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Peach processing by-product (*Prunus persica*): An upcycling approach using green techniques

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"*Na vida, não existe nada a temer, mas a entender*."

Marie Curie

RESUMO

O pêssego (*Prunus persica*), pertencente à família Rosaceae, é altamente valorizado por seu sabor, textura, suculência e valor nutricional. O processamento do pêssego gera uma quantidade considerável de coprodutos, como as subutilizadas sementes e bagaço de pêssego, sendo que a semente é rica em ácidos graxos e proteínas. Um dos capítulos desse estudo teve como objetivo investigar diferentes técnicas de extração para recuperação proteica de sementes de pêssego bem como avaliar a funcionalidade da fração proteica. As sementes de pêssego foram desengorduradas por extração com fluido supercrítico (SFE-CO_2), resultando na recuperação de óleo de até 29,53 % ± 0,45. Em seguida, a fração proteica foi recuperada utilizando os seguintes métodos: extração com agitação térmica (HSE) como método convencional e, como procedimentos alternativos, extração assistida por micro-ondas (MAE), extração por líquido de alta pressão (PLE) e extração assistida por ultrassom (UAE) usando solução de NaOH como solvente. O PLE proporcionou o maior teor de proteína (95,10 % ±0,98), seguido pela técnica HSE (55,09 %±0,35). Portanto, sugere-se que no método PLE (10 min) recuperou uma proteína isolada com melhores propriedades funcionais, proporcionando resultados promissores para purificação proteica. Na sequência, foi desenvolvida a formulação de um análogo da maionese, os resultados deste estudo destacaram que a proteína isolada de pêssego (PSP) utilizada correspondia estreitamente ao perfil de cor da maionese vegana comercial (CM1). A análise de textura demonstrou que a amostra PSP partilha propriedades físicas semelhantes às proteínas da soja e do soro de leite em termos de dureza, adesividade e resiliência, enquanto a sua coesão é comparável à da proteína da ervilha. Além disso, exibiu perfil reológico próximo ao da formulação controle (CM1). A análise de microestrutura revelou a presença de gotículas lipídicas aprisionadas na fase aquosa da formulação, característica crucial para emulsões essenciais no preparo de maionese. Estas descobertas sugerem que a proteína isolada de pêssego pode servir como uma alternativa viável na elaboração de análogos de maionese, oferecendo características semelhantes aos produtos convencionais veganos. Por fim, foi avaliado o bagaço de pêssego, um subproduto do processamento industrial da polpa, rico em compostos bioativos e pectina. Este estudo teve como objetivo realizar uma extração líquida pressurizada (PLE) sequencial para o fracionamento de bagaço de pêssego, recuperando extratos fenólicos e pectina. Estas frações separadas foram avaliadas e novas propriedades funcionais foram determinadas. O método alternativo (PLE) foi comparado às extrações sequenciais de baixa pressão conduzidas por Soxhlet (SOX) e extração com agitação térmica (HSE), como procedimentos padrão. Os resultados indicam que o PLE a 40°C proporcionou maior rendimento de fenólicos (10,31 mg GAE/g), exibindo potencial antioxidante, por DPPH, ABTS e FRAP. O rendimento de pectina foi de 20 % por HSE, enquanto o PLE atingiu rendimento máximo de 14 %. As propriedades funcionais da fração pectina de ambas as amostras apresentaram boa capacidade e estabilidade de emulsificação e formação de espuma, com maior valor para a amostra HSE. Portanto, o PLE pode ser considerado um método alternativo para agregar valor aos subprodutos de pêssego, promovendo o conceito de upcycling. Este estudo apresenta inovações para o aproveitamento da semente e bagaço de pêssego e esclarece as propriedades tecno-funcionais da fração rica em proteína e pectina, que se mostraram promissoras para aplicações em formulações alimentícias.

Palavras-chave: Propriedades funcionais; coprodutos; técnicas emergentes; pectina; proteína; análogo de maionese.

ABSTRACT

Peach (*Prunus persica*), belonging to the Rosaceae family, is highly valued for its flavor, texture, juiciness and nutritional value. Peach processing generates a considerable amount of co-products, such as underused peach seeds and pomace. The seed is rich in fatty acids and proteins. One of the chapters of this study aimed to investigate different extraction techniques for protein recovery from peach seeds and evaluate the protein fraction's functionality. Peach seeds were defatted by supercritical fluid extraction (SFE-CO_2), resulting in oil recovery of up to 29.53 % ±0.45. Then, the protein fraction was recovered using the following methods: heat steering agitation extraction (HSE) as a conventional method and, as alternative procedures, microwave-assisted extraction (MAE), high-pressure liquid extraction (PLE) and ultrasound-assisted (UAE) using NaOH solution as solvent. The PLE provided the highest protein content (95.10 % ±0.98), followed by the HSE technique (55.09 % ±0.35). Therefore, it is suggested that the PLE method (10 min) recovered an isolated protein with better functional properties, providing promising results for protein purification. Subsequently, the formulation of a mayonnaise analog was developed; the results of this study highlighted that the peach isolated protein (PSP) used closely corresponded to the color profile of commercial vegan mayonnaise (CM1). Texture analysis demonstrated that the PSP sample shares similar physical properties to soy and whey proteins in terms of hardness, adhesiveness and resilience. At the same time, its cohesion is comparable to pea protein. Furthermore, it exhibited a rheological profile close to the control formulation (CM1). Microstructural analysis revealed the presence of lipid droplets trapped in the aqueous phase of the formulation, a crucial characteristic for emulsions essential in the preparation of mayonnaise. These findings suggest that peach protein isolate can serve as a viable alternative in preparing mayonnaise analogs, offering characteristics similar to conventional vegan products. Finally, peach pomace, a co-product of industrial pulp processing, rich in bioactive compounds and pectin, was evaluated. This study aimed to perform a sequential pressurized liquid extraction (PLE) for the fractionation of peach pomace, recovering phenolic extracts and pectin. These separated fractions were evaluated and new functional properties were determined. The alternative method (PLE) was compared to sequential low-pressure extractions conducted by Soxhlet (SOX) and thermal agitation extraction (HSE) as standard procedures. The results indicate that PLE at 40°C provided a higher yield of phenolics (10.31 mg GAE/g), exhibiting antioxidant potential, by DPPH, ABTS and FRAP. The pectin yield was 20 % by HSE, while PLE reached a maximum yield of 14 %. The functional properties of the pectin fraction, both samples showed good emulsification and foaming capacity and stability, with a higher value for the HSE sample. Therefore, PLE can be considered an alternative method to add value to peach by-products, promoting the concept of upcycling. This study presents innovations for peach seeds and pomace and clarifies the techno-functional properties of the fraction rich in protein and pectin, which have shown promise for applications in food formulations.

Keywords: Functional properties; co-products; emerging techniques; pectin; protein; mayonnaise analog.

RESUMO EXPANDIDO

Introdução

O pêssego (*Prunus persica*), pertencente à família Rosaceae, é amplamente valorizado por suas qualidades sensoriais, como sabor, textura e suculência, além de seu elevado valor nutricional. No entanto, o processamento industrial desse fruto gera uma quantidade significativa de coprodutos, como sementes e bagaço, que são subutilizados. As sementes de pêssego são ricas em ácidos graxos e proteínas, enquanto o bagaço é uma fonte abundante de compostos bioativos, como fenólicos e pectina, que possuem propriedades funcionais importantes para a indústria alimentícia.

Objetivo

O principal objetivo deste estudo foi valorizar os subprodutos do pêssego por meio da utilização de métodos de extração verde, aplicando uma abordagem de upcycling, visando o aproveitamento sustentável desses materiais.

Metodologia

A composição centesimal dos subprodutos foi determinada de acordo com os procedimentos oficiais da AOAC, abrangendo análises de umidade, cinzas, lipídeos, proteína, fibra bruta e carboidratos (determinados por diferença). Para o desengorduramento das sementes, foram utilizadas a extração com fluido supercrítico (SFE) e o método Soxhlet (SOX). A composição das frações lipídicas foi analisada por cromatografia gasosa (GC). As características morfológicas das sementes antes e após o tratamento com SFE foram avaliadas por microscopia eletrônica de varredura (MEV). Após o desengorduramento, diversas técnicas de extração proteica foram aplicadas, incluindo extração assistida por micro-ondas (MAE), extração com líquido pressurizado (PLE), extração assistida por ultrassom (UAE) e extração com aquecimento e agitação (HSE). O teor de proteína foi determinado pelo método Kjeldahl, e suas propriedades funcionais foram avaliadas por meio de análises de turbidez, ponto isoelétrico, solubilidade, emulsão, formação de espuma, grupos sulfidrila livres, hidrofobicidade,

espectroscopia no infravermelho com transformada de Fourier (FTIR) e calorimetria exploratória diferencial (DSC).

No capítulo sobre a formulação de maionese, diferentes versões do produto foram desenvolvidas e avaliadas quanto às características qualitativas, incluindo cor, textura, microestrutura e viscosidade. No capítulo que abordou o bagaço de pêssego, as técnicas de extração PLE e HSE foram aplicadas, e os extratos foram analisados quanto ao conteúdo de compostos fenólicos, flavonoides e capacidade antioxidante (utilizando os métodos DPPH, ABTS e FRAP). Além disso, foram quantificados carotenoides e açúcares redutores, e os compostos fenólicos foram caracterizados por cromatografia líquida de alta eficiência (HPLC). A fração de pectina foi analisada quanto ao grau de esterificação, solubilidade em água e óleo, capacidade emulsificante, formação de espuma, teor de ácido galacturônico e viscosidade,

Resultados e Discussão

As sementes de pêssego foram desengorduradas utilizando extração com fluido supercrítico (SFE-CO_2), resultando na recuperação de até 29,53%±0,45 de óleo. Posteriormente, a fração proteica foi extraída usando os métodos convencionais de agitação térmica (HSE) e as técnicas alternativas MAE, PLE e UAE, com solução de NaOH como solvente. O método PLE proporcionou o maior teor de proteína (95,10%±0,98), seguido pelo HSE (55,09%±0,35), indicando que o PLE, com apenas 10 minutos de extração, foi mais eficiente na recuperação de proteínas com melhores propriedades funcionais, mostrando potencial para purificação proteica.

Na formulação de um análogo de maionese, os resultados indicaram que a proteína isolada de pêssego (PSP) apresentou um perfil de cor semelhante ao da maionese vegana comercial (CM1). A análise de textura revelou que a amostra PSP compartilha características físicas comparáveis às proteínas de soja e soro de leite em termos de dureza, adesividade e resiliência, enquanto sua coesão foi similar à da proteína de ervilha. O perfil reológico da PSP também se aproximou ao da formulação controle (CM1). A análise de microestrutura demonstrou a presença de gotículas lipídicas aprisionadas na fase aquosa da formulação, característica essencial para emulsões no preparo de maionese. Esses resultados sugerem que a proteína

isolada de pêssego pode ser uma alternativa viável na produção de análogos de maionese, com propriedades semelhantes aos produtos veganos convencionais.

Em relação ao bagaço de pêssego, rico em compostos bioativos e pectina, o estudo utilizou extração líquida pressurizada (PLE) sequencial para recuperar extratos fenólicos e pectina. As frações foram avaliadas e novas propriedades funcionais foram identificadas. O método alternativo PLE, comparado às extrações de baixa pressão por Soxhlet (SOX) e HSE, apresentou maior rendimento de fenólicos (10,31 mg GAE/g) e potencial antioxidante pelos métodos DPPH, ABTS e FRAP. O rendimento de pectina foi de 20% por HSE, enquanto o PLE atingiu 14%, ambos com boa capacidade de emulsificação e formação de espuma, sendo a amostra HSE superior.

Considerações Finais

Conclui-se que o PLE é um método promissor para agregar valor aos subprodutos do pêssego, promovendo o conceito de upcycling. Este estudo contribui para o aproveitamento sustentável de sementes e bagaço de pêssego, elucidando as propriedades tecnofuncionais das frações ricas em proteína e pectina, que apresentam grande potencial para aplicações em formulações alimentícias.

LIST OF ABBREVIATIONS

ABTS - 2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid
DTNB - 5,5-dithio-bis-2-nitrobenzoic acid - Ellman's Reagent
ANS - 8-anilino-1-naphthalene sulfonic acid
DNS - acid 3,5 dinitrosalicylic
CER - Constant Extraction Rate
DES - Deep Eutectic Solvent
DE - degree of esterification
DSC - Differential Scanning Calorimetry
DC - Diffusion Controlled phase
DPPH - by 2,2′-Diphenyl-1-picrylhydrazyl antioxidante
DTNB - Ellman's Reagent- 5,5-dithio-bis-2-nitrobenzoic acid
EAI - emulsion activity index
ESI - emulsion stability index
FER - Falling Extraction Rate
FRAP - ferric reducing-antioxidant power
FS - Foam stability
FA - Foaming ability
FTIR - Fourier Transform Infrared Spectroscopy
GAE - galacturonic acid
GC - Gas Chromatography
HSE - heat steering agitation
HPLC - high-performance liquid chromatography
Ho - hydrophobicity
MAE - Microwave-assisted extraction
MC - methylation content
ORC - oil retention capacity
PSP - peach seed protein
PLE - Pressure Liquid Extraction
RS - reducing sugars
SEM - Scanning Electron Microscopy
SDS - sodium dodecyl sulfate
SOX - Soxhlet
SFE - Supercritical Fluid Extraction
SDG - Sustainable Development Goals
TPA - Texture perfil analysis
TCC - total carotenoids content
TFC - total flavonoids content
TPC - total phenolics content
TRS - total reducing sugar
UAE - Ultrasound Assisted Extraction
UN - United Nations
WRC - water retention capacity

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CONCEPTUAL DIAGRAM

Why?

- Peach (*Prunus persica*) is a relevant fruit in terms of world production volume;
- Peach processing generates a large number of by-products such as pomace and stone (seed and seed shell);
- Studies indicate that peach by-products are rich in oil, bioactive compounds, and protein, presenting relevant biological activities;
- Novel extraction techniques may be an alternative to minimize environmental impacts and add value to peach by-products;
- By-products reuse contribute to the concepts of circular economy, biorefinery, and upcycling;
- Product design methodology can contribute to developing new food products based on the food industry by-products.

What has been done?

- The published literature about peach by-products is mostly related to the extraction of bioactive compounds from peach pomace and seeds;
- Oil extraction from peach by-products using supercritical fluid extraction (SFE);
- Some works were found related to protein extraction using other seeds cakes, and none were found using microwave assisted extraction (MAE), pressurized liquid extraction (PLE) and ultrasound assisted extraction (UAE) using seed peach;
- The functional properties of different seed cakes have been studied, but properties of peach proteins recovered by alternative extraction methods are still not evaluated;
- Several studies have been exploring using other by-products concerning the concept's circular economy, biorefinery, and upcycling, but we were not found for by-products from peach;
- Only few works were found concerning the extraction of valuable compounds from peach pomace;
- Techno functional properties of pectin extract obtained from pomace not yet evaluated;

Hypotheses

- It is possible to extract proteins using microwave assisted extraction (MAE), pressure liquid extraction (PLE), and ultrasound assisted extraction (UAE) techniques;
- Functional properties of proteins can be improved if alternative extraction techniques are used for the recovery of this relevant components;
- It is possible to elaborate a plant-based product from protein extracted from peach seeds
- Peach pomace can be an alternative source of valuable components such as phenolic compounds and pectin.

CHAPTER 1– INTRODUCTION AND OBJECTIVES

1.1 INTRODUCTION

The search for sustainable industrial processes has guided the production of chemicals and fuels from biomass-based economies (ARORA et al., 2018). However, millions of tons of by-products from the food processing industry are currently generated worldwide, being disposed of in landfills, used in composting or for animal feed (RICO et al., 2020). According to PLAZZOTTA *et al.*, (2020), around 2.4 million tons of peach by-products are generated annually worldwide, while in Brazil, considering the peach production from 2019, about 18 tons of peach waste were generated. Additionally, the industry is dealing with increasing interest in naturalness as a current and future trend to global trades (BATTACCHI et al., 2020). The companies, aware of the consumer's demands, are continuously looking for innovation in the recovery processes to maintain the natural characteristics of the obtained products. Therefore, elucidating the product design concept is of high relevance to contribute for the development of food products, warranting natural attributes.

Besides, it is essential to change the form of production, converting the linear economy into a sustainable circular bioeconomy (AWASTHI et al., 2021) .The philosophy of the dominant economic model of “take, make and dispose” must be urgently transformed into a sustainable model (MAINA; KACHRIMANIDOU; KOUTINAS, 2017). For that, the concept of upcycled foods, which is the use of food ingredients or processed food materials, that would, otherwise, be discarded, must be properly understood (GOODMAN-SMITH et al., 2021). Applying the concepts of circular economy and biorefinery is decisive to recover high-added value molecules, since the growing demand for processed foods increases the generation of residues or by-products from the processed foods (BANERJEE et al., 2018).The biorefinery concept arises from the need to reduce the existing dependence on fossil-based resources to cover the rising demand for energy, fuels, chemicals, polymers and oil (DE LA TORRE et al., 2019). Then, food by-products or residues are appealing biomasses with valuable chemical compounds, i.e., proteins, lipids, pectin, phenolic compounds, among other substances. Mostly, these biomasses are not fully exploited, with insufficient knowledge about their nutritional and economic values (BANERJEE et al., 2018). Some fruit residues have already been studied considering the biorefinery concept, such as

pineapple, orange, mango, and banana (ARORA et al., 2018; BANERJEE et al., 2019; DE LA TORRE et al., 2019; NARANJO; CARDONA; HIGUITA, 2014). Otherwise, investigations related to peach residues are mostly focused on the extraction of bioactive compounds and oil associated with bioactivities. Therefore, information is still lacking on improving the use of peach by-products, for instance for the extraction of proteins from the seeds and pectin from the pomace, aspects of interest from the present research associated with alternative extraction methods, and biorefinery and product design approaches. These aspects show the innovativeness and relevance of the present research proposal.

1.1.1 Main objective

The main objective of this study is the valorization of peach by-products using green extraction methods and an upcycling approach.

1.1.2 Specific objectives

- To determine the proximate composition of the peach processing by-products (seed and pomace);
- To recover the lipid fraction from peach seeds using supercritical fluid extraction (SFE) and Soxhlet (SOX) method, and to determine the oily fractions composition by gas-chromatography (GC);
- To recover protein fraction from peach seeds by using the alternative techniques MAE, UAE, and PLE, with alkaline water as a solvent, and compare with the traditional heat stirred extraction (HSE) method;
- To determine the protein content (Kjeldahl method) and the protein yield from peach seeds recovered by MAE, UAE, PLE and HSE techniques;
- To evaluate the chemical, physical and functional properties of the protein fractions from peach seeds;
- To develop five formulations of mayonnaise from peach seed protein (PSP), pea, soy, whey and compared with commercial vegan mayonnaise;
- To analyze functional properties: color, rheological, texture profile and microstructure of vegan mayonnaise;

- To recover the phenolic compound and pectin fraction from peach pomace using PLE and HSE using ethanol and citric acid as solvents, respectively;
- To determine the total phenolics content (TPC) and profile by LC-MS/MS methods from the extracts recovered from the peach pomace by PLE and HSE;
- To characterize the pectin fraction (pomace) Physico-chemical and functional properties of the pectin-rich;
- To evaluate the composition of the pectin fraction recovered.

