coalescence by rheology, laser diffraction and multiple light scattering. **Colloids and Surfaces B: Biointerfaces**, v. 159, p. 405–411, 2017. Elsevier B.V. Disponível em: <http://dx.doi.org/10.1016/j.colsurfb.2017.08.015>. .
SANTOS, P. H.; BAGGIO RIBEIRO, D. H.; MICKE, G. A.; VITALI, L.; HENSE, H. Extraction of bioactive compounds from feijoa (Acca sellowiana (O. Berg) Burret) peel by low and high-pressure techniques. **Journal of Supercritical Fluids**, v. 145, n. December 2018, p. 219–227, 2019. Elsevier. Disponível em: <https://doi.org/10.1016/j.supflu.2018.12.016>. .
SCHUBERT, M. A.; MÜLLER-GOYMANN, C. C. Characterisation of surface-modified solid lipid nanoparticles (SLN): Influence of lecithin and nonionic emulsifier. **European Journal of Pharmaceutics and Biopharmaceutics**, v. 61, n. 1–2, p. 77–86, 2005.
SHETTA, A.; KEGERE, J.; MAMDOUH, W. Comparative study of encapsulated peppermint and green tea essential oils in chitosan nanoparticles: Encapsulation, thermal stability, in-vitro release, antioxidant and antibacterial activities. **International Journal of Biological Macromolecules**, v. 126, p. 731–742, 2019. Elsevier B.V. Disponível em: <https://doi.org/10.1016/j.ijbiomac.2018.12.161>. .
SHRESTHA, M.; HO, T. M.; BHANDARI, B. R. Encapsulation of tea tree oil by amorphous beta-cyclodextrin powder. **Food Chemistry**, v. 221, p. 1474–1483, 2017. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.foodchem.2016.11.003>. .
DE SOUZA, C. G.; RODRIGUES, T. H. S.; E SILVA, L. M. A.; RIBEIRO, P. R. V.; DE BRITO, E. S. Sequential extraction of flavonoids and pectin from yellow passion fruit rind using pressurized solvent or ultrasound. **Journal of the Science of Food and Agriculture**, v. 98, n. 4, p. 1362–1368, 2018.
STASSE, M.; LAURICHESSE, E.; RIBAUT, T.; et al. Formulation of concentrated oil-in-water-in-oil double emulsions for fragrance encapsulation. **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, v. 592, n. February, p. 124564, 2020. Elsevier. Disponível em: <https://doi.org/10.1016/j.colsurfa.2020.124564>. .
TSUKUI, A.; JÚNIOR, H. M. S.; OIGMAN, S. S.; et al. Microwave-assisted extraction of green coffee oil and quantification of diterpenes by HPLC. **FOOD CHEMISTRY**, v. 164, p. 266–271, 2014. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.foodchem.2014.05.039>. .
TURASAN, H.; SAHIN, S.; SUMNU, G. Encapsulation of rosemary essential oil. **LWT - Food Science and Technology**, v. 64, n. 1, p. 112–119, 2015. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.lwt.2015.05.036>. .
VAZQUEZ-ROIG, P.; PICÓ, Y. Pressurized liquid extraction of organic contaminants in environmental and food samples. **TrAC - Trends in Analytical Chemistry**, v. 71, p. 55–64, 2015. Elsevier B.V. Disponível em: <http://dx.doi.org/10.1016/j.trac.2015.04.014>. .
VEISI, H.; DADRES, N.; MOHAMMADI, P.; HEMMATI, S. Green synthesis of silver nanoparticles based on oil-water interface method with essential oil of orange peel and its application as nanocatalyst for A3 coupling. **Materials Science and Engineering C**, v. 105, n. September 2018, p. 110031, 2019. Elsevier. Disponível em: <https://doi.org/10.1016/j.msec.2019.110031>. .