CHAPTER 2 – LITERATURE REVIEW

From biorefinery to food product design: peach (*Prunus persica*) by-products deserve attention

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2.1 INTRODUCTION

The search for sustainable industrial processes has guided the production of chemicals and fuels from biomass-based economy (ARORA et al., 2018). However, millions of ton of by-products from food processing industry are currently generated worldwide, being disposed of in landfills, used in composting or for animal feed (RICO et al., 2020). The industry is dealing with the increasing interest in naturalness as current and future trends in global trades. The companies, aware of the consumers demands, are continuously looking for process innovations to maintain the natural characteristics of the products. Therefore, understanding the concept of product design is highly relevant for the development of food products, warranting naturalness attributes. Firstly, the highest challenge for companies is understanding the customer's necessities and capture their attention, transforming the consumer's ideas into chemical and physical parameters of the final product, improving its appeal (TAIFOURIS et al., 2020). Therefore, in order to attend these demands, the companies must follow modern and innovative technologies to provide competitive and sustainable products, resulting in optimized food production with less water and energy consume, and also better use of edible and non-edible parts of food raw materials and supplies. Consequently, the companies need to improve the design, optimization, and development of different formulations and techniques, before introducing new food products to the market (GRANATO et al., 2020).

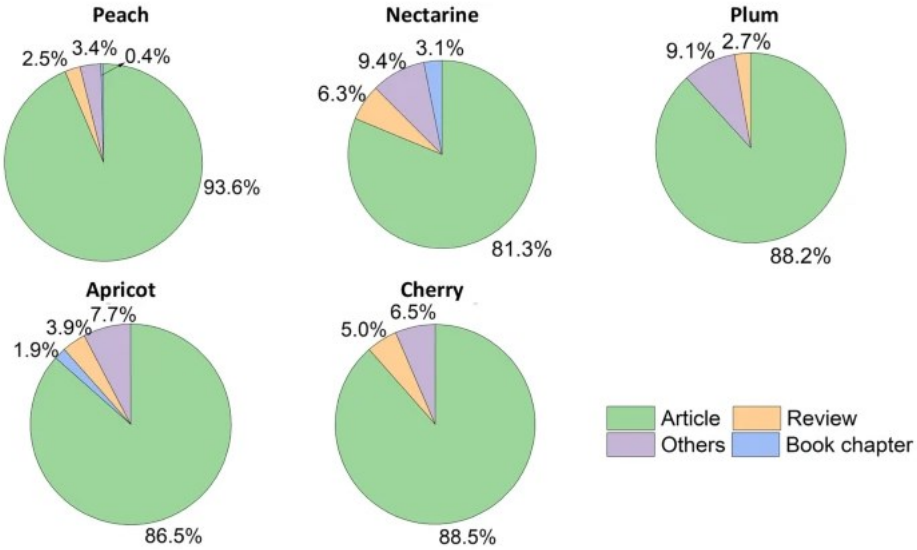
Besides, it is essential to change the form of production, converting the linear economy into a sustainable circular bioeconomy. The philosophy of the dominant economic model of “take, make and dispose” must be urgently transformed into a sustainable model (MAINA; KACHRIMANIDOU; KOUTINAS, 2017). For that, the concept of upcycled foods, which is the use of food ingredients or processed food materials, that would, otherwise, be discarded, must be properly understood (GOODMAN-SMITH et al., 2021). Applying the concepts of circular economy and biorefinery is decisive to recover high-added value molecules since the growing demand for processed foods increases the generation of residues or by-products from the processed foods (BANERJEE et al., 2018, 2019). Mostly, these biomasses are not fully exploited, with insufficient knowledge about their nutritional and economic values (BANERJEE et al., 2018). Some fruit residues have already been studied considering the biorefinery concept, such as pineapple, orange, mango, and banana (BANERJEE et al.,

2018; DE LA TORRE et al., 2019; NARANJO; CARDONA; HIGUITA, 2014). However, no similar data have been found for peach by-products. Therefore, a search of Scopus database (http://www.scopus.com) was performed using a different set of Booleans concerning peach and its residues or by-products from studies published from 2010 to 2021. The search considered the title, abstract and keywords of the published works, and the results are presented in

Figure *2-1*.

Regarding documents, the first search included the terms “peach” AND *Prunus* AND residue OR waste OR “by-product", showing 236 documents, represented by research articles (93.6 %), book chapters (0.4 %), review articles (2.5 %), book chapter (0.4 %) and other (3.4 %). In order to expand the search and comparing with other fruits of the same genus such as nectarine, plum, apricot, and cherry, and using the same Booleans (Figure *2-1*), the results showed the peach fruit represented highest quantity of article (93.6 %), followed cherry (88.5 %) and plum (88.2 %). These searches demonstrate the growing interest in peach by-products, although, there is still a lack of reviews to support the researches in this area. Those studies found from this search are related to wood residues from the peach plantation and the removal of pharmaceutical compounds using systems with different stone residues. Therefore, the present review deals with the contribution of peach by-products bringing a combined product design approach to the biorefinery concept considering its use as biomass, and as a potential source of bioactive compounds, highlighting *in vivo* studies.

Figure 2-1 - Publications about peach, nectarine, cherry, apricot and plum from 2010 to 2022 according to SCOPUS Database Platform (www.scopus.com).



Source: Elaborated by the author.

Food production suffered major transformations in recent years, mostly stimulated by the increasing demand for sustainably processed foods, which must be considered safe, fresh, natural and with high-nutritional value (GRANATO et al., 2020). Due to the COVID-19 pandemic, the search for natural components with bioactivities such as anti-inflammatory has deeply increased because they may play a crucial role in many diseases, including viral infections. This is explained by the use of products enriched with bioactive compounds, which can contribute to human health and the immune system (BENVENUTTI et al. 2021).

2.2 PEACH FRUIT AND ITS INDUSTRIAL PROCESSING

Customers widely appreciate peach fruits (*Prunus persica*) for their taste, texture, juiciness, and nutritional value. According to genomic and phenotypic evidence, some reports accredit its origin in southwest China (PATRA; BAEK, 2016). Belonging to the *Rosaceae* family (NOWICKA; WOJDYŁO, 2019), it is estimated that there are currently more than 400 cultivars worldwide (LI; WANG, 2020). The peach is one of the most variable fruit species representing diverse international germplasm. This fruit has different shapes, sizes, seeds, peel, and pulp colors (red, white, or yellow flesh). Peach

has a climacteric peak, indicating it continues the respiration process after harvest and ethylene increase readily. Then, because it is highly perishable, cold storage after harvesting is an important ally in reducing respiration rates and maturity (MINAS; TANOU; MOLASSIOTIS, 2018). The peach fruit is divided into three parts. The first is the pulp or mesocarp, corresponding to 75.2 % of the fruit weight. The pulp is juicy, yellow and usually has an acid and sweet taste, varying widely, and with pH on average from 3.50 to 4.00. (FEATHERSTONE, 2015) The second part is the peel (exocarp), which represents 22.5 % of the fruit. The last part is the stone, the endocarp, which is formed by the seed (inside), covered by a hard shell, denominated seed shell or kernel shell. The seed represents 5 to 12.5 % of the fruit weight depending on the peach specie (DE FRANÇA SOUSA; PALMEIRA GOMES, 2018; NOWICKA; WOJDYŁO, 2019). In general, the stones consist an average of 6 % of seed and 94 % of seed shell (UYSAL et al., 2014). According to the Food and Agriculture Organization Corporate Statistical Database (FAOSTAT 2020), the world production of peaches and nectarines in 2020 was over 24.5 million tones. Peaches and nectarines are very similar, and belong to the same specie (*Prunus persica)* and family (Rosaceae). For that reason, the data are presented combined (CARRASCO et al., 2013). In 2020 production of the five continents were: Asia 73.6 %, Europe 14.9 %, Americas 7%, Africa 4.3 %, and Oceania 0.3 %, where China, Spain, Italy, Türkiye, Greece, Iran and USA, are the world leader countries in peach production (FAOSTAT 2020). In 2020, Brazil occupied the 15th world position, with peaches and nectarines production of 201,880 ton.

Besides the economic importance, peaches have several nutritional benefits due to their high content of vitamin A, potassium and organic acids, sugars, and minerals conferring interesting nutritional value (MANZOOR et al., 2012) This fruit is highly consumed and destined for different food products such as jellies, sweets, and tea, among others. Besides, peaches are also used in formulations of various cosmetic products (VÁSQUEZ-VILLANUEVA; MARINA; GARCÍA, 2015a). Canned peaches in syrup cover 93 % of the total processed peach products, while peach jam covers 6 % and peach juice only 1 % (KAMENIDOU; RINI ZIMITRA-KALOGIANNI; MATTAS, 2002).

The peach industrial process depends on the final product, with the most popular represented by peach in syrup (canned or glass vessels) and concentrated peach puree, where the last is used as a ingredient for formulations such as baby food, juice, jams, pulp

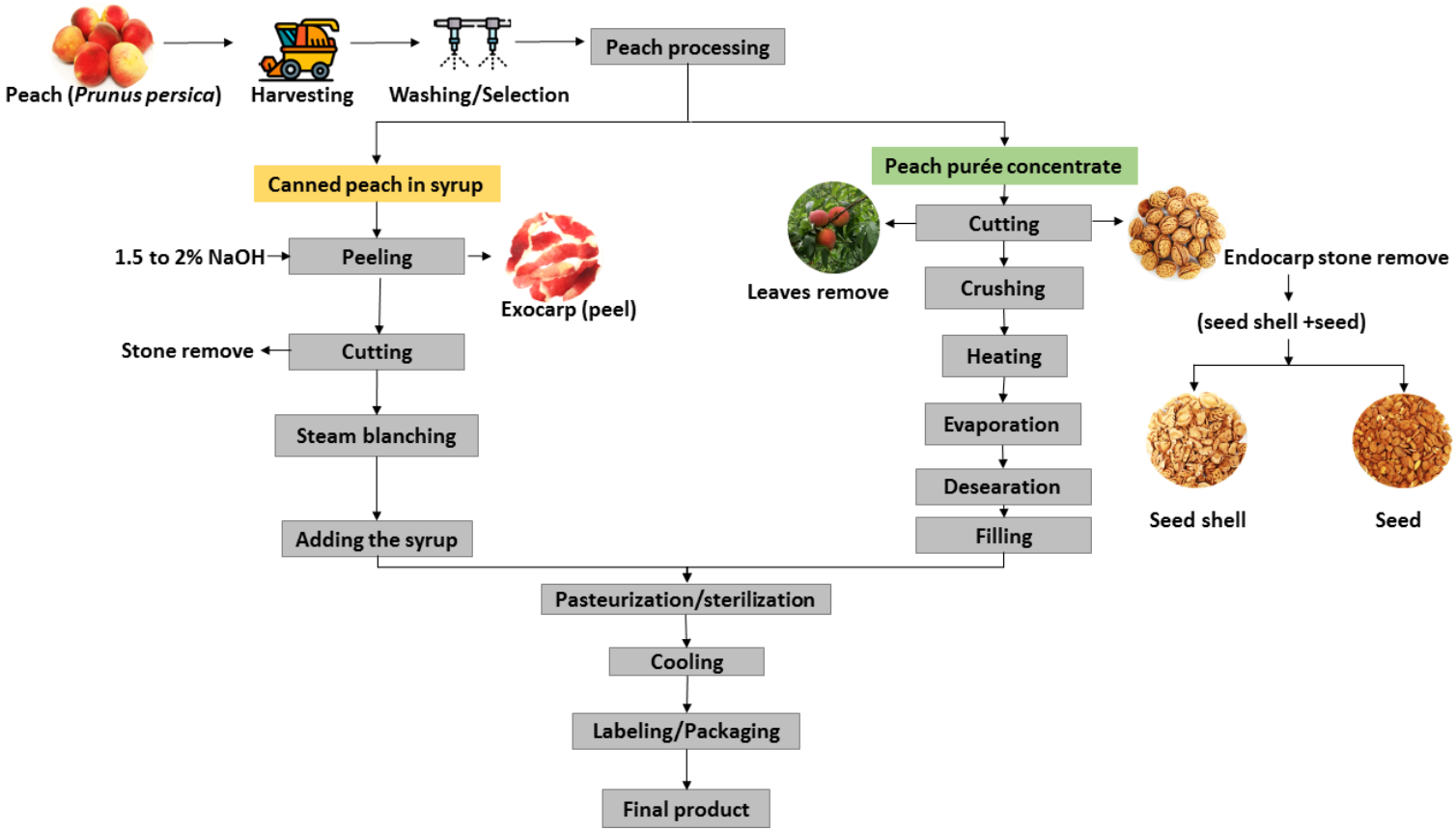
and yogurt (AKTAĞ; GÖKMEN, 2021). Figure 2-2 shows a flowchart of peach processing for these featured products and their respective generated by-products.

Basically, the processing of peaches in syrup consists of: harvesting, selection, peel chemical removal (sodium hydroxide solutions from 1.5 to 2 % concentration, near boiling temperature) (FEATHERSTONE, 2015), cutting stone removal and steam blanching. It is followed by filling the syrup at the glass or canned packaging, for final pasteurization. Peach purée concentrate is obtained by washing/selection, and removing the leaves and stone formed by seed shell and seed, (Figure 2-2), crushing, heating (90 - 95 ºC), evaporation (60 - 75 ºC), deaeration, filling and sterilization (105 - 120 ºC) and purée concentration (AKTAĞ; GÖKMEN, 2021), which is ready for packaging and commercialization.

The main residues generated from peach processing are peel, pomace, and stone. According to (PLAZZOTTA et al., 2020), around 15 million metric ton of peach are processed worldwide each year to produce juices, with approximately 10 % of discard, depending on the fruit's ripeness. Then, based on the annual global production, and considering 10 % of residues or by-products, according to (PLAZZOTTA et al., 2020), above 2.4 million ton of peach by-products are generated each year globally. Then, considering the previously mentioned Brazilian production in 2020, about 20 thousand ton of peach by-product are generated each year in Brazil. The increase in fruit harvesting and processing, and the lack of proper handling methods and infrastructure, also increase the generation of by-products, which are poorly explored worldwide. Studies demonstrate that peach by-products contain oils (seed), anthocyanins (pomace), pectin (peel and pomace) , and proteins (seed) (FARAVASH; ASHTIANI, 2008; PAGÁN et al., 2001; VÁSQUEZ-VILLANUEVA; MARINA; GARCÍA, 2015a).

The by-products from peach processing are disposed in landfills and or used as animal feed, burned, or used in steam production. In order to improve the value of peach by-products. New strategies have been developed and applied to obtain relevant substances from peach by-products. This way, bioactive compounds, vitamins, pectin, phenolics could be recovered for further use in pharmaceutical, food, and cosmetic industry. Therefore, the following topics discuss relevant aspects for better use of peach by-products, and are valuable for applying of product design methodology to obtain derived products from the peach processing chain.

Figure 2-2- Processing steps of peach (purée concentrate and canned peach in syrup) and their by-products obtained.



Source: Elaborated by the author.

2.3 CHEMICAL COMPOSITION

Considering the peach by-products represented mostly by peel, pomace and stone (seed + seed shell), it is important to investigate the components from these constituents, to evaluate their potential uses.

Different studies have shown that fruit peels present several beneficial substances, such as flavonoids, hydroxycinnamic acids, flavanols, anthocyanins and carotenoids, which are highly accumulated in the peel compared to the pulp of various fruits (MICHAILIDIS et al., 2021). (PATRA; BAEK, 2016)detected peach peel compounds such as chlorogenic acid, catechin, epicatechin, rutin, and cyanidin-3-glycoside. In another study, SAIDANI et al., (2017), detected the many phenolic compounds from peach peel for exemple: Flavonols (quercetin-3-galactoside; quercetin-3-O-glucoside plus quercetin-3-o-rutinoside; kaempferol-3-O-glucoside), Hydroxycinnamic acid (neochlorogenic acid; p-coumaroylquinic acid; chlorogenic acid; 4-caffeoylquinic acid; caffeoylquinic acid derivative) and anthocyanin (cyanidin-3-O-glucoside). According to (SAIDANI et al., 2017) the content of flavonoids found in peach peels ranged from 39.3 to 245 mg equivalent of catechins/100 g of fresh weight (fw) in nine different peach cultivars while in pulp these values were lower, ranging from 8.18 to 112 catechins/100 g of fresh weight. In this study, Chlorogenic acid was the main hydroxycinnamic acid found in the peel, and the quantified value ranged from 6.74-31.2 mg/100 fw. In sequence, neochlorogenic acid, whose value was 1.02 -7.98 mg/100 fw, and lastly, the identified compounds were the anthocyanins that went from 0.24-17.6 mg cianidine-3-glycoside/100 g fw. The authors observed that those detected compounds were more in the peel than in the peach fruit pulp. In addition, another interesting molecule found by REDONDO et al., (2021) verify higher amounts of quercetin in the peach peel (7.1 mg 100/g fw) than in fruits such as plum (5.8 mg 100/g fw) and apricot (6.4 mg 100/g fw).

On the other hand, NOWICKA; WOJDYŁO, (2019) evaluated the seeds of 20 different peach varieties. The authors detected a (+)-catechin amount that ranged from 49.49 - 250.79 mg/100 g dry matter (dm). The flavan-3-ols dimers, procyanidin B1 and procyanidin B2, were detected in most seeds, with average content of 150.65 and 28.12 mg/100 g dm, respectively. The group of hydroxycinnamic acids was the second main polyphenolic group detected in peach seeds, after flavan-3-ols. These phenolic acids

ranged from 130.94 mg to 2275.95 mg/100 g. In addition, another important compound It is responsible for the yellow color of the fruit is the carotenoid compound. LARA et al., (2020) mentioned the main carotenoids found were β-carotene and xanthophylls (mono- or di-hydroxylated carotenoids), zeaxanthin, β-cryptoxanthin, and violaxanthin.

The peach pomace is formed of pulp and peel a by-product from the juice processing containing the fruit peel and pulp. Several studies have shown that peach pomace is rich in pectin (FARAVASH; ASHTIANI, 2008), while peach pulp has phenolic compounds and anthocyanins, with contents varying with peach species (CEVALLOS-CASALS et al., 2006). Caffeic acid, protocatechuic, and gallic acid were also detected in peach pomace (EL DARRA et al., 2018). For instance, the methanolic extract presented from 100 to 449 mg of chlorogenic acid equivalent (CGA)/100 g of fresh pulp weight. While main anthocyanins identified was cyanidin 3-glycoside. The values varied from 6 to 37 mg of cyanidin 3-glucoside equivalents/100 g fresh pulp weight. LIU et al., (2018), evaluated the antioxidant potential from peel and pulp of four varieties of peach from China. Methanol/acetone extracts showed that peels have higher phenolic content, from 45.5 to 64.8 %, compared to the pulp. ABIDI et al., (2015) evaluated the antioxidant capacity, and aroma compounds from nectarine *Prunus persica* (L. Batsch) genotypes. The results indicated that the fruit is a good source of phenolic compounds, especially ascorbic acid. The authors identified more than 60 volatile compounds, including 10 carboxylic acids, 10 aldehydes, 5 alcohols, 3 esters, 12 ketones, 8 lactones, and 12 terpenoids.

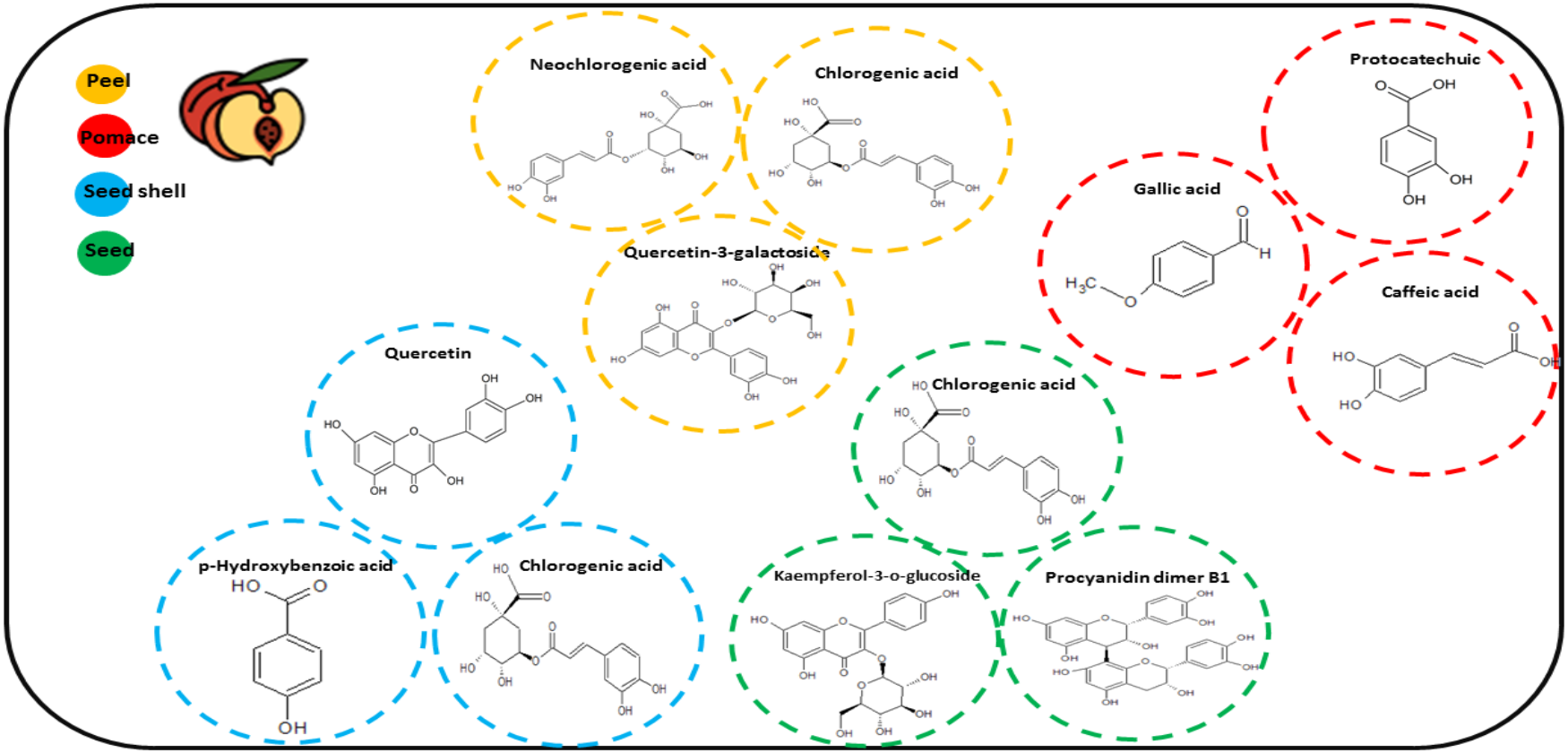
Finally, the lignocellulosic composition of peach stone consists mainly of lignin, cellulose, and hemicellulose, forming complex bonds on cell walls, where the lignin provides physical protection around the sugar fraction (BURATTI et al., 2018). UYSAL et al., (2014), indicated peach stone (seed shell + seed) composed of 46 % cellulose, 14 % hemicellulose, and 33 % lignin. For Instance, HONG et al., (2021)characterized the phenolic acids from the ethanolic extract (70 %, 20 mL) from peach seed shell. Identified the main components Hydroxycinnamic acids group were p-Coumaric acid 4-O-glucoside, 3-Caffeoylquinic acid, 3-Feruloylquinic acid, 3-p-Coumaroylquinic acid, m-Coumaric acid, Hydroxycaffeic acid. The second group was Hydroxybenzoic acids: Ellagic acid acetyl-xyloside, Protocatechuic acid 4-O-glucoside, 2-Hydroxybenzoic acid, 2,3-Dihydroxybenzoic acid, 3-O-Methylgallic acid. The third group was Hydroxyphenylpropanoic acids: 3-Hydroxy-3- (3-hydroxyphenyl) propionic acid. The

fourth group was Hydroxyphenylpropanoic acids: Dihydrocaffeic acid 3-O -glucuronide and 3-Hydroxy-3-(3-hydroxyphenyl) propionic acid. Regarding the anthocyanins, the main detected one was Cyanidin 3-O- (2-O- (6-O- (E) -caffeoyl-D glucoside) -D-glucoside) -5-O-D-glucoside. The results could suggest the applications of these seed shell fruit wastes in other food, feed, nutraceutical, and pharmaceutical industries.

The main phenolic compounds already detected from the peach by-products (peel, pomace and seed and seed shell) are highlighted in Figure 2-3, based on the works by (EL DARRA et al., 2018; HONG et al., 2021; NOWICKA; WOJDYŁO, 2019; SAIDANI et al., 2017). The main groups of detected compounds were: hydroxycinnamic acids, flavonols, hydroxybenzoic acids and procyanidins, which were detected by various researchers on peach by-products.

Besides, some studies have evaluated that peach seeds are also rich in fatty acids and protein. For instance, (HAO et al., (2019) used gas chromatography–mass spectrometry (GC-MS) to define the fatty acid composition from peach seed oil, indicating 86 % of unsaturated fatty acids, within the total fatty acids content. The main unsaturated fatty acids were oleic (55.2 %) and linoleic (30.8 %) acids; while the main saturated fatty acids were palmitic (7.97 %), stearic (2.37 %), and α-linolenic (0.11 %) acids. Peach seed extracts were obtained by SHUKLA; KANT, (2020a) used Soxhlet with different solvents, petroleum ether, chloroform, ethyl acetate, ethanol, and water. The results provided 29.36 % of protein and 7.48 % of crude fat from the dry seed. In addition to the high protein and fat contents, peach seeds are also rich in phenolic compounds (GONZÁLEZ-GARCÍA; MARINA; GARCÍA, 2016; SÁNCHEZ-VICENTE et al., 2009).

Figure 2-3- The main compounds and their respective chemical composition structures of the by-products (pomace, peel and seed).



Source: Elaborated by the author.

Then, peach by-products are considerable sources of nutrients such as lipids, proteins, fibers, and carbohydrates, and the centesimal composition for peach by-products (pomace, peel and stone) is summarized in Table 2-1. For instance, the oil and protein content from peach seeds represents 48.4 % and 26.7 %, respectively, (GETTENS, 2016; (LAZOS, 1991; RAHMA; EL-AAL, 1988) while the fiber content from the peach peel represents 57 % (DE ESCALADA PLA et al., 2012; YANGILAR, 2016), which makes seed and peel as an interesting by-products. Besides, the most recent and relevant works about the chemical composition of peach by-products are summarized in Table 2-2. It shows the highest relevance of peach peel as a research subject, compared to other peach by-products, with most compounds evaluated by various liquid chromatography methods.

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Table 2-1 Average centesimal composition of peach by-products, in percentage (%) of dry samples.

Fruit by-product	Carbohydrate	Fiber	Protein	Lipids	Ashes	Reference
Pomace	13.06	38.85	5.32	0.37	2.13	(PAGÁN; IBARZ, 1999; PAGÁN *et al.*, 2001; SARKAR; CHOUDHURY; SARKAR, 2014)
Peel	31.00	57.48	9.01	2.88	4.30	(PLA *et al.*, 2012; YANGILAR, 2016)
Seed	12.91	4.0	26.77	48.41	3.82	(GETTENS, 2016; LAZOS, 1991; RAHMA; EL-AAL, 1988)

Table 2-2 Summarizes data obtained from the chemical composition from peach by-products and the identification methods used. The data comprises publications last recent studies from 2015 to 2021.

Source	Work proposal	Identification technique *	Chemical composition	References
Peel	Identify anthocyanins from peach peel; the regulatory role of 1-methylcyclopropene and ethylene on anthocyanin accumulation, and the mechanism of ethylene-mediated inhibition of anthocyanin biosynthesis in the fruit.	HPLC-TOF-MS	Cyanidin 3-Glucoside, Cyanidin 3-Galactoside, Cyanidin 3-Rutinoside, Delphinidin-3-Glucoside, Petunidin-3-Galactoside and Petunidin-3-Glucoside.	(ZHANG *et al.*, 2022b)
	Identify and quantify the phenolics profile	UPLC-MS	Quercetin-3-Galactoside (FO1), Quercetin-3-O-Glucoside Plus Quercetin-3-O-Rutinoside (FO2) And Kaempferol-3-O-Glucoside.	(SAIDANI *et al.*, 2017)
	Determine the polyphenols profile and antioxidant capacity from peach peel	HPLC-DAD	Flavonols (Quercetin-3-Rutinoside, Quercetin-3-Glucoside, Quercetin-3-Rhamnoside and Kaempferol-3-Rutinoside).	(STOJANOVIC *et al.*, 2016)

	and pulp, of 6 different peach cultivars and one nectarine cultivar			
	Report the phenolic and vitamin C composition, in vitro antioxidant potencies and metal chelating activity of pulp and peel for five peach cultivars	HPLC-DAD	Hydroxybenzoic Acid (Protocatechuic Acid), Two Hydroxycinnamates (Chrologenic Acid, Neo-Chlorogenic Acid), One Flavan 3-Ols ((+)-Catechin) And One Flavonol (Quercetin-3-Rutinoside)	(LIU; CAO; JIANG, 2015)
	Determine a time course of UV-B-stimulated transcription of genes involved in UVR8 signaling, in phenolics biosynthesis and their transcriptional regulators; phenolics quantification from peach peel.	UHPLC-ESI-QTOF-MS	Cyanidin (2-(3,4-Dihydroxyphenyl) Chromenyl-3,5,7-Triol; Anthocyanins), (+) - Catechin (Flavanols), Luteolin (3 ′, 4 ′, 5,7-Tetrahydroxyflavone; Resveratrol (3 ,4 ′, 5-Trihydroxy-Trans-Stilbene; Stilbenes), 5-Pentadecylresorcinol (Alkylphenols), Hydroxycinnamic Acids, Sesamine (Furofuran Lignans) and Matairesinol (Dibenzylbutyrolactone and Dihydroxydibenzylbutane Lignans.	(SANTIN *et al.*, 2019)
	Recover phenolic compounds from the stone from peach (Prunus persica L.), nectarine (Prunus nucipersicaL.), plum (Prunus domesticaL.) and apricot (Prunus armeniaca L.).	HPLC-PDA; LC-ESI-QTOF-MS/MS	Gallic acid, Protocatechuic acid, p-Hydroxybenzoic acid, Chlorogenic acid, Caffeic acid, Catechin, Epicatethin, Epicatechin gallate, Quercetin and Kaempferol	(HONG *et al.*, 2021)
Seeds	Separate components from *Prunus persica* kernel for possible development of anti-inflammatory, analgesic, and antipyretic medicinal agents from natural resources.	LC-ESI-MS/MS	Amigdalin, Prunasin, Apigenin O-pentoside, Methylated flavonoid haxoside and Naringenin O-hexoside	(ELSHAMY *et al.*, 2019)
	Identify the peptides from peach seeds and their relation with the protective effect of genotypes in which they were identified.	RP-HPLC-ESI-Q-TOF	Peptides contained high amounts of hydrophobic amino acids and imidazole-containing amino acids	(HERNÁNDEZ-CORROTO; MARINA; GARCÍA, 2018)

	Evaluate the oxidative stability, thermal behavior, antioxidant activity, phenolic content, and physicochemical properties of *Prunus persica* kernel oil	GC	Palmitic acid, Palmitoleic acid, Stearic acid, Oleic acid and Linoleic acid	(SODEIFIAN; SAJADIAN, 2021)
	Peptides in fraction PSH-3 kilodaltons (kDa) from peach seeds	LC-Q-TOF-MS/MS	Peptide isoleucine–tyrosine–serine–proline–histidine (IYSPH)	(VÁSQUEZ-VILLANUEVA; MARINA; GARCÍA, 2015)
Pomace	To assess the binding capacity of the soluble peach fiber (SPF) as influenced by the microfluidization pretreatment and cellulase hydrolysis.	HPAEC-PAD	Lactose, glucose, xylose,mannose, and fructose, rhamnose and anrabinose	(XU *et al.*, 2015)

***** HPLC-TOF-MS (high-performance liquid chromatography coupled mass spectrometry time-of-flight); UPLC–MS (Ultrahigh-pressure liquid chromatography coupled mass spectrometry); HPLC DAD (high-performance liquid chromatography diode array detection); UHPLC/QTOF ((Ultrahigh-pressure liquid chromatography coupled quadrupole-time-of-flight); UHPLC-ESI-QTOF-MS (Ultrahigh-pressure liquid chromatography coupled to a quadrupole-time-of-flight high-resolution mass spectrometer via an electrospray ionization system); HPLC-PDA LC-ESI-QTOF-MS/MS (high-performance liquid chromatography photodiode array and liquid chromatography electrospray ionization quadrupole-time-of-flight mass/mass spectrometry); LC-ESI-MS/MS (liquid chromatography electrospray ionization mass/mass spectrometry); RP-HPLC-ESI-Q-TOF (reversed-phase high-performance liquid chromatography coupled to electrospray-ionization quadrupole-time-of-flight mass spectrometry); GC (gas chromatography); LC-Q-TOF-MS/MS (liquid chromatography quadrupole-time-of-flight mass/mass spectrometry); HPAEC-PAD (high performance anion exchange chromatography coupled with a pulse amperometric detector).

2.4 RECOVERY OF VALUABLE COMPONENTS FROM PEACH BY-PRODUCTS

Extraction methods are the most common operations used to obtain natural extracts from raw materials, for further use as a food ingredient (Zhang et al. 2018). Several extraction techniques can be used, from classical, or conventional procedures, to emergent or alternative methods. Maceration and Soxhlet (SOX) are within the conventional and well established most used methods. Otherwise, pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) are within the alternative procedures (RUDKE; DE ANDRADE; FERREIRA, 2020). Nowadays, various studies report the use of emergent tools due to their advantages over conventional methods, such as high yields or selectivity, and use of solvents generally recognized as safe (GRAS). Besides, these alternative methods are fast, have low energy and solvent consumption, and can be very selective towards target compounds. Then, these combined advantages can designate these methods as ecological processes with less environmental impact than traditional procedures (MARTINS *et al.*, 2020). Various studies involving different extractions methods for peach by-products are analyzed as follows.

Plazzotta et al., (2020) studied the pomace from peach juice processing. Microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE), both methods using ethanol: water (70:30) at a 80°C as solvent, were applied for the recovery of antioxidant compounds from the peach juice pomace. The optimization of the processes indicates the best extraction conditions as 540 W during 50s for MAE, and using 23 % amplitude during 120s for UAE. The extract samples recovered from the frozen peach residues provided higher flavonoids, anthocyanins, and vitamin C, compared to extracts from dry residues. Plazzotta et al., (2021) used two extraction methods with ethanol (70 %) to obtain unpurified bioactive compounds for peach pomace, a conventional thermal extraction, at 50 ºC for up to 90 min, and the pulsed electric field (PEF) technology with an energy input of 0.0014 to 2.88 kJ/kg. The processing time required for PEF was much lower, in the order of microseconds (μs), compared to conventional thermal extraction (average time of 40 min, reaching similar extraction

efficiency than PEF). The bioactive fraction recovered by PEF from peach pomace reached total flavonoid content (TFC) of 86 mg QE/100 dm, total phenolics content (TPC) of 386 mg GAE/100 g dm, and DPPH value of 80 mg TE/100 g dm. Otherwise, the conventional thermal treatment, set at 50°C, provided maximum values for TPC, TFC and DPPH of 524 ± 27 mg GAE/100 g dm, 108 ± 1 mg QE/100 g dm and 143 ± 7 mg TE/100 g dm, respectively. Then, although the traditional extraction method shows samples with the highest values of TPC, TFC, and DPPPH, the PEF method is a promising technology because it has lower energy costs, than traditional methods, and recovers valuable compounds.

Adil et al., (2007) recovered polyphenols from peach pomace by subcritical extraction with CO_2 added with ethanol. The effect of the process parameters, pressure (20 - 60 MPa), temperature (40 - 60°C), ethanol concentration (14 – 20 % by weight), and extraction time (10 - 40 min), were studied. The results indicate that the increase in all parameters significantly contributes to the recovery of total phenolics content (TPC), and DPPH performance to evaluate the antioxidant activity. The optimum conditions were 51 MPa, 52.3°C, 20 % ethanol content and 40 min of extraction. TPC values were up to 0.26 mg of gallic acid equivalent/g of dried sample, with AE of 1.5 mg of DPPH /mg dried sample. Faravash and Ashtiani, (2008) recovered pectin from peach pomace in an acid medium (with HCL at 60°C) followed by precipitation with ethanol (96 %). The experiments were carried out using different HCl volumes (35, 65, 105, and 130 mL), acid washing times of 30, 60, and 180 min and ethanol:extract ratios of 0.5, 1, and 1.5. The maximum pectin recovery yield was 9.94 ± 0.2 %, obtained using 65 mL of HCl, the extraction yield of 1.5, and the acid washing time of 120 min.

Mezzomo et al., (2010) recovered the oily fraction from peach seed (also called peach almond) by different methods: Soxhlet with different solvents (n-hexane, dichloromethane, ethyl acetate and ethanol), hydrodistillation, and ethanol maceration followed by partitioning with the solvents n-hexane, dichloromethane, ethyl acetate and ethanol. Compared the traditional low-pressure methods with Supercritical Fluid Extraction (SFE) at 30, 40 and 50°C and at 100, 200 and 300 bar, carried out with CO_2 and ethanol as co-solvent in concentrations of 2 % and 5 % (w/w). The Soxhlet with ethanol provided higher yield values compared to SFE. The main fatty acids detected from the oily fraction were oleic and linoleic acids, particularly for the samples recovered by Soxhlet with ethanol: water mixture (50:50 v/v) and SFE with 5 % ethanol at 50°C/300

bar. The authors concluded that peach seeds are an important source of edible oil with high content of unsaturated fatty acids that have health benefits.

Peach seeds were used by (GONZÁLEZ-GARCÍA; MARINA; GARCÍA, 2016) González-García et al., (2016) for the recovery of protein. The seeds were first defatted with hexane and then washed with MeOH/H_2O and acetone/H_2O. The extraction, using a native buffer and a denatured buffer as solvents, was conducted by applying sonication for 10 min, and then with gentle shaking overnight. The recovered protein was precipitated from the extract with acetone, in a 1:2 ratio (extract:acetone), and then stored in freezer overnight. The method of Combinatorial Peptide Ligand Libraries (CPLLs) aids the reduction of the dynamic range of protein concentration, contributing to identify a higher number of proteins than conventional methods. Thus, the results enable identifying of 97 unique genetic products from peach seeds. In addition, 14 peptides previously identified as bioactive peptides were ascertained within protein sequences. The results from this work suggest that the bioactive peptides recovered from peach seeds are valuable and worth investigating, as proteins are important for human nutrition and present high added value.