VELDERRAIN-RODRÍGUEZ, G. R.; ACEVEDO-FANI, A.; GONZÁLEZ-AGUILAR, G. A.; MARTÍN-BELLOSO, O. Encapsulation and stability of a phenolic-rich extract from mango peel within water-in-oil-in-water emulsions. **Journal of Functional Foods**, v. 56, n. December 2018, p. 65–73, 2019. Elsevier. Disponível em: <https://doi.org/10.1016/j.jff.2019.02.045>. .
VÉLEZ-ERAZO, E. M.; CONSOLI, L.; HUBINGER, M. D. Mono and double-layer emulsions of chia oil produced with ultrasound mediation. **Food and Bioproducts Processing**, v. 112, p. 108–118, 2018. Institution of Chemical Engineers. Disponível em: <https://doi.org/10.1016/j.fbp.2018.09.007>. .
VIGANÓ, J.; BRUMER, I. Z.; BRAGA, P. A. DE C.; et al. Pressurized liquids extraction as an alternative process to readily obtain bioactive compounds from passion fruit rinds. **Food and Bioproducts Processing**, v. 100, p. 382–390, 2016. Institution of Chemical Engineers. Disponível em: <http://dx.doi.org/10.1016/j.fbp.2016.08.011>. .
VIROT, M.; TOMAO, V.; COLNAGUI, G.; VISINONI, F.; CHEMAT, F. New microwave-integrated Soxhlet extraction An advantageous tool for the extraction of lipids from food products. **Journal of Chromatography A**, v. 1174, p. 138–144, 2007.
VISENTIN, A.; RODRÍGUEZ-ROJO, S.; NAVARRETE, A.; MAESTRI, D.; COCERO, M. J. Precipitation and encapsulation of rosemary antioxidants by supercritical antisolvent process. **Journal of Food Engineering**, v. 109, n. 1, p. 9–15, 2012. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.jfoodeng.2011.10.015>. .
WEISS, J.; HERRMANN, N.; MCCLEMENTS, D. J. Ostwald ripening of hydrocarbon emulsion droplets in surfactant solutions. **Langmuir**, v. 15, n. 20, p. 6652–6657, 1999.
WORAKITKANCHANAKUL, W.; IMURA, T.; FUKUOKA, T.; et al. Phase behavior of ternary mannosylerythritol lipid/water/oil systems. **Colloids and Surfaces B: Biointerfaces**, v. 68, n. 2, p. 207–212, 2009.
XHAXHIU, K.; KORPA, A.; MELE, A.; KOTA, T. Ultrasonic and Soxhlet Extraction Characteristics of the Orange Peel from “Moro” Cultivars Grown in Albania. **Journal of Essential Oil-Bearing Plants**, v. 16, n. 4, p. 421–428, 2013.
YANG, S. J.; WU, J. J.; WANG, Y. C.; et al. Encapsulation of propolis flavonoids in a water soluble polymer using pressurized carbon dioxide anti-solvent crystallization. **Journal of Supercritical Fluids**, v. 94, p. 138–146, 2014. Elsevier B.V. Disponível em: <http://dx.doi.org/10.1016/j.supflu.2014.07.009>. .
YU, M.; LIU, Z.; ZENG, G.; et al. Characteristics of mannosylerythritol lipids and their environmental potential. **Carbohydrate Research**, v. 407, p. 63–72, 2015. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.carres.2014.12.012>. .
ZAMBAUX, M. F.; BONNEAUX, F.; GREF, R.; et al. Influence of experimental parameters on the characteristics of poly(lactic acid) nanoparticles prepared by a double emulsion method. **Journal of Controlled Release**, v. 50, n. 1–3, p. 31–40, 1998.
ZHAO, J.; XIANG, J.; WEI, T.; YUAN, F.; GAO, Y. Influence of environmental stresses on the physicochemical stability of orange oil bilayer emulsions coated by lactoferrin-soybean soluble polysaccharides and lactoferrin-beet pectin. **Food Research International**, v. 66, p. 216–227, 2014. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.foodres.2014.09.019>. .

APÊNDICES

APÊNDICE I

Table A – Extraction SFE times, weights, accumulated weights and yield of the kinetic experiments for orange bagasse at 200 bar, 40 °C and 17 ± 2 g CO_2.min^{-1}.

Time (min)	Weight (g)	Accumulated weight (g)	Yield (%)
15	0.0164	0.0164	0.0820
20	0.1157	0.1321	0.0660
25	0.1206	0.2527	0.1263
30	0.2410	0.4937	0.2468
35	0.3702	0.8639	0.4319
45	0.2327	1.0966	0.5482
55	0.1591	1.2557	0.6278
65	0.0892	1.3449	0.6724
75	0.0866	1.4315	0.7157
85	0.0244	1.4559	0.7279
95	0.0304	1.4863	0.7431
100	0.0209	1.5072	0.7535
120	0.0084	1.5156	0.7577
140	0.0033	1.5486	0.7742
160	0.0362	1.5848	0.7923

Table B – Extraction PLE times, weights, accumulated weights and yield of the kinetic experiments for orange bagasse at100 bar, 60 °C and 3 ± 0,2 ml.min^{-1}.

Time (min)	Weight (g)	Accumulated weight (g)	Yield (%)
3	0.1523	0.1523	3.0296
6	0.1651	0.3174	6.3138
9	0.0949	0.4123	8.2015
12	0.0584	0.4707	9.3633
15	0.0559	0.5266	10.4752
20	0.1003	0.6269	12.4704
25	0.07	0.6969	13.8629
30	0.0631	0.76	15.1181
35	0.0571	0.8171	16.2539
40	0.0625	0.8796	17.4972
45	0.0403	0.9199	18.2988
50	0.0434	0.9633	19.1621
55	0.0352	0.9985	19.8623
60	0.0308	1.0293	20.4750