Several extraction methods have been studied to obtain different valuable fractions from peach by-products. These procedures are summarized in Table 2-3, which contains the extraction data applied to the peach by-products, the solvents and the operating conditions used, and the main results obtained. The data from Table 2-3 represent the most recent publications on this subject. However, these available works have not explored the concept of biorefinery applied to peach by-products, nor discussed the food design approach to value the recovered fractions from peach by-products or residues. These aspects, addressed to improve the peach processing chain, are presented in the next sections.

Table 2-3 Summarized data from extraction procedures applied for peach by-products: methods, solvents, operational conditions; main results. The data comprises publications last recent studies.

Source	Protocol of extraction	Solvent	Parameters of extraction	Compound	References
Peel	Maceration	Methanol and acetone	One gram of the sample and 20 mL of acidic methanol-water (50:50, v/v, pH 2) at room temperature for 1 h. Then 20 mL of 70 % aqueous acetone were added to the residue, followed by stirring, shaking and centrifugation.	Phenolic compounds, flavonoid and L-ascorbic acid and antioxidant assay.	(LIU; CAO; JIANG, 2015)
	Ultrasound	HCl concentrations (0, 0.1, 1 or 2 %)	30 mL of 80 % (v/v) acetone solution and 60 min	Polyphenolic compounds	(STOJANOVIC et al., 2016)
	Maceration	Adding 5 mL of 5 % HPO_3; 10 mL of a mixture of MeOH/H_2O/formic acid (60:38:2 v/v/v); 10 mL of water.	Adding 5 mL of 5 %; (60:38:2 v/v/v); 10 mL	Ascorbic acid, total phenolics, flavonoids, polyphenols and antioxidant capacity and sugar.	(SAIDANI et al., 2017)
	Maceration	1mL +1 mL 95 % EtOH centrifuged 4500 RPM 10 min diluted with 50 % EtOH	Extracted at 25°C by ethanol (absolute, ≥ 99.5 %) (400, 1500 and 250 mL, respectively) (3×72 h)	Total phenol, flavonoids and carotenoids	(LOIZZO et al., 2015)
Seed	Extraction Soxhlet	Petroleum ether, chloroform, ethyl acetate, ethanol, and water	60 cycles of siphoning was completed with each solvent and extraction was continued until siphon tube became colour less	Alkaloids,carbohydrate, glycosides, inulin, protein, steroids/triterpenoids, fixed oils and fats, flavonoids, phenolic,	(SHUKLA; KANT, 2020)

				compound and tannins, gums and mucilage	
Pomace	Sonication (10 min)	MeOH/H_2O (80:20), acetone/H2O (80:20)	Extract:acetone ratio of 1:2 and centrifuged (30 min, 13,400 rpm)	Bioactive peptides	(GONZÁLEZ-GARCÍA; MARINA; GARCÍA, 2016)
	Ultrasound	5 mL of extraction buffer (100 mM Tris–HCl (pH 7.5)	1 min at 30 % of amplitude, after centrifugation at 4000 g for 10 min, the supernatant was collected, added to 10 mL of cold acetone, and kept in the fridge for 15 min	Peptides	(VÁSQUEZ-VILLANUEVA; MARINA; GARCÍA, 2015b)
	Microwave (MAE) and ultrasound assisted extraction (UAE)	Ethanol:water (70:30)	Optimal MAE (540 W, 50 s) and UAE (23 %, 120 s)	Polyphenols, flavonoids, anthocyanins, and vitamin C	(PLAZZOTTA et al., 2020)
	Pulsed electric fields (PEF) and Conventional thermal treatment (CTT)	Ethanol:water (70:30)	PEF at 50°C and 90 min and PP dispersions were thus treated at 0.8–10 kV/cm, using 4–30 monopolar pulses of 4 μs	Bioactive extracts	(PLAZZOTTA et al., 2021)

2.5 BIOREFINERY CONCEPT FOR THE PROCESSING OF PEACH BY-PRODUCTS

The biorefinery concept arises from the need to reduce the existing dependence on fossil-based resources to cover the rising demand for energy, fuels, chemicals, polymers and oil (TORRE *et al.*, 2019). Biomass and food by-products are also considered a potential source of high-added value chemicals such as fermentable sugar, furfural, citric acid, pectin, proteins, lipids, phenolic compounds, among other substances (MACIEL-SILVA; MUSSATTO; FORSTER-CARNEIRO, 2019; ORTIZ-SANCHEZ et al., 2021). The food by-products are abundant, renewable and inexpensive bio-residues, or biomasses, which can be fully processed to obtain valuable bioactive compounds, as an interesting alternative from economic and environmental aspects (GULLÓN et al., 2020). Besides, the adequate processes for the recovery of these valuable compounds, allied with the appropriate pre-treatments to modify the cellulose, lignin, and hemicellulose, converting the lignocellulosic biomasses, are of upmost relevance, contributing to efficient conversion of underestimated by-products, such as peach residues (MANARA et al., 2014). These methods can be applied, individually or combined, in a biorefinery approach, preferably represented by ecological and sustainable processes to attend the Sustainable Development Goals (SDG) of the United Nations (UN). The growing interest in this area expands the application of new technologies, enabling the development of innovative products and businesses, expanding the portfolio of the existing companies, and contributing to achieving new markets.

Newest approaches have been showing the use of a deep eutectic solvent (DES) as a relevant alternative for the recovery of valuable phytochemicals from food products (BENVENUTTI; ZIELINSKI; FERREIRA, 2019).ŞAHIN; BILGIN, (2022) used a lactic acid/glycerol mixture (1/1) and the addition of water (10, 30 and 50 % v/v) to recover bioactive compounds from the peach peel. The results showed that with the use of DES with the addition of 50 % water, at the optimized condition of 90 s and 8400 rpm, the recovered extract presented a polyphenol content of 10.68 mg-GAE/g-dried and chlorogenic acid 2.69 mg/g-dried. SKIBA; VOROBYOVA, (2022) used a green solvent named NADES (natural deep eutectic solvent) composed of lactic acid, glucose, and water (5:1:3) to obtain phenolic compounds from peach pomace, and the main identified compounds were chlorogenic acid (1.04 mg/g extract), gallic acid (1.04 mg/g extract),

and ferulic acid (0.115 mg/g extract). This extract was compared with plasma-assisted extraction with water, which provided higher antioxidant activity in terms of 50 % inhibition concentration (IC_{50} of 1.05 mg/mL) compared to the extract recovered by NADES (IC_{50} of 1.3 mg/mL).MÁRMOL et al., (2021) evaluated the extraction of bioactive compounds from peach pomace using ethanol: water (w/w) ratio (70:30) as a solvent, and followed by homogenization at 1600 rpm for 2 min. The results showed that the most representative phenolic component recovered was the ellagic acid (28.26 mg/100 g). These works indicate that peach presents interesting phytochemical compounds detected in peach by-products, such as peach peel and pomace. Using green solvents at different extraction methods is an alternative to promote the valorization of these biomasses, which is in line with the biorefinery concept. Nevertheless, the optimization of a selected extraction procedure, preferably performed on a laboratory scale, is high relevance for the industrial processing of agro-industrial by-products (MARTINS et al., 2020).

In Brazil, there is a huge generation of residual biomasses, mostly agro-industrial residues or by-products, which has not yet received much attention as a direct energy source, such as biodiesel. Biodiesel is a fuel from vegetable oils, animal fats, or residual vegetable oils(LONGATI; BATISTA; CRUZ, 2020). Then, according to Ribeiro et al., (2018), the Southern Brazilian region has the higher concentration of biodiesel processing plants, and the most used raw materials are soybean oil (70 %), beef fat (17 %), and used frying oil (1 %). These examples show the limited use of other biomasses, although several studies have been indicating other abundant and renewable plant-based biomasses as valuable alternatives for the application of the integrated biorefinery concept. Among these commodities and important fruits, we can mention: soy, orange, corn, tamarind and mango (LONGATI; BATISTA; CRUZ, 2020; MA et al., 2021; MARTINS et al., 2020; MONTEIRO et al., 2021; ORTIZ-SANCHEZ et al., 2021). As already mentioned, because peach is an important worldwide commodity, some researchers have already proposed the use of peach residues as a source of high-value products. However, there is a lack of studies related to the use of integrated paths, in a biorefinery concept, for the valorization of this residue. Therefore, besides biofuel production, the biorefinery concept involves biomass conversion to produce biofuels, energy, and also valuable chemicals (CHERUBINI, 2010). Consequently, following the concept of circular bioeconomy (principles related to reuse, reduction and recycling), the process integration, allows the

recovery of different products from the original biomass and also improves sustainability. Yet, the industrial plants have this considerable challenge in combining these two concepts.

2.6 BIOACTIVITIES ASSOCIATED TO PEACH BY-PRODUCTS

Most literature studies about peach by-products are related to the bioactive compounds and antioxidant activity associated with peel, pomace, seeds and seed shell. To justify the significance of these peach by-products, as sources of valuable biological active components. The most recent and relevant studies from literature concerning peach by-products are presented and discussed below, highlighting the main biological activities associated, such as antimicrobial, antioxidant, antidiabetic, anti-obesity, anti-inflammatory and anti-cerebral ischemia.

2.6.1 Antioxidant activity

Hong et al., (2021) evaluated the antioxidant activity and the phenolics content of the ethanolic extract (70 %, 20 mL) from the shell of the peach seed. The results show the total phenolics content (TPC) of 0.47 (mg gallic acid equivalents (GAE)/g), total flavonoids content (TFC) of 0.18 (mg quercetin equivalents (QE)/g), total tannins content (TTC) of 0.07 (mg catechin equivalents (CE)/g), while the antioxidant activity by 2,2′-Diphenyl-1-picrylhydrazyl antioxidant assay (DPPH) was 0.98 (mg ascorbic acid equivalents (AAE)/ g), while by ferric reducing-antioxidant power (FRAP) assay was 0.54 (mg AAE/g), and by 2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay was 0.43 (mg AAE/g), with total antioxidant capacity (TAC) of 0.27 (mg AAE/g), estimated by phosphomolybdate method. According to Hong et al., (2021), these results show higher radical scavenging capacity compared to other seed shells such as nectarine (0.23 mg AAE/g), plum (0.21 mg AAE/g), and apricot (0.25mg AAE/g).

Another relevant study by Nowicka and Wojdyło, (2019) show that the total polyphenol content of 20 different peach seed cultivars ranged from 3.8 to 12.7 g/100 dry matter. At the same time, the cyanogenic glycoside ranged from 17.4 to 245.7 mg/100 of dry matter. The main polyphenols compounds found were Flavan-3-ols group: (+)-catechin, (−)-epicatechin, (−)-epicatechin gallate; Polymeric procyanidins group: (B1, B2 and procyanidin A2). In addition, the major phenolic acid was found Hydroxycinnamic

acid: (chlorogenic acid, neochlorogenic acid, 3-O-p-coumaroyluinic acid and cis-5-p-coumaroylquinic acid, 2-O-caffeoyl-L-malate, phenylpropanoid o-diphenol phaselic acid) and Hydroxybenzoic acid: ellagic acid, dilactone of hexahydroxydiphenic acid. The peach seeds were also characterized by high antioxidative potential and the ability to inhibit enzymes linked to obesity, type 2 diabetes and Alzheimer's disease. Generally, confirmed that peach seed could be a valuable source of polyphenols used by the pharmaceutical industry.

Redondo et al., (2021) detected and quantified the polyphenols and evaluated the antioxidant activity from peach pulp, peel and seeds, from fruits in commercial maturity. The evaluated extracts were recovered by methanol: water solution (80:20; v:v). The results from peel extract showed the presence of quercetin 6.2 mg/100g, chlorogenic acid 3.9 mg/100g, and neochlorogenic acid 7.1 mg/100g, while from seed extract, the detected and quantified compounds were quercetin (5.5 mg/100g), chlorogenic acid (4.0 mg/100g), neochlorogenic acid (4.9 mg/100g), and ferulic acid (5.7 mg/100g). As for the pulp, the results detected were: quercetin (5.4 mg/100g), chlorogenic acid (1.2 mg/100g), and neochlorogenic acid (4.8 mg/100g). These values suggest that the peach seeds present more valuable phenolic compounds than other fruit parts. The antioxidant activity provided by the extracts also confirmed this behavior. For instance, the results by FRAP method provided the highest antioxidant capacity for peach seed (3.3 mmol Trolox/100 FW), while peach peel was 2.2 mmol Trolox/100 FW and pulp 0.2 mmol Trolox/100 FW. However, it is essential to note that the values vary with the conditions used to recover the extracts, such as type of solvent, extraction technique, fruit maturity, and harvest conditions (soil, geographical aspects and plant variety). The study by Redondo et al., (2021) reveals the potential of peach by-products as a source of bioactive compounds with different potential uses, such as in pharmaceutical, cosmetic, food, or animal feed formulations. It is also important to highlight that the pomace by-product contains peach pulp and. El Darra et al., (2018) recovered polyphenols from peach pomace by solid-liquid ratio 1:10 (w:v) extraction using β-cyclodextrin and ethanol as solvent. Identified the compounds Gallic acid, Caffeic acid, and Protocatechin from the recovered extract. The mixture of β cyclodextrin and ethanol selectively enhances the extraction of gallic and caffeic acids with yields 220 and 328μg/g dry matter, respectively. However, ethanol recovers more the protocatechinic (4899μg/g dry matter).

Mokrani; MADANI, (2016) investigated the effect of extraction parameters (solid/liquid) of peach fruit (*Prunus persic*a *L*): solvents (ethanol, methanol, acetone and water), time (30 - 450 min) and temperature (25 - 70°C), for the recovery of total phenolic compounds (TPC), total flavonoid compounds (TFC) and antioxidant capacity. Optimized extraction conditions show high levels of TPC, 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity (DPPH-RSA) and FRAP from the peach fruit extracts, with values of 363 GAE/100 g, 48 % inhibition percentage and 317 AAE/100 g respectively. Then, it is possible to highlight that the peach by-products have relevant bioactive compounds compared to the edible part of the fruit. Thus, further studies should be carried out to enhance the use of these by-products as a source of relevant bioactive compounds and add value to these biomasses.

2.6.2 Antimicrobial activity

Patra; Baek, (2016) evaluated the biological synthesis of gold nanoparticles (AuNPs) generated from peach peel aqueous extract. The extract showed strong antibacterial synergistic activity when combined with the positive control substances kanamycin (inhibition zone from 9.38 - 20.45 mm) and rifampicin (inhibition zone from 9.52 - 25.23 mm), and also synergistic anticandida activity when combined with amphotericin B (inhibition zone from 10.09 - 15.47 mm) for five pathogenic *Candida* species. In addition, the extract presented strong antioxidant potential by elimination of 1,1-diphenyl-2-picrilhidroxil radical, nitric oxide, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical. Although detected the significant antimicrobial potential for peach peel, there is a lack of information related to this important bioactivity associated with peach by-products, which should be further investigated.

2.6.3 Anti-diabetic and anti-obesity activity

Kan et al., (2020) investigated the effects of peach peel phenolic extract in preventing lipid accumulation and intestinal microbiota composition of mice with high-fat diet-induced obesity. Extracts were obtained with methanol by ultrasound technique (400 W, 40°C) for 3 hours. The recovered extracts showed that peach peel significantly inhibited lipid accumulation in mice fed a high-fat diet. Meanwhile, treatment with peach

peel phenolic extract regulated the expression levels of mRNA involved in lipid metabolism. Furthermore, the results showed that the extract regulated the intestinal microbiota composition, increasing the relative abundance of *Lactobacillus, Bacteroides, Lachnospiraceae, Prevotellaceae Alloprevotella, Akkermansia, Roseburia* and *Ruminococcus*. This effect might be attributed to the phenolic content and composition of the peach peel extracts, showing the relevance of this by-product to provid a functional food additive to improve lipid metabolic abnormalities associated with obesity. Based on what has been discussed so far, few studies have evaluated the potential of by-products activities. Thus, some studies are still focused on the edible part of the fruit. For instance, Nowicka et al., (2018) provide important insights extract properties from the pulp of *Prunus persica* L. Batsch, obtained by sonication with methanol:H_2O (80:20 %, v/v) + 1 % HCl at 20°C for 15 min. Results from *in vitro* analysis show the potential to inhibit enzymes relevant for the management of type 2 diabetes (α-amylase, α-glucosidase) and obesity (pancreatic lipase). The yellow peach extract, with high carotenoids content (varieties Harrow Diamond and Harrow Beauty), showed high inhibitory activity toward porcine pancreatic lipase.

Sharma et al., (2018) evaluated the antidiabetic activity of extract from leaves of *Prunus persica*. The extract prepared by percolation with 90 % ethanol at room temperature, was concentrated under reduced pressure at 50–55°C and used at *in vivo* assays with adult male rats. The results show that 200 mg $_{extract}$/kg provided significant anti-hyperglycemic activity. Improvement in rat body weight and lipid profile were also observed by streptozotocin induced diabetic rat model. The extract good performance suggests its possible use as a natural drug candidate for diabetes mellitus treatment.

Song et al., (2019) investigated the anti-obesity effect of extracts from flowers of *Prunus persica* L. Batsch, obtained with distilled water at 100°C for 2 hours under reflux. The results from *in vivo* assays using peach flower extract show a significant reduction in body weight, abdominal fat mass, serum glucose, alanine transaminase and aspartate aminotransferase levels, and liver and spleen weights from mice. Besides, reduced the levels of glucose, cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and leptin, while the levels of AST/ALT and adiponectin/leptin ratios and adiponectin were increased. The results suggest an anti-obesity effect of the extract in obese mice, which may improve the hepatic lipid metabolism by reducing lipogenesis and increasing fatty acid oxidation.

Wang et al., (2017) evaluated the antidiabetic activity potential of peach gum polysaccharides, from peach tree trunks and fruits. The peach gum exudate was dissolved in boiling water at a ratio of 1:100 (w/v), and filtered to obtain the supernatant solution. The concentrated solution was precipitated overnight in four folds of ethanol, for purification, and the resulting polysaccharides were dried. The peach gum polysaccharide reduced significantly (42.76 %) the postprandial blood glucose in streptozotocin-induced diabetic mice. Histology and immunohistochemistry results confirmed that the polysaccharide, consisting of arabinose, xylose and galactose, partially restored the pancreatic islets in diabetic mice, and enhanced the expression of pancreatic duodenal homeobox-1, insulin and hexokinase-1. The results may suggest the peach gum-derived polysaccharide as a relevant non-insulin therapy for diabetes treatment, probably due to the presence of the detected compounds.

2.6.4 Anti-inflammatory potential

Elshamy et al., (2019) prepared seed extract from nectarine (*Prunus persica*,variety *nucipersica*) by maceration with methanol 70 %, a fruit from the same family as peach. The acute anti-inflammatory effect of the extract was evaluated by a carrageenan-induced rat hind paw edema test. The nectarine seed extract was administrated in rats at doses of 50 and 100 mg/kg reducing significantly the paw edema by 11 and 47 %, respectively. The results suggest that alcoholic nectarine seed extract is a safe natural anti-inflammatory, providing analgesic activity through central and peripheral mechanisms. Furthermore, the authors evaluated the content of total polyphenols (55.91 ± 5.78 mg/g) and flavonoids (29.89 ± 0.55 mg/g), indicating that the extract is a promising source of these constituents, which are possibly related to the anti-inflammatory effect.

Another interesting study concerning the edible part of the fruit was conducted by Kim et al., (2017) which prepared white-flesh peach extract (WFPE) using ethanol 80 % for 48 hours at room temperature. The study evaluated the effect of the extract on the excretion of nicotine and 1-hydroxypyrene metabolites in smokers and chronic nicotine-induced tissue damage in mice. WFPE inhibited nitrotyrosine expression and inflammatory responses in liver, kidney, and lung tissues of nicotine-treated mice. The results showed that peach extract increased the metabolism of toxic smoke components

of tobacco in smokers and protected normal tissues against nicotine toxicity in mice. Conducted this research for the peach edible part and, although no anti-inflammatory studies were conducted using the by-products, the peach flesh is also present at the pomace, which should be further investigated to observe if it contains similar activity.

Seo et al., (2020) evaluated the anti-inflammatory potential of the methanolic extract from *P. persica* on lipopolysaccharide stimulated glial cells. Glial cells are immune cells from the central nervous system, with a key role in numerous neurodegenerative diseases, including Alzheimer's, Parkinson's and Huntington's disease. Used different parts of the peach fruit (leaves, fruits, and twigs) to obtain the methanolic extracts, prepare by sonication (1,500 W, 40 kHz) frequency for 15 min at 2 h intervals for 3 days. The results indicated that the combined methanolic extracts reduced the transcription of several pro-inflammatory enzymes (nitric oxide synthase and cyclooxygenase-2) and cytokines (tumor necrosis factor-α, interleukin (IL)-1β and IL-6) in lipopolysaccharide-stimulated cells. Furthermore, the extract inhibited the activation of nuclear factors and several mitogen-activated protein kinases necessary for the transcription of the pro-inflammatory mediator. It can be used as a potential therapeutic agent for neurodegenerative diseases and neurotoxicity by suppressing glial cell activation. The evaluated extract presented chlorogenic acid, catechin, quercetin, and quercetin-3-O-glycoside, and the anti-inflammatory effect on PPB on BV2 cells may be due to the presence of phenolics and flavonols.

2.6.5 Cerebral ischemia

Li et al., (2020) studied aqueous extract mixtures from peach seeds and rhubarb (*Rheum palmatum* L) rhizome, and its effect on cerebral ischemia/reperfusion injury (I/R). The extracts with different mixture proportions (peach seeds and rhubarb) (m/v), 1:0, 2:3, 1:1, 3:2 and 0:1. Procedure first soaked for 30 min and decocted for 1 hour then filtered. The results demonstrate that the extracts effectively reduced the neurobehavioral defect scores, areas of cerebral infarction caused by cerebral ischemia and pathological brain tissue injury, and inhibit abnormal increases in inflammatory factors. Also, the extracts alleviated the I/R damage by altering the uncontrolled expression of the ADORA2A protein. The extract that provided the best regulatory effect was obtained from a 1:1 ratio of peach seed: dried rhubarb root.

2.6.6 Other applications

Different applications from peach by-products have been studied and presented in the literature. For instance, dicussed applications in pharmaceuticals, cosmetics, food products, and energy industries. Fratebianchi et al., (2017) used peach pomace as a source of carbon and energy to produce polygalacturonase in submerged cultures using *Aspergillus Sojae*. (PAPAIOANNOU; LIAKOPOULOU-KYRIAKIDES, 2012), studied peach peels as a carbon source for *Blakeslea trispora* culture for carotenoid production. The results indicate 76 % of β-carotene production, and this high performance suggests using this residue as substrate cultures for carotenoids production on industrial scale.

Li et al., (2018) evaluated the chemical characteristics of peach seed shells, the sugar yields and the impact of Deep Eutectic Solvent (DES), formed by choline chloride 1:2: lactic acid, as biomass (seed shell) pre-treatment, affecting physical and chemical properties and lignin extraction. The results from this biomass indicate high apparent density and significant hardness. Enzymatic saccharification of peach seed shells treated with DES provided a high glucose yield (above 90 %). DES pretreatment removed 70.2 % of lignin from the peach seed shell, while the pretreatment with dilute acid and alkali recovered 22.2 % and 48.7 % of lignin. Besides, the enzymatic hydrolysis Cellic® CTec2 and HTec2 recovered up to 90 % sugar yield from the sample pretreated with DES, while the sample pretreated with alkali provided 57.3 % sugar yield and dilute acid was 49.7 %. Thus, this is interesting biomass for the conversion of biofuels and chemicals, with DES presenting a great influence on lignin fractionation.

Uysal et al., (2014) produced activated carbon from peach stone (seed +seed shell) with zinc chloride activation. The first step was carried out at different temperatures, 300°C and 400°C, to obtain the bio-oil. Then, the second step was activating the pre-carbonized coal after impregnation with zinc chloride. The results showed that the temperature had a significant effect on the surface area of the activated carbon and activation temperature, and the bio-oil had fungicidal activity against the *Coriolus versicolor*. In addition, the adsorption capacities of activated carbons for phenol and methylene blue were 51.6-64.9 mg/g and 104.2-121.9 mg/g, respectively.

2.7 PRODUCT DESIGN: FUNCTIONAL FOOD PRODUCT

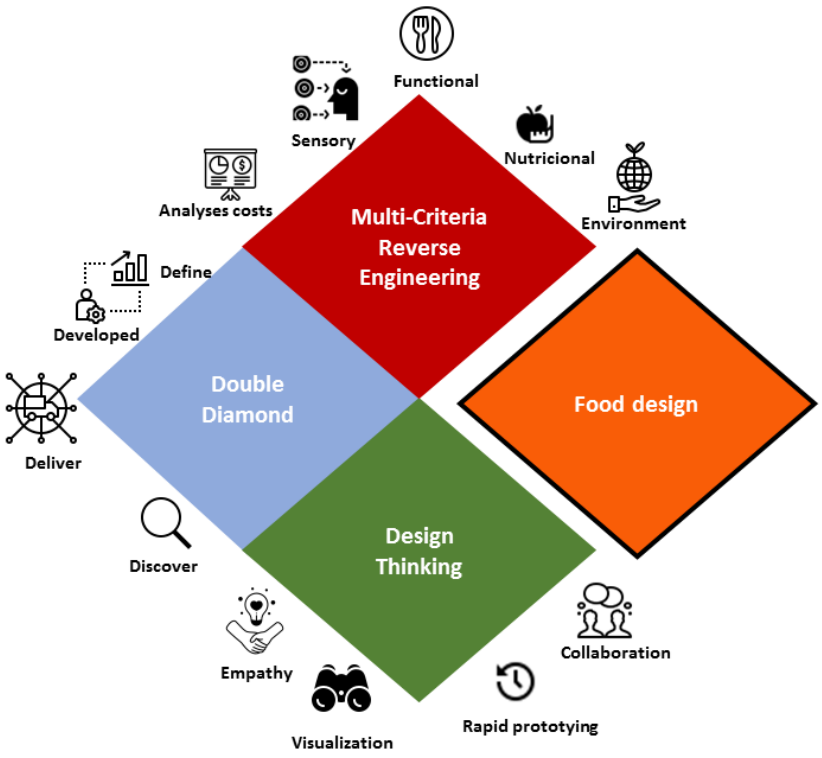
Functional foods are natural or industrially processed food products that, when regularly consumed at satisfactory levels in a diversified diet, have positive health effects beyond basic nutrition (GRANATO *et al.*, 2020). The Functional Food Center (FFC) from USA (Texas, USA) is committed to leading research on functional foods as well as bringing the functional foods to the mainstream worldwide food markets, and introducing a new approach for the definition of functional foods (MARTIROSYAN; SINGH, 2015). This new concept describes functional foods containing biologically active compounds in effective and non-toxic quantities, derived from natural or processed food sources, and clinically tested, based on specific biomarkers, providing health benefits for prevention, management or treatment of chronic diseases or their symptoms (ALONGI; ANESE, 2021) .Therefore, functional foods must be regulated by the Food and Drug Administration (FDA) before approval, and are suitable if they are constituted by compounds Generally Recognized as Safe (GRAS).

Given the above assumptions about functional foods, the increase in various types of chronic diseases has motivated the food industry to develop food products that help consumers to prevent these diseases and provide other health benefits (ALONGI; ANESE, 2021). According to Micheli et al., (2019), the design thinking concept could be utilized in different areas including public policy, education, healthcare, politics, and social and economic development. This concept has emerged in sustainability literature to generate positive economic, social, and environmental impacts, in association with a business transition, food innovation, and applied business innovation (MASSARI et al., 2022). Meanwhile, the use of design thinking, double diamond and multi-criteria reverse engineering methodologies (Figure 2-4) is fundamental to meeting the new consumer's needs, and to understanding the industrial challenges for the use of by-products as a source of bioactive and functional compounds. Therefore, the health benefits from peach by-products (discussed previously) may suggest its industrial use to provide functional foods. Although, there is a long way to be crossed, which can be eased by product design methodology, as discussed below.

The design thinking method is based on solving problems and has become an important approach for food design. According to Olsen, (2015), this methodology can contribute to food innovation using empathy, visualization, rapid prototyping, and

collaboration (Figure 2-4**)**. According to Tkaczewska et al., (2021), the method to create products must be economically and technologically viable, following the aspects: (I) observation and synthesis, (II) visualization and rapid prototyping, and (III) revising and refining. For Batat and Addis, (2021), the design thinking concept is a high contribution to food innovation, when designing sustainable food products and services that respond to the needs of consumers.

Figure 2-4 Methodologies Design thinking, double diamond, and multi-criteria reverse engineering approach in food design.



Source: elaborated by the author.

Another methodology proposed for food design is Multi-Criteria Reverse Engineering (MCRE). This technique simultaneously considers several criteria of the product's properties, increasing the complexity of the problem. For instance, MCRE enables the selection of adequate process conditions for designing or redesigning food, food processes, and food-related systems (THOMOPOULOS et al., 2019). Dima et al. (2020) describe the use of MCRE as a design method for functional foods, taking into

account the following aspects: developing a nutritional, sensorial, and functional evaluation of the food product, attending to the consumer's needs; selecting target bioactive components, its nutraceuticals distribution systems, encapsulation technique, food matrix, or the excipient; choose the packaging method, analyze costs, impacts on the environment, analyze the perception of the food product, and, finally, test the bioavailability of nutraceuticals incorporated into the food product (Figure 2-4), therefore, combining several specific criteria for functional food design.

The Double Diamond model, another product design methodology also known as 4D (SIJTSEMA; FOGLIANO; HAGEMAN, 2020) consists of four steps: discover, define, develop and deliver (Figure 2-4). The first step (Discover) is essential to establish new opportunities, markets and processes, with a search aided by literature. The second step, “Define”, is fundamental to select insights to help demarcate and redefine the study topic. This step enables the establishment of the technologies, ingredients, and packages for use. The third step is to “Develop” the design refine it, and teste it with multidisciplinary teams. This stage involves of developing samples/concepts with different processing, ingredients, packaging, target groups, and sensory research. The last step, “Deliver”, is the final prototype (food design), packaging and distribution, which are tested, produced and launched.

These models can help the product design by combining different tools that can be a solution to obtain innovative products aligned with sustainability, reducing agro-industrial by-products or improving their use and value. Then, based on what has been discussed so far, peach by-products (pomace, peel, seed shell and seed) are relevant biomasses with high application potential (components with valuable bioactivities associated). For instance, could use the recovered products in food, pharmaceutical, and cosmetic formulations. Besides, using these bioactive compounds implies in several processing steps, such as recovery, purification, isolation, identification and protection. Moreover, it is necessary to evaluate the production chain and the different sectors involved, such as logistics, transport, and packaging, where the by-products are present and could be directed to further processing. For instance, by-product handling can consist of drying, milling, extraction, fractionation, and others, to provide different products, which must be evaluated in terms of quality and processing yield (efficiency). Therefore, carefully evaluation of all these “product design” steps may provide the adequate feasibility for the “new process”.

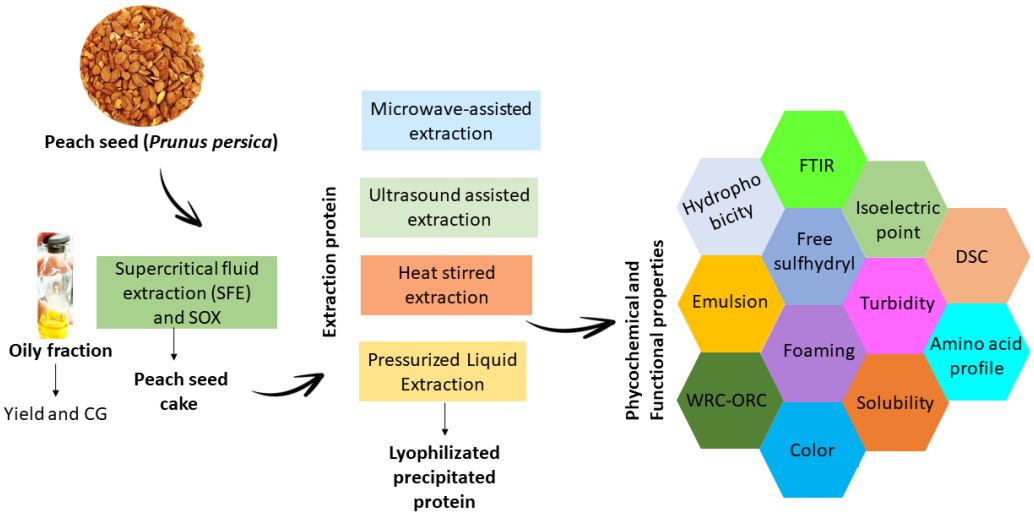
Because of the strong relevance of the 17 SDG from ONU, there is a growing search for greener processes and protocols to obtain bioactive substances from industrial food by-product. To achieve these objectives, it is still necessary to study extraction methods capable of reducing solvent and energy consumption and, also increasing the overall eco-sustainability of the food life cycle.

Some existing business models can serve as inspiration. For instance, the company *Rubian Extracts*, a Brazilian startup, recently developed natural extracts by supercritical CO_2 technology, recovering bio compounds from by-products. Combining biorefinery concepts and circular economy is the key for the sustainable recovery of by-products, for application as food ingredient, with functional properties. Therefore, governmental policies may incentive partnerships between private companies, and researchers as a fast solution for food-product design, seeking benefits for society.

2.8 STATE OF ART

From the literature review presented in this chapter, it is clear that the studies related to peach by-products are focused on the extraction of bioactive compounds, and the recovery of lipids and fibers. In addition, *in vivo* assays using the recovered fractions from the peach by-products have already been studied, such as anti-inflammatory, anti-obesity, and anti-cerebral ischemia activities associated with the peach components. Therefore, there is still a gap to be explored related to peach seed protein extraction using emerging techniques such as PLE, UAE and MAE, and also the evaluation of the functional properties of the recovered protein fraction. In addition, due to the relevant pectin content from peach pomace, it should also be better explored, and few studies focused on improving extraction yields and techno functional properties. So far, no study has been found regarding an approach to the concept of upcycling for better use of these valuable peach fractions to add value to these by-products to expand applications in food design.

CHAPTER 3 – *COMPARING GREEN EXTRACTION METHODS FOR THE RECOVERY OF PROTEIN-RICH FRACTION FROM PEACH SEEDS (PRUNUS PERSICA)*



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3.1 INTRODUCTION

The food industry has increased its interest in alternative protein sources (KAHRAMAN; PETERSEN; FIELDS, 2022). According to RIVERA DEL RIO et al., (2022), the plant-based protein market could constitute up to 15 % of the total protein market until 2035. Moreover, the consume of plant-based proteins from different sources is related to ethnic, cultural, and sustainable aspects (RIVERA DEL RIO *et al.*, 2022). The growth of this market also contributes to increaser the search for alternative protein sources. Besides, more efficient protein production has been focused on plant coproducts as interesting and low-cost sources of protein (ALVAREZ-OSSORIO et al., 2022). As to indicate the seeds as an alternative processing material, it is important to observe the world production of peaches and nectarines in 2020, which was over 24.5 million tones (FAOSTAT, 2020) representing the high amount of underused agro-industrial coproduct. Then, peach seed may be an attractive material due to its high protein content of 27 % (FARAG; BAHAA ELDIN; KHALIFA, 2022).

It is also important to establish the most appropriate and effective method for protein recovery, improving the extraction yield and the quality of the compounds of interest. Emergent extraction technologies have been considered alternative methods to reduce the use of organic solvents, which may contribute to the formation of lysinoalanine, an undesirable component, and also to the increase in Maillard reactions, darkening the resulting product (melanoidin) (CONSTANTINO; GARCIA-ROJAS, 2022). Besides, the extraction and fractionation methods also affect the functional properties of the recovered protein fraction, and help to establish good industrial uses of this fraction as a multipurpose ingredient for food product formulations (KAHRAMAN; PETERSEN; FIELDS, 2022). Some relevant functional properties are hydrophobicity, emulsifying, sulfhydryl groups and foaming characteristics. These properties are decisive attributes in developing new protein-based food products, its characterization for new plant protein sources remains a challenge that must be elucidated. Different methods have been applied to extraction protein vegetable and also improve their functional properties (KAHRAMAN; PETERSEN; FIELDS, 2022). The prevalent approach involves

precipitation, which consists of reaching the protein's isoelectric point using an acidic solution. From this, it is possible to obtain an isolated or concentrated protein, which may add nutritional value if used as an ingredient in a food product (KAMAL et al., 2021; SINGH et al., 2022).

Recent studies have investigated new emerging extraction technologies and their impact on the functional properties of protein fractions recovered from different plant-based sources. Ultrasound Assisted Extraction (UAE) was applied for protein recovery from guamuchil *(Pithecellobium dulce)* seeds and from plum (*Domestica L.*) seeds (GONZÁLEZ-GARCÍA et al., 2014; THALÍA FLORES-JIMÉNEZ et al., 2022), while GUZMÁN-LORITE et al., (2022) used Pressurized Liquid Extraction (PLE) for pomegranate seeds. Microwave Assisted Extraction (MAE) was also applied for protein recovery from sesame bran and pumpkin seeds (GÖRGÜÇ et al., 2020; LIU et al., 2017). These alternative methods are associated with different extraction principles, for instance, cavitation, high-pressure, and electromagnetic waves, respectively (MATHEWS et al., 2023), and it has been demonstrated that they provide high yield performance, improved quality and functional properties of the protein fraction compared to traditional methods like heat-stirred extraction (HSE) (CHANG; JIANG; LIU, 2022). Furthermore, these technologies have lower environmental impact as they use minimal chemicals and mostly non-harmful solvents (KUMAR et al., 2021), compared to traditional methods. Nonetheless, peach seeds have not yet been evaluated by non-traditional methods. Lining up, the concepts of upcycling and biorefinery suggest different routes for using agro-industrial coproducts to obtain different bio-products sustainably (UBANDO; FELIX; CHEN, 2020). Therefore, this study aims to recover protein from peach seed cake using non-conventional methods (MAE, UAE, and PLE) with alkaline water as a solvent and compare the results with the traditional HSE. The effect of the different extraction methods on the quality of the protein fraction was also evaluated in terms of physical, chemical, and functional properties.

3.2 MATERIALS AND METHODS

3.2.1 Raw material

The peach coproduct, represented by the peach stone (endocarp + seed), was kindly provided by Villa Puree Company, located in Santo Antônio do Paraíso, PR, Brazil (coordinates 23°30'42' 'S and 50°38'43"W). First, the seed was separated manually from the stone, and the seed was dried in an oven with air circulation (De Leo, Porto Alegre/RS, Brazil) at 40°C. That stone was dried for 24 hours. Finally, the seeds were ground in a knife mill (De Leo, model EDB-5, Porto Alegre/RS, Brazil), and the average particle diameter obtained was 0.80 mm.

3.2.2 Proximate composition

The proximate composition of the raw material was determined considering the AOAC methods (AOAC, 2012): moisture content (925.09), lipid content (920.39), ash (923.03), crude fiber (962.09) and protein content by Kjeldahl method (954.01) based on a determination of the total nitrogen matter from a sample (AOAC, 2005). The carbohydrate content was estimated using 3,5-dinitrosalicyclic acid (DNS) following the procedure described by MONTEIRO et al., (2021). The results were expressed as a total reducing sugar based standard glucose curve. The proximate composition analyses were performed in triplicate, and the standard deviation was presented.

3.2.3 Defatting the peach seeds

The Supercritical Fluid Extraction (SFE) assays was carried out using the integral grounded peach seeds to obtain the oily fraction and the defatted cake. The SFE was carried out in a high-pressure unit, following the procedure and conditions presented by MEZZOMO et al., (2010). The SFE assays were conducted at 300 bar and 40°C for 150 minutes, with a solvent flow rate of 0.8 $kg.h^{-1}$, using 15 grams of dried and ground peach seeds, and using carbon dioxide (CO_2) as a non-polar solvent (SFE-CO_2) to recover the oily fraction from peach seed. For comparison purposes, the peach seeds were also

defatted using the Soxhlet method with hexane as solvent (SOX-hexane), following the methodology lipid content (920.39) described by AOAC (2005). The defatted assays (SFE-CO_2 and SOX-hexane) were performed in duplicate, and the results expressed as mean and standard deviations.

3.2.4 Characterization of the lipid-rich fraction from peach seeds

The oily fractions from the peach seeds, recovered by SFE-CO_2 and by SOX-hexane, were analyzed by gas-chromatography-mass–mass spectrometry (GC–MS). The samples were prepared according to MARTINS et al., (2022). The equipment model was USA GC 7890 A/MS 5975C GC (Agilent Technologies, California, USA), with 5 % Phenyl Methyl Siloxane column (HP-5MS 30 m x 250 µm x 0.25 µm; Agilent). Firstly, the oily samples (SFE-CO_2 and SOX-hexane) were submitted to esterification, which consisted of 20 µL of the oily fraction hydrolyzed in KOH (1 N) and MeOH for 1.5 h at 55°C. Then, the esterified samples were submitted to methylation through catalysis, performed with H_2SO_4 24 N for 1.5 h at 55°C, and then 3 mL of hexane was added. Finally, the oily samples were centrifuged for 5 minutes, and 1 mL aliquot of the upper phase (hexane) was transferred to the flask and stored (4°C) until GC–MS analysis. The compounds were identified by comparison with a database from the National Institute of Standards and Technology (NIST, 2011), and also based on literature data. Linear retention indexes were determined by comparing the retention times of n-alkane standard samples, submitted to the same chromatographic conditions as the peach seed oily samples. The conditions involved the use of helium as carrier gas at 1.2 $mL \cdot min^{-1}$ flow rate, an injection volume of 1 µL in split mode (1:50) at 250°C, coupled with a solvent delay of 5 min. The initial oven temperature was set at 60°C for 3 min, followed by a gradual increase at 5°C·min^{-1} until reaching 220 °C. The transfer line temperature was held steady at 240°C. The mass spectrometer was operated in EI positive mode at 70 eV, scanning a mass range from 55 to 550 m/z. The entire analytical procedure was completed within a total time frame of 40 min.

3.2.5 Scanning electron microscopy (SEM)

The morphological structure of the integral peach seeds (raw material) and of the defatted peach seed cake (PSC), the resulting solid material after SFE-CO_2 and after SOX-hexane (PSC-SFE and PSC-SOX, respectively), was evaluated by Scanning Electron Microscopy (SEM) as described by FERRO et al., (2019). Briefly, the dried and ground samples are distributed in carbon tapes on the surface of the stubs and then coated with a thin layer of gold. The SEM analyses will be performed in a JEOL (JSM 6390LV) with a tungsten electron source, secondary electron detector, and 10 kV.

3.2.6 Protein fraction recovery by different extraction methods

Different extraction routes were applied for the recovery of protein-rich fractions from peach coproduct (peach seeds), with the extraction methods described as follow.

3.2.6.1 Heat stirred extraction (HSE)

The defatted peach seeds (after SOX-hexane) were used to produce the protein-rich fraction by heat-stirred extraction (HSE). The HSE was carried out as described by KARIMI et al., (2022). NaOH solution was used as solvent at pH 10 (0.2 mol/L NaOH) with a solid-to-solvent ratio of 1:20 at 40°C under continuous stirring for 10 min. Then, the extract was recovered (supernatant) for protein precipitation and yield determination (section 3. 2.6.5). The combination of SOX-hexane followed by HSE represents the low-pressure route (route 1), providing two extract fractions from the peach seeds: an oily fraction and a protein-rich fraction.

3.2.6.2 Pressurized Liquid Extraction (PLE)

The pressurized liquid extraction (PLE) was applied to recover the protein-rich fraction from the peach seed cake defatted by SFE (PSC-SFE). The PLE procedure included packing a jacketed stainless-steel reactor (AISI 316 stainless) with an internal volume of 90 mL as described by GONÇALVEZ-RODRIGUES et al. (2019), which was

kept at 40 ºC by a thermostatic bath. The conditions used were: 1 g of peach seed cake, defatted by SFE, called PSC-SFE, was placed inside the reactor, and the reactor was completed with 60 g of glass beads to avoid preferred paths, and to keep the fixed bed in place inside the extraction vessel. NaOH solution was used as solvent at pH 10 (0.2 mol/L NaOH) and added into the reactor until the pressure of 100 bar (± 2 bar) by an HPLC pumped (Costech SSI Series III) to obtain a solid-liquid ratio of (1:20). The pressure was kept constant by intermittent pumping of water into the reactor for 10 minutes in static mode. Then, the extract was recovered (supernatant) for protein precipitation and yield determination (section 3.2.6.5). The combination of SFE-CO_2 followed by PLE represents the high-pressure extraction route (route 2).

3.2.6.3 Microwave-assisted extraction (MAE)

Microwave-assisted extraction was performed for the peach seed cake after SFE (PSC-SFE) following the procedure described by Torres, et al., (2022). The extractions were conducted in a microwave reactor (MonowaveTM 200, Anton Paar, Austria) using distilled water as solvent, with pH adjusted to 10 (0.2 mol/L NaOH), and a solid/liquid ratio of (1:20). The extraction was carried at 40 ºC for 10 min. Firstly, the PSC-SFE (1 g) was added, and a magnetic stirring bar was placed inside the borosilicate glass flask (G30), and the mixture with the solvent, the rector was closed and placed at the MAE unit. Then, the extract was recovered (supernatant) for protein precipitation and yield determination (section 3. 2.6.5). The experiments were performed in triplicate, and results presented as mean with standard deviations. The combination of SFE-CO_2 followed by MAE represents another alternative extraction route (route 3).

3.2.6.4 Ultrasound-assisted extraction (UAE)

Ultrasound assisted extraction was conducted on the peach seed cake after SFE (PSC-SFE) using the methodology outlined by Prandi et al., (2022) with some modifications. UAE was performed in an ultrasonic bath (Ultrasonic cleaner Unique,

USC-4880, Brazil) operating at 40 kHz, and 220 Watts for 10 minutes, and temperature of 40°C. The defatted seed protein (PSC-SFE) was added to the Becker, and distilled water with pH 10 (0.2M NaOH) was added as a solvent and maintained a solid/liquid ratio (1:20). The liquid samples (extract) were collected in falcon tubes for protein precipitation and yield determination (section 3.2.6.5). The experiments were conducted in triplicate, and results presented as mean with standard deviations. The combination of SFE-CO_2 followed by UAE represents another alternative extraction route (route 4).

3.2.6.5 Protein precipitation and yield

The protein-rich fractions (extracts), obtained by HSE, PLE, MAE, and UAE were centrifuged for 20 min at 3400 rpm (Quimis, Q22TM, Diadema-SP, Brazil), and the supernatant was collected. The pH of the supernatant obtained from each method was adjusted to 4.7 (Kasvi, model K39-0014PA, São José dos Pinhais-PR, Brazil) using HCl of 0.25 M following the procedure described by KARIMI *et al.*, (2022), and then centrifuged for 20 min at 3400 rpm to collect the precipitate. The precipitate was resuspended in 20 mL of distilled water, and the pH adjusted to 7.0 using 0.2 mol/L NaOH. The recovered protein was freeze-dried in a vacuum freeze-drying machine (Liotop, L101, São Carlos-SP, Brazil) and stored at −20°C until use. The protein content from the extract samples was calculated according to Ahluwalia et al., (2020). The experiments were conducted in duplicate, and the results presented as mean with standard deviations.

$$Recovered\ protein\ \% = \frac{M_1\ \times P_{f\%}}{M_2\ \times\ P_i} x100 \qquad \text{Equation 3-1}$$

Where: M_1 is Dry mass extract precipitated (g); M_2 the initial weight of seed cake (g); $P_{f\%}$ is the Protein content from the extract samples (by Kjeldahl method %), and P_i is the Protein content from the peach seed cake (PSC).

3.2.7 Protein fraction characterization

3.2.7.1 Water solubility

The solubility in water of the peach seed protein (PSP) fractions (the protein-rich extracts), recovered by HSE, PLE, MAE and UAE was assessed following Wang et al., (2023) method. Each PSP sample was dissolved in water at 1.0 mg/mL, and the solution was adjusted to pH 7. After, the PSP samples were hydrated for 2 hours, and centrifuged at 5000 g for 15 minutes. The resulting supernatant was collected, and the protein content was determined by Kjeldahl method (AOAC, 2005). The experiments were conducted in triplicate, and results presented as mean with standard deviations. Solubility was calculated using the following equation:

$$Solubility\ (\%) \frac{supernatant\ protein\ \ content}{total\ protein\ content} \times 100 \qquad \text{Equation 3-2}$$

3.2.7.2 Water and Oil Retention Capacity

The water retention capacity (WRC) and the oil retention capacity (ORC) of the PSP samples, obtained by HSE, PLE, MAE, and UAE, were determined in accordance with the methodology by SÁ et al., (2022). Initially, each PSP sample was dissolved in soy oil or in water at a concentration of 0.1 g/mL, for the determination of WRC and ORC, respectively. The solutions were vortexed six times for 1 minute each and settle for 5 minutes. Subsequently, the samples were centrifuged for 15 minutes at 5000 g, and the supernatant was carefully removed and weighed. The experiments were conducted in duplicate, and results presented as mean with standard deviations. Equation 3 was used to calculate WRC and ORC.

$$WRC\ or\ ORC\ (g/g) = \frac{final\ weight\ (g)}{initial\ \ weight\ (g)} \times 100 \qquad \text{Equation 3-3}$$

3.2.7.3 Turbidity

The PSP turbidity was determined using the method described by Chang et al., (2022). The PSP samples were dissolved (1 mg/mL) in a phosphate buffer solution and the absorbance read at 500 nm with a UV-Spectrophotometer to indicate the sample turbidity. The phosphate buffer was used as a control, and the turbidity was defined as the absorbance read. The experiments were conducted in triplicate, and results presented as mean with standard deviations.

3.2.7.4 Color

The color of the PSP samples was evaluated in a colorimeter (Delta Vista, model 450 G SN 7012003357, São Leopoldo-RS, Brazil). The parameters L*, a*, and b* were used to calculate hue angle (H*), whiteness index (WI), and chroma (C) as described in the equations below according to Naik et al., (2022).

$$Hue\ angle\ (H^*) = 180 + Tan^{-1}\left(\frac{b^*}{a^*}\right) \quad \text{Equation 3-4}$$

$$Whiteness\ index\ (Wi) = L^* - 3c^* \quad \text{Equation 3-5}$$

$$Chroma\ (C) = \sqrt{(a^*)^2 + (b^*)^2} \quad \text{Equation 3-6}$$

Where L* represent lightness; + a* is redness and - a* the greenness, while +b* is the yellowish and -b* the blueness.

3.2.7.5 Emulsion activity index and stability activity index

The emulsion activity index (EAI) and the emulsion stability index (ESI) from the PSP samples were determined according to Wang *et al.*, (2023) with some modifications. Firstly, the PSP samples were lyophilized and dissolved in distilled water (10 mg/mL). After, 12 mL of PSP solution and 3 mL soybean oil were homogenized using ultra-turrax equipment (IKA, model T25 digital, São Paulo-Brazil) equipment for 2 min at 12000 rpm.

Afterward, a 30 μL aliquot of the prepared emulsion was mixed completely with 3.0 mL of 0.1 % sodium dodecyl sulfate (SDS) solution (w/v), and the absorbance was measured at 500 nm at 2 min (A_2) and at 30 min (A_{30}). Then, the EAI and ESI were calculated as follows:

$$EAI\ (m^2/g) = 2 \times 2.303 \times \frac{2 \times 2.303 \times A_2 \times D}{C \times (1 - \varphi) \times 10000} \quad \text{Equation 3-7}$$

$$ESI\ (min) = \frac{A_{30}}{A_2} \times 30 \quad \text{Equation 3-8}$$

Where C means protein concentration (0.01 g/mL), D is the dilution factor (100), and φ is the oil volume fraction (20 %).

3.2.7.6 Foaming Ability and Stability

The foaming ability (FA) and foam stability (FS) were determined for the PSP samples according to the method described by Wang *et al.*, (2023) with some modifications. The PSP samples were solubilized in distilled water to achieve suspensions (10 mg/mL) and homogenized ultra-turrax equipment (IKA, model T25 digital, São Paulo-Brazil) at 20.000 rpm for 2 min at room temperature. After homogenization, the foam volume was measured after 2 min of standing and was labeled as V_2, and the foam volume after 30 min was labeled V_{30}. The foaming ability and stability were calculated according to the following equations below:

$$FA\ \% = \frac{V_2}{10} \times 100 \quad \text{Equation 3-9}$$

$$FS\ \% = \frac{V_{30}}{V_2} \times 100 \quad \text{Equation 3-10}$$

3.2.7.7 Isoelectric point

The isoelectric point of the PSP samples was measured through the equipment Malvern, Zetasizer Nanosizer-ZS (United Kingdom) according to described by

QUINTERO-QUIROZ et al., (2022). The PSP was diluted in distilled water at 0.1 % (w/v), and the pH of the solution was adjusted (2-10) using 0.1 M NaOH and HCl solutions. A zeta potential versus pH curve was constructed, and the isoelectric point determined by the curve correspondent to the point where the potential becomes 0 mV.

3.2.7.8 Free sulphydryl (SH) group

The concentration of free sulfhydryl group from the PSP samples was measured according to Wang *et al.*, (2023). Briefly, the PSP samples were dissolved (5 mg/mL) in tris-glycine buffer (0.09 M glycine, 4 mM EDTA, Tris 0.086, pH 8.0). Then, 50 µL of the solution was added with Ellman's Reagent- 5,5-dithio-bis-2-nitrobenzoic acid (DTNB) (10 mmol/L), and the absorbance was measured at 412 nm after 15 min. The concentration of free sulfhydryl groups of the PSP samples was calculated using the equation below:

$$SH(nmol/mg.protein) = \frac{A \times D}{\varepsilon \times C} \times 100 \qquad \text{Equation 3-11}$$

A = Absorbance measured at 412 nm, D = Dilution factor =1, ε = 13600 (M^{-1} cm^{-1}), C = Protein concentration (mg/mL).

3.2.7.9 Surface hydrophobicity (Ho)

The hydrophobicity of the PSP samples was measured according to Mota da Silva et al., (2021). The PSP samples were dissolved in 4mL in phosphate buffer (5 mM, pH 7.0) using different concentrations, from 0.004 to 0.03 (w/v). After, an aliquot (20 µL) of 8-anilino-1-naphthalene sulfonic acid (ANS) solution (8 mM ANS in 5 mM phosphate buffer, pH 7.0) was added to the PSP solutions. The solutions were stored in the dark for 15 min and measured in a fluorescence spectrophotometer (Multileader Infinite TECAN, M200, Switzerland). The excitation and emission wavelengths were 360 nm and 480 nm, respectively. The hydrophobicity (H_o) is expressed as the initial slope of the plot of fluorescence intensity versus protein concentration.

3.2.7.10 Fourier- transform infrared (FTIR) spectroscopy

The FTIR analysis of the PSP samples (obtained by HSE, PLE, MAE, and UAE) was performed in a spectroscopy (Agilent Technologies, model CARY 660, CA, USA), following the methodology by HADIDI et al., (2021). The analysis was conducted to evaluate the chemical structure of the protein, and the FTIR spectra samples were recorded from 400 to 4000 cm^{-1}. Besides, 32 scans were made at 4 cm^{-1} resolutions for each spectrum. Briefly, the samples were mixed with KBr powder and ground in an agate mortar, and subsequently compressed forming a pellet. The KBr pellet was then used to record the reference spectrum.

3.2.7.11 Differential scanning calorimetry (DSC)

The temperature for maximum denaturation of the PSP samples was evaluated by DSC (Jade-DSC Model; Perkin Elmer, SP, Brazil) thermograms following the procedure by WANG et al., (2021). The PSP samples were heated from 25°C to 120°C, at a rate of 10°C/min, and the denaturation temperature was identified as the highest temperature peak from the DSC curve, which corresponds to the calories absorbed during the protein denaturation process.

3.2.7.12 Amino acids profile of the PSP samples

The amino acids profile of the PSP samples recovered by HSE, PLE, and MAE was determined by high-performance liquid chromatography (HPLC) using an Alliance 2695 appliance (Waters, Ma., USA) equipped with a fluorescence detector (Alliance 2475, Waters, USA) and Pico Tag column (4 µm, 3.9 × 150 mm, Waters, USA), following the method by WHITE et al., (1986). The identification and quantification of the amino acids were performed using the standards from the AccQ Tag^{TM} kit (Waters, MA, USA). Briefly, the binary elution system consisted of Mobile Phase A (140 mmol/L sodium acetate and 17 mmol/L triethanolamine) and Mobile Phase B (60 % acetonitrile). The gradient used was 0 % B (0 min), 2 % B (0.5 min), 7 % B (15 min), 9 % B (19 min), 12 % B (22 min), and 30 % B (33 min) followed by washing and reconditioning the column.

Excitation at 250 nm and emission at 395 nm was used for fluorescence detection. The peaks were identified by comparing the retention time with those of authentic standards. The individual amino acids with 99 % purity were quantified based on peak area and calibration curves derived from authentic standards (at least five concentrations).

3.2.8 Statistical Analysis

The results were statistically evaluated by using Statistica v.13.5 software (TIBCO Software Inc., Palo Alto, CA, USA) considering 5 % as the level of significance to identify the significant differences ($p < 0.05$). Assays were duplicated, and the results were expressed as average and standard deviation.

3.3 RESULTS AND DISCUSSION

3.3.1 Proximate composition of the peach seeds

The proximate composition of the raw material (peach seeds) is presented in Table 3-1. The oily fraction (lipid content) is the main portion from the peach seeds (33.40 %) followed by the protein fraction (29.37 %). The lipid content was similar to that reported by PELENTIR et al., (2011) and by Maikhuri et al., (2021), which ranged from 23 to 48 %, and the protein content also follows literature results (26-29 %) (MAIKHURI et al., 2021; SHUKLA; KANT, 2020b). The protein content from peach seeds is higher than reported for other food coproducts such as guava seeds (6-10 %) (ANGULO-LÓPEZ et al., 2021), avocado seeds (4.9 %) (TESFAYE et al., 2022), and grape seeds (10-11 %) (ALVAREZ-OSSORIO et al., 2022) indicating the relevance to consider peach seeds as possible source of protein-rich extracts. The determination of total reducing sugars was 3.28 % were lower than reported by EL-ADAWY & EL-KADOUSY, (1995) who obtained 8.60 %. The value of crude fiber was 19 % higher than that reported by EL-ADAWY & EL-KADOUSY, (1995), who mentioned 5.80 %. This discrepancy can be justified by the maturity of the cultivar, climate, geographic and fruit cultivation

conditions (BENTO et al., 2020). The value of ashes was found 3.82 % according to Lima et al., (2014) that reported 3.9 %.

Table 3-1 Proximate composition of seed.

Composition	Peach seeds Dry weight basis (%)
Moisture	9.86 ± 0.35
Lipids	33.40 ± 2.0
Protein	29.37 ± 1.30
Crude fiber	19.78 ± 0.04
Total reducing sugar (as glucose)	3.28±0.06
Ashes	3.82 ± 0.05

**Analyzed by difference and *Dry weight basis and milled (%).

3.3.2 Oil fraction recovered from peach seeds

The extraction yield of the lipid fraction s by SOX and SFE was 33.4 % ± 2.0 and 29.53 % ± 0.89, respectively. The highest yield obtained from SOX-hexane can be explained by the high solubility of the lipids in hexane, and also to the high Soxhlet temperature, close to the solvent boiling point, which reduces the surface tension and viscosity, increasing the extraction yield (MARKOM et al., 2007; MESQUITA et al., 2021). Nevertheless, high temperatures can also affect the quality of the extract due to degradation of thermolabile components, and the solvent hexane is considered toxic to the environment and demands further separation step, after extraction. Therefore, SFE with CO_2 for the recovery of the lipid fraction presents advantages because it is renewable, reduces energy consumption, and improves the quality of the extracts (TEIXEIRA et al., 2021).

The fatty acids profile of peach seeds oil is presented in Table 3.2. The results indicate the presence of six fatty acids: palmitoleic acid, palmitic acid, linoleic acid, oleic acid, stearic acid, arachidic acid, and cis-11-eicosenoic acid, with similar fatty acids profile between SOX and SFE lipid fractions. The main fatty acid from the peach seed oily fraction is the oleic acid (SOX: 59 %; SFE: 58 %), followed by linoleic acid (SOX:

36 %; SFE: 28 %) and palmitic acid (SOX-10.01 %; SFE-9.47 %). The minoritarian fatty acids are the palmitoleic acid (SOX: 0.09 %; SFE: 0.13 %), stearic acid (SOX:3.13 %; SFE: 3 %), arachidic acid (SOX: 0.11 %, not detected at SFE sample), and cis-11-eicosenoic acid (not detected at SOX sample; SFE: 0.04 %). These results are according to MAIKHURI et al., (2021) in relation to oleic acid (58 %) and linoleic acid (30.8 %), but higher than palmitic acid content (5.85 %) for peach seeds. SÁNCHEZ-VICENTE et al., (2009) obtained peach seed oil by SFE with CO_2 at 40°C, and 198 bar, and found oleic acid and linoleic acid as the main fatty acids, with area percentages of 72.6 % and 17.7 %, respectively.

Table 3-2 Lipid profile of the oily fractions recovered from the peach seeds

RT (min)	**Fatty acid**	**CAS**	**SFE-CO_2 (300 bar, 40°C and 2.5 h)**	**SOX (Hexane and 6 h)**
			Peak (area %)	
34.19	C16:1 - Palmitoleic acid	001120-25-8	0.13 ± 0.01	0.09 ± 0.05
34.73	C16:0 - Palmitic acid	000112-39-0	9.47 ± 0.01	10.01 ± 0.50
38.67	C18:2 - Linoleic acid	000112-63-0	28.89 ± 0.10	36.36 ± 0.27
38.89	C18:1n9c - Oleic acid	000112-62-9	58.31 ± 0.03	59.83 ± 1.12
39.37	C18:0 - Stearic acid	000112-61-8	3.00 ± 0.19	3.13 ± 0.11
43.06	C20:1 - cis-11-Eicosenoic acid	000112-61-8	0.04 ± 0.00	nd
43.64	C20:0 - Arachidic acid	001120-28-1	nd	0.11 ± 0.03

Ni: not identified; Rt: Retention Time; CAS: Chemical Abstracts Service; SFE: Supercritical Fluid Extraction; SOX: Soxhlet.

The main identified compounds from peach seeds oily fraction were oleic acid and linoleic acid, which are essential unsaturated fatty acids, necessary for human metabolism and not synthesized by human body (SÁNCHEZ-VICENTE et al., 2009). Some studies have shown that oleic acid consumption reduces cholesterol levels,

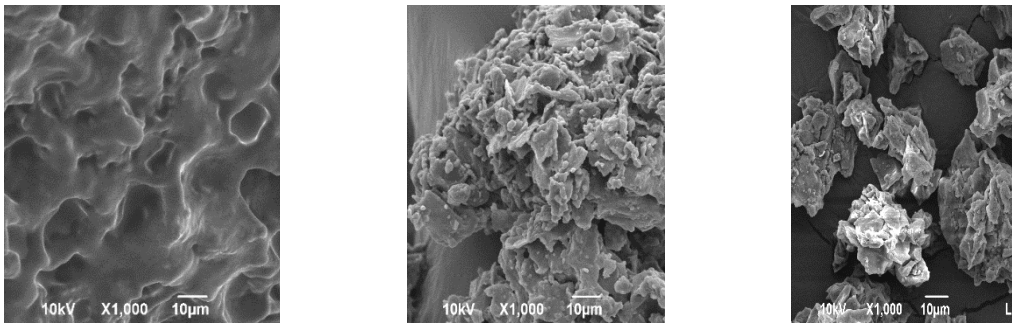
decreases the risk of coronary heart disease, and prevents type 2 diabetes and inflammatory diseases (XIAO et al., 2022). In addition, oleic acid is the most abundant compound found in olive oil, which is considered a healthy product (STAWARSKA et al., 2020). Therefore, the fatty acids profile of the peach oil suggests this fraction as an attractive product for different applications.

3.3.3 Scanning electron microscopy (SEM)

The morphology of the peach seed particles (from the raw material and the defatted samples by SOX and SFE) were evaluated by SEM (Figure 3-1). In Figure 3-1-A, the raw material (*in natura*) sample shows a smooth structure with few fragments. Figure 3-1-B shows the SOX defatted sample, containing structure with open pores and agglomerated particles. The sample defatted by SFE is presented at Figure 3-1-C, with more particle's fragmentation, compared to the previous samples. This profile is expected due to the effect of pressure variation (300 bar to atmospheric pressure) applied to SFE samples (section 2.3), as also observed by FERRO et al., (2019).

The SFE samples present more fragmented particles, and more pores compared to the raw material and to the SOX sample, which is interesting when using the defatted solid in sequential steps, since the extraction matrix will be more exposed to the solvent in the following extraction step, increasing the solute recovery. The protein recovery from the defatted PSC (by SOX or by SFE) is improved (higher yield), compared to non-defatted peach seeds (section 3.4). Sequential extractions are relevant for the fractionation of biomass, following the biorefinery concept by maximizing the use of the raw material to obtain different coproducts (TORRES, et al., 2022).

Figure 3-1 Scanning electron microscopy (SEM) images: peach seed raw material (A), peach seed solid after SOX defatting (B) and after SFE defatting (C).



Raw material (A) **SOX (B)** **SFE (C)**

3.3.4 Peach seed proteins obtained by different extraction methods

The protein content from the peach seed cake (PSC), after defeating by SOX, was evaluated by Kjeldahl method which is based on the determination of total nitrogen content in a sample, is considered a reliable and accurate analytical technique (HAYES, 2020). The result indicates a protein content of 29.37 % ± 1.30 in relation to the mass of PSC. Then, the protein-rich fraction was recovered from the PSC by different extraction methods (HSE, PLE, MAE and UAE), and the extracts recovered enabled the concentration of the protein fraction (measured by Kjeldahl method), as compared at Figure 3-2. The picture from the different samples of protein lyophilized are presented through in Figure 3-3.

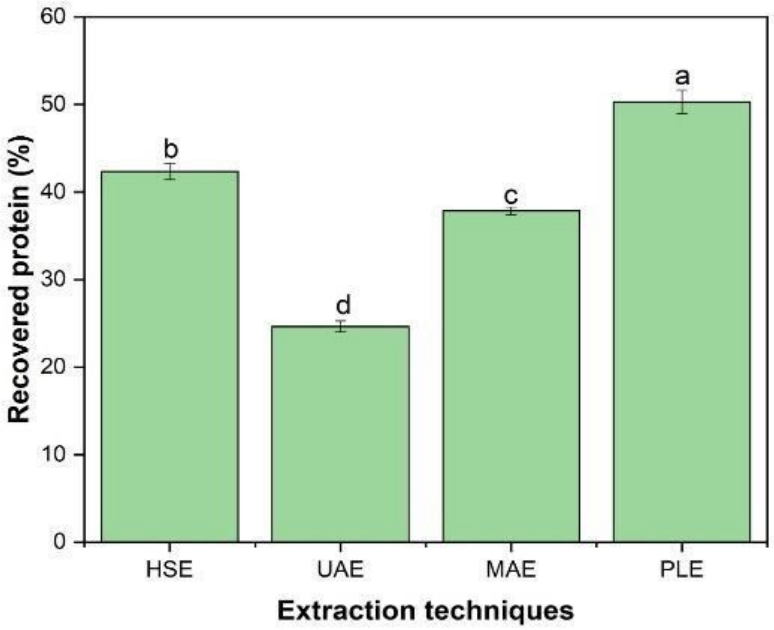
The highest protein concentration was observed for the PLE sample (95.10 % ± 0.98), followed by HSE sample (55.09 % ± 0.35), while MAE and UAE samples showed lower protein content values. Although HSE is one of the most common methods for protein extraction because of its low cost, simplicity, and high efficiency due to protein-solvent interactions by deprotonation of amine and ionizing carboxyl groups (GUZMÁN-LORITE; MARINA; GARCÍA, 2022; PHONGTHAI; LIM; RAWDKUEN, 2016), the

best PLE performance may be explained by the high-pressure and temperature involved, improving the solvent penetration into the matrix, increasing mass transfer (GALVÁN et al., 2022) and protein recovery. Otherwise, the mechanisms involved in MAE and UAE are related to microwave irradiation and cavitation phenomenon, respectively (NÁTHIA-NEVES; ALONSO, 2021; PRANDI et al., 2022). The protein content of the raw material (PSC) was 29 %, which can be concentrated by extraction, producing a protein-rich fraction (extract). This concentration reached a protein content as high as 95 % of the extract (by Kjeldahl) from the PLE sample, which suggests that the current study is very promising for the recovery of a protein concentrate fraction from PSC using high-pressure method, indicating also the PLE method as highly recommended for protein recovery.

Similarly, and for clarity purpose, the protein content from the extract samples (by HSE, PLE, MAE and UAE) were also converted in % of recovered protein (equation 1). The results reached 50.3 % ± 1.33 (PLE sample), followed by 42.36 % ± 0.94 (HSE sample), and then MAE and UAE samples with 37.85 % ± 0.42 and 24.64 % ± 0.63, respectively. These results indicate the efficiency of protein recovery from the PSC by the different methods.

Guzmán-Lorite et al., (2022) applied PLE and UAE to recover protein from pomegranate seed cake, with yields of 15.3 % and 10.2 %, respectively, while GONZÁLEZ GARZA et al., (2017) obtained a protein-rich fraction from *Moringa oleifera* seeds using HSE, with a yield of 10 %. Comparing these raw materials with the present work, it is observed that peach seed cake (PSC) provided extracts with higher protein fraction, suggesting its potential use as a valuable coproduct with relevant content of non-animal protein. Nevertheless, for further applications of this PSP extract, the characteristics of the protein fraction must be elucidated (section 3.4).

Figure 3-2 Protein recovered yield, obtained by different techniques (HSE, UAE, MAE, and PLE).



Source: Elaborated by the author.

HSE: Heat stirred extraction, UAE: ultrasound-assisted extraction, MAE: microwave-assisted extraction and PLE: pressure liquid extraction. Mean ± standard deviation; Different letters indicate a significant difference between the means ($p < 0.05$).

Figure 3-3 Images of protein lyophilizate obtained using different techniques (MAE, UAE, PLE, and HSE).

Source: Elaborated by the author.

3.4 PROTEIN FRACTION CHARACTERIZATION

The results for protein fractions characterization are presented at Table 3-3 for the extract samples recovered by HSE, PLE, MAE and UAE, from the peach seeds cakes. The discussion of the results are presented as follows.

3.4.1 Water Solubility

The water solubility of the peach seed protein (PSP) samples varied between 2.62 % to 4.97 % (Table 3-3), with the high solubility performance from the UAE sample,

followed by HSE sample (3.49 %), while the lower solubility values were detected for PLE and MAE samples, with no significant difference. The water solubility if the PLE sample was close to that reported for isolated rice protein (2.75 %) (IGARTÚA et al., 2024). The lower water solubility value (PLE sample of 2.62 % ±0.18) could be attributed to the higher protein concentration (up to 95.10 % ± 0.98 by Kjeldahl method). Conversely, higher water solubility was provided by the UAE sample, submitted to the cavitation phenomenon, which likely played a role in disrupting non-covalent bonds (such as electrostatic and hydrophobic interactions) responsible for increased solubility (WANG et al., 2021; FLORES-JIMÉNEZ et al., 2021).

3.4.2 Water and oil retention capacity

The water retention capacity (WRC) ranged from 5.72 to 3.35 g water/g from the PSP extract obtained by different methods (Table 3-3). MAE sample provided the highest WRC value (5.72 g water/g), while PLE, HSE and UAE samples exhibited lower values, with no statistical difference observed. PHONGTHAI et al., (2016) used MAE for protein extraction from rice bran, resulting in WCR of 3.80 g water/g. Similarly, MATHEWS et al. (2023) recovered sesame isolate protein by MAE, and the extract provided WRC of 2.16 g water/g. The result from PSP obtained by MAE (Table 3-3) suggests that the higher WRC value for MAE sample (5.72 g water/g PSP) may represent promising applications for the extract (PSP), compared to the white rice protein and sesame isolate protein, for instance, for applications such as in adjusting the texture of food products.

The oil retention capacity (ORC) of the PSP extracts varied from 4.4 to 8.0 g oil/g (Table 3-3), with the highest value (8.01 g oil/g PSP) from HSE sample, and the lowest from PLE sample (4.4 g oil/g), with statistically significant differences compared with other methods. Comparing these results with those from other defatted sources, such as sesame, the PSP performance was better than reported by MATHEWS et al., (2023), which provided ORC of 2.22 g oil/g for sesame isolated protein obtained by MAE, and of 3.15 g oil/g obtained by UAE. The good PSP performance in this importante attribute (ORC) suggests its use as an interesting ingredient for future food applications.

The WRC values were higher than ORC for PSP samples recovered by different methods (Table 3-3), which can be attributed to high exposure of nonpolar groups on the protein surfaces, making the concentrated protein extracts promising for use in food products (LOPES et al., 2020).

3.4.3 Turbidity

There was not significant difference in the turbidity parameter between MAE and HSE treatments (Table 3-3), while higher turbidity was detected from the PLE sample. This result could be related to varying the chain lengths of the components, the intra/intermolecular bonding, and the cross-linking (NAZIR; WANI, 2021). Lowest turbidity was observed for UAE sample, probably due to the cavitation effect that may have reduced the particles size, reducing turbidity. According to MIR et al., (2021), smaller particles increase the light scattering and reduce turbidity, which was also reported by FLORES-JIMÉNEZ et al. (2022) that used ultrasound treatment and reported a reduction in turbidity for *guamuchil* seed, with a value of 0.78 (400 W/15 min).

3.4.4 Color

The PLE sample had a significantly different L* (lightness) value (79.19) ($p<0.05$) compared to other methods (Table 3-3). HSE sample showed the highest values of a* (redness) and b* (yellowness) (6.73 and 20.63, respectively), while PLE provided the lowest (4.22 and 14.18, respectively). According to MA et al., (2022), high luminosity (L*) and low a* and b* values are preferred, which indicate that the sample has white appearance with low coloring components. However, the protein fraction recovered by PLE showed better color properties confirmed by the highest whiteness index (Wi) of 36.67, while HSE sample provided the lowest Wi (12.66). The highest whiteness index from the PLE sample may be associated to the higher protein content obtained by PLE method (section 3.3.4), providing a more purified protein, compared to other samples. It can also be observed that the values of H* hue angle showed no significant difference among the samples. The Chroma (C) value was the highest for the HSE samples (21.7)

($p<0.05$), followed by UAE (17.97) and MAE (16.67) samples. From these results, it is possible to suggest the PSP application as ingredient in food products. Each product has a different appearance, and each PSP can distinctly influence food formulations. This may be related to the extracted protein content according to the corresponding samples.

3.4.5 Emulsion activity index (EAI) and stability index (ESI)

PLE sample showed the highest EAI value (2.72 m^2/g) followed by the samples by MAE (2.04 m^2/g), UAE (1.20 m^2/g) and HSE (1.04 m^2/g) (Table 3-3). The stability index (ESI) did not show a significant difference among all extraction methods. Many factors can affect these properties, including particle size, pH of the aqueous phase, and the presence of carbohydrates (SHI et al., 2023). Besides, the presence of proteins with hydrophilic groups reduces the interaction between the protein and the oily phase, decreasing the stability (LIU et al., 2022). The emulsion property is closely related to changes in protein structures due to the effects of the different extraction methods. Once the protein is partially unfolded, more hydrophobic groups will be exposed, increasing the affinity for the oily (nonpolar) phase, contributing to the stability.

3.4.6 Foaming Activity and Stability

The highest value of foaming activity was observed for the PLE sample (16.33 %), followed by MAE (12 %), while the lowest value was for UAE sample (6.33 %), as also observed at Table 3-3. The results demonstrated that the UAE technique obtained the lowest foaming capacity. The low performance of the UAE may be attributed to insufficient ultrasound intensity for the globular protein's denaturation, which increases the protein surface activity and consequently the foaming properties (HADIDI; AGHABABAEI; MCCLEMENTS, 2024). Otherwise, the high-pressure and microwave effects (PLE and MAE respectively) may have provided protein denaturation for high foaming performance. Besides that, the foaming performance is associated to protein content, which was higher for PLE and MAE, and lower for UAE (Figure 3-2), corroborating with the foaming performance. Although the foaming activity values were

low, the foam stability was considerably high, indicating the percentage volume of foam that remains after a specified period of time, in relation to the initial foam volume (NAIK et al., 2022). Regarding the foam stability, the highest value was obtained by HSE sample, and the lowest by PLE, UAE and MAE samples, without significant differences. The foaming property of protein is associated with the liquid/vapor interphase, by protein molecules forming continuous intermolecular polymers to envelope the air bubbles. Stable foams are obtained due to intermolecular cohesion and elasticity (HOU et al., 2017). The foaming activity of the PSP seems promising, which may suggest the possible replacement of egg white (relevant food ingredient due to its high foam-forming capacity) by PSP.

3.4.7 Isoelectric point

The isoelectric point of the PSP samples ranged from 4.53 to 5.09 (Table 3-3), depending on the extraction method used. The results are in agreement with other studies. For instance, isolate sesame protein had neutral charge at pH 4-5 (YÜZER; GENÇCELEP, 2023). Bao et al., (2022) detected an isoelectric point of 4.7 for *Cinnamomum camphora* seed protein. The isoelectric point represents the pH where the protein electrical charge is zero, implying zeta potential (ZP) equal to zero because the proteins electric charges have the same sign as the zeta potential (LOPES et al., 2022). In foods, this aspect can affect the structure, texture, flavor, color, and other important properties in the formulation of a food product (CANO-SARMIENTO et al., 2018).

3.4.8 Sulfhydryl content

The free sulfhydryl (SH) values were higher for the PLE sample (0.47 nmol/mg.protein), followed by MAE (0.35 nmol/mg.protein) and UAE (Table 3-3). The PLE method combines high pressure and temperature, which may have contributed to the protein denaturation, justifying the highest free SH content, which became easily available for new intermolecular disulfide bonds due to thiol-disulfide exchange reactions (MIR et al., 2021). Another possibility is the electrolysis process, when water is oxidized

to hydrogen + and converted to adsorbed hydrogen with electrons, inducing the reduction of S–S in the protein to form a large amount of –SH (WANG et al., 2023). According to ZHANG et al., (2022), the sulfhydryl group participates in weak secondary bonds (disulfide bonds), which are important in stabilizing the protein conformation.

Table 3-3 Protein fraction characterization (PSP)

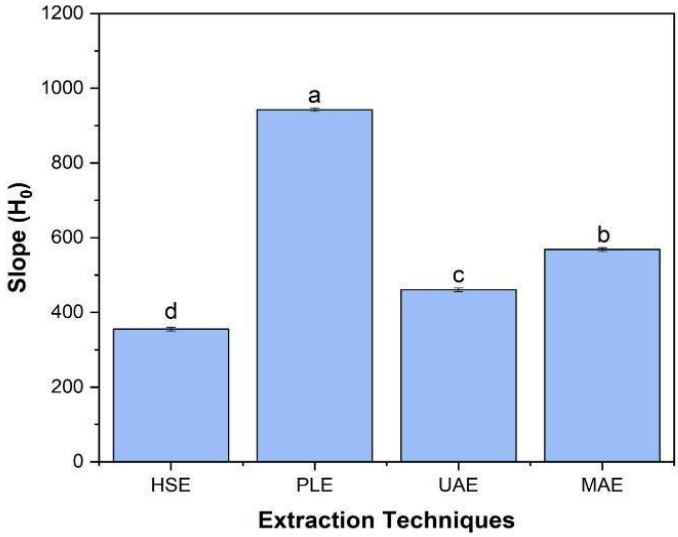
Properties	**PSP samples recovered by different methods**			
	HSE	**MAE**	**UAE**	**PLE**
Solubility (%)	3.49 ± 0.32^{b}	3.19 ± 0.41^{bc}	4.97 ± 0.37^{a}	2.62 ± 0.18^{c}
Water Retention Capacity- WRC (g water/g PSP)	4.08 ± 0.24^{b}	5.72 ± 0.37^{a}	4.0 ± 0.10^{b}	3.35 ± 0.19^{b}
Oil Retention Capacity-ORC (g oil/g PSP)	8.01 ± 0.30^{a}	6.0 ± 0.08^{b}	5.4 ± 0.07^{b}	4.4 ± 0.09^{c}
Turbidity (500 nm)	0.86 ± 0.01^{b}	0.85 ± 0.06^{b}	0.74 ± 0.01^{c}	0.94 ± 0.02^{a}
L*	74.54 ± 0.06^{b}	74.65 ± 0.20^{b}	75.54 ± 0.30^{b}	79.19 ± 0.95^{a}
a*	6.73 ± 0.38^{a}	4.91 ± 0.58^{ab}	5.48 ± 0.57^{ab}	4.22 ± 0.26^{b}
b*	20.63 ± 0.35^{a}	15.92 ± 0.13^{b}	17.12 ± 0.54^{b}	14.18 ± 0.05^{c}
Hue (H*)	181.26 ± 0.01^{a}	181.27 ± 0.04^{a}	181.26 ± 0.02^{a}	181.28 ± 0.02^{a}
Whiteness index (wi)	12.66 ± 1.10^{c}	26.89 ± 0.18^{b}	24.19 ± 1.94^{b}	36.67 ± 0.80^{a}
Chroma (C)	21.70 ± 0.45^{a}	16.67 ± 0.05^{b}	17.97 ± 0.69^{b}	14.79 ± 0.03^{c}
Emulsion activity index (m^2/g)	1.04 ± 0.08^{c}	2.04 ± 0.14	1.20 ± 0.09^{c}	2.72 ± 0.25^{a}
Emulsion stability index (min)	28.02 ± 1.63^{a}	27.25 ± 2.01^{ab}	22.36 ± 0.43^{b}	25.53 ± 1.17^{ab}
Foaming activity (%)	11.00 ± 1.00^{bc}	12.00 ± 3.46^{ab}	6.33 ± 0.58^{c}	16.33 ± 1.15^{a}
Foaming stability (%)	54.85 ± 5.01^{a}	33.75 ± 5.45^{b}	36.51 ± 5.50^{b}	24.58 ± 6.15^{b}
Isoelectric point	4.80 ± 0.57^{a}	5.02 ± 0.73^{a}	4.53 ± 0.66^{a}	5.09 ± 0.14^{a}
Free sulfhydryl (SH) group (412 nm)	0.35 ± 0.01^{c}	0.40 ± 0.02^{b}	0.35 ± 0.01^{c}	0.47 ± 0.01^{a}

Different superscripts in each line represent a significant difference ($p < 0.05$) and ± standard deviations. L (lightness), a* (redness greenness),b*(yellowness-blueness).

3.4.9 Surface hydrophobicity (Ho)

Figure 3-4 provides the hydrophobicity property that evaluates the amount of hydrophobic amino acid residues exposed on the protein surface (WANG et al., 2023). PLE provided the highest hydrophobicity slope (942), followed by MAE (568.37), while and the lowest was from HSE sample (360.6). The PLE, which combines high-pressure and temperature, may have induced the protein unfolding, loosening its structure and becoming unstable. Therefore, more exposed hydrophobic groups increase the hydrophobicity value. This result is according to CHEN et al., (2019), which also detected hydrophobicity increase for the cumin seed isolated protein obtained by high-pressure.

Figure 3-4. Surface hydrophobicity values of PSP extracted by HSE, UAE, MAE, and PLE.



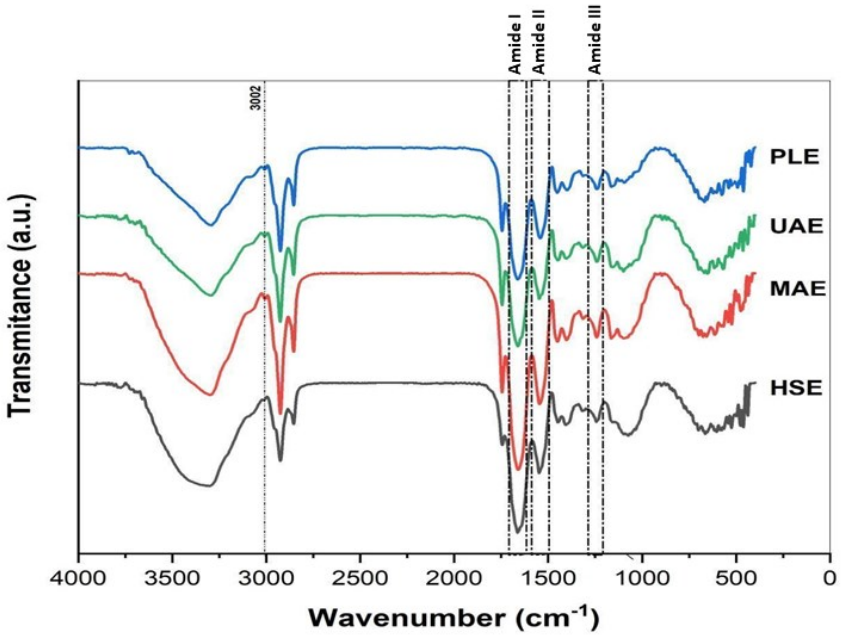
Source: Elaborated by the author.

HSE: Heat stirred extraction, UAE: ultrasound-assisted extraction, MAE: microwave-assisted extraction and PLE: pressure liquid extraction. Mean ± standard deviation; Different letters indicate a significant difference between the means ($p < 0.05$).

3.4.10 Fourier-transform infrared (FTIR) spectroscopy

The proteins have three characteristic FTIR bands, highlighted in Figure 4: region between 3000–3500 cm^{-1}, represented by N–H bending and O–H stretching vibrations related to hydrogen bonds on the main polypeptide chain (KELANY; YEMIŞ, 2023), band between 3000 and 2800 cm^{-1} associated to protein hydrophobic region caused by C-H vibrational stretching (LIU et al., 2022); and the band between 1700-1600 cm^{-1}, as the most sensitive region of the structural amino group 1, common component for secondary structure of protein. The secondary structure of proteins is composed of elements such as α-helix, β-sheet, β-turn and random coil, which have characteristic bands at 1660–1648 cm^{-1}, 1640–1610 cm^{-1}, 1670–1665 cm^{-1} and 1648–1640 cm^{-1}, respectively. Deviations in these band regions imply changes in the secondary structure of proteins, which affect directly the protein interactions with other molecules (XU et al., 2024), and as a consequence, affecting the solubility an important functional property of the protein. For instance, the FTIR result (Figure 3-5) for UAE sample showed a very discreet change in relation to other samples at the Amide I group, which was probably due to ultrasonic cavitation and bubble implosion, as also observed by NAIK et al., (2022). Also, it was possible to observed similar functional groups and chemical bonds concerning FTIR analysis for PSP recovered by different methods.

Figure 3-5. FTIR spectroscopy analysis.



Source: Elaborated by the author.

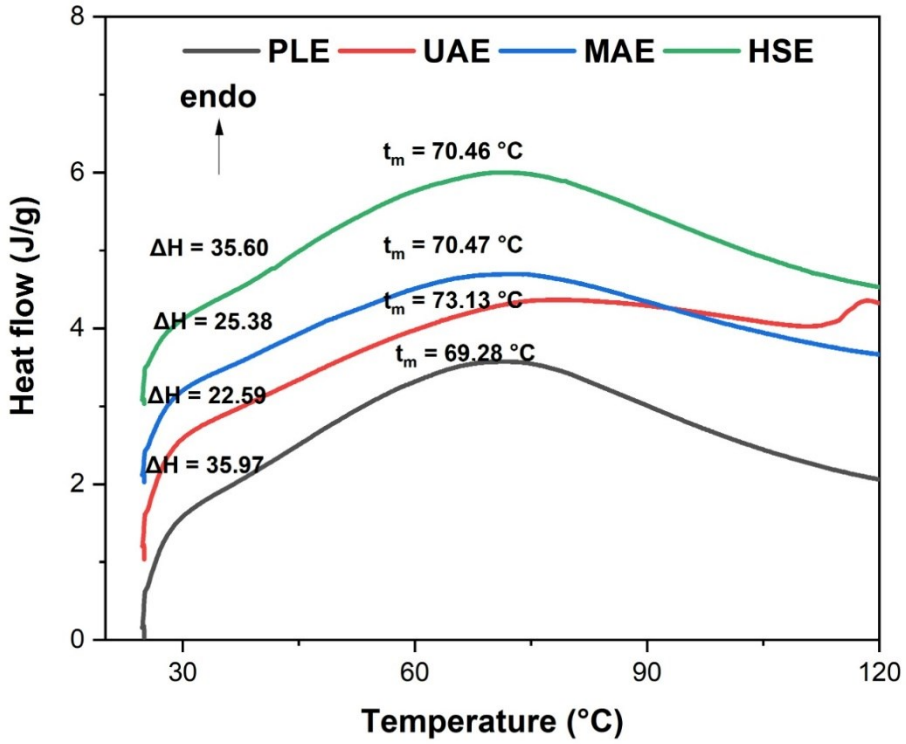
HSE: Heat stirred extraction, UAE: ultrasound-assisted extraction, MAE: microwave-assisted extraction and PLE: pressure liquid extraction.

3.4.11 Differential scanning calorimetry (DSC)

From DSC analysis provided the values of midpoint (T_m) and enthalpy (ΔH), as presented at Figure 3-6, which indicate the protein denaturation temperature and the heat amount required for protein denaturation, respectively (KELANY; YEMIŞ, 2023). The midpoint temperature (T_m) ranged from 69.28°C to 73.13°C (Figure 3-6), with the highest value for UAE sample, which suggest higher thermal stability, compared to other samples. The cavitation and bubble implosion phenomenon (UAE) may have affected the protein secondary structure (NAIK et al., 2022), increasing the T_m value. The DSC results were similar to reported by FEYZI et al., (2018) for pea grass isolated protein (T_m from 71 to 80°C). The ΔH results ranged from 33.31 to 43.98 J/g. Lower ΔH values may represent less hydrophobic interactions, while higher values may represent higher protein

aggregation, which requires more energy for denaturation (WANG et al., 2021). Therefore, the Tm value must be considered before the protein use in formulations of thermally processed foods, in order to maintain the functional characteristics of the final product (YUE et al., 2021).

Figure 3-6 Differential scanning calorimetry (DSC).



Source: Elaborated by the author.

HSE: Heat stirred extraction, UAE: ultrasound-assisted extraction, MAE: microwave-assisted extraction and PLE: pressure liquid extraction.

3.4.12 Amino acid composition

Table 3-4 presents the amino acid composition for the PSP recovered by HSE, MAE, and PLE methods. The decision to evaluate was based on the highest yield of the

assays. The isolated protein obtained by PLE presented essential amino acids, with highest content in valine, isoleucine, leucine, and phenylalanine. These results were similar to obtained from HSE sample. Besides, the main non-essential amino acids were glutamic acid, arginine, and aspartic acid. The composition of the amino acids from the samples is important to define the nutritional quality of the protein fraction recovered. These results are similar to reported by EL-ADAWY & EL-KADOUSY (1995) for protein from peach kernel meal. The results are promising, suggesting that PSP presents a valuable amino acids profile, and do not compete with traditional sources of vegetable proteins such as soy, beans, and peas (SÁ; MORENO; CARCIOFI, 2020). Then, increase the diversity of protein sources, especially high in essential amino acids, contributes to a healthy diet, and PSP may be suggested as a complementary source of essential nutrients for human nutrition.

Table 3-4 Amino acid composition

Amino acid composition (%)	**Samples**		
	HSE	**MAE**	**PLE**
Essential amino acid			
Histidine	1.52	1.04	1.45
Threonine	1.86	1.23	1.79
Valine	3.27	2.18	3.23
Isoleucine	2.59	1.72	2.55
Leucine	5.06	3.37	4.99
Lysine	1.08	0.84	1.04
Methionine	0.24	0.19	0.21
Phenylalanine	3.91	2.56	3.82
Nonessential amino acid			
Aspartic acid	8.72	5.74	8.40
Alanine	3.33	2.22	3.26
Glutamic acid	19.72	12.91	19.54
Serine	2.80	1.88	2.70
Glycine	3.78	2.61	3.69
Arginine	8.21	5.35	8.08
Proline	2.33	1.48	2.25
Tirosine	2.33	1.48	2.25
Cystine	0.65	0.49	0.63
Hydroxyproline	0.08	0.09	0.07

Source: Elaborated by the author.

3.5 CONCLUSIONS

Peach seed cake (PSC) is an agro-industrial coproduct that can be considered an interesting source of oil and protein. Overall, the oily fraction reached and yield of 33 % and 29 % when recovered by SOX and by SFE, respectively. The oily fraction was rich in oleic and linoleic acids, important as beneficial unsaturated fatty acids that participate in essential metabolic functions of the human body. Subsequently, the emerging techniques of MAE, UAE, and PLE showed interesting results in the yield and quality of the protein fraction. PLE provided the highest protein recovery (95 %), followed by HSE (55 %). Also, the PLE protein fraction showed good functional properties, with the highest foaming activity, hydrophobicity and whiteness index. In addition, the isolated protein is rich in essential amino acids such as leucine, phenylamine, and valine. Therefore, the PSC can be an alternative source of protein using emerging extraction techniques that can open new paths for industrial use as an alternative and sustainable source of proteins.

CHAPTER 4 – UPCYCLING INNOVATION: ELABORATION OF MAYONNAISE ANALOGUE USING ALTERNATIVE PROTEIN RECOVERED BY PRESSURIZED LIQUID EXTRACTION FROM PEACH SEEDS

4.1 INTRODUCTION

Mayonnaise is one of the most popular sauces in the world, especially in North America and Western Europe. There is controversy over the etymological origin of the word “mayonnaise”. The most accepted one is related to the place where the sauce was created, in Mahón, the capital of Menorca (Spain) (MENEZES et al., 2022). The global mayonnaise market was valued at around US$11.80 billion in 2021, with a prospect of estimation to grow at a rate of 4.13 % from 2022 to 2027 (SU et al., 2023), which demonstrates the relevance of this product for consumers and the market. According to the Food and Drug Regulatory Agency in Brazil (ANVISA, 2005), mayonnaise is defined as a creamy product in the form of a stable emulsion, oil in water, prepared from vegetable oils, water, eggs, and other ingredients, which may be added as long they do not distort or acidify the product.

Over the last few years, the plant-based market has grown substantially and transformed from a niche market to a consolidated segment. Also, there is a growing demand for more sustainable products and new ingredients that meet the preferences of many consumers with restrictions on products of animal origin (BATISTA et al., 2023). This tendency widens the exploration of new plant-source ingredients or constituents for use in food formulations. Recently, Brazil elaborated a new normative Secretariat of Agricultural Defense, Ministry of Agriculture, Livestock and Food Supply SDA/MAPA (2023), establishing strict quality and identity requirements for food products of plant origin. According to this normative regulation, plant-based products must be commercialized with the label including explicitly the expression "plant analogue", and covering a variety of items, from foods to beverages, all formulated exclusively with ingredients from plant sources. Specifically, for mayonnaise, a traditional egg-based product, finding a replacement for the animal-based ingredients, but maintaining the product's characteristics, stands out as an interesting challenge. The egg imparts distinct sensory characteristics, playing an essential role in the emulsion formation, with the oil droplets dispersed into a continuous phase (aqueous phase) (GHOUSH et al., 2008). In the literature, several studies have already explored new substitutes for the formulation of vegan mayonnaise, including pea protein (CHOI et al., 2023), potato protein (LI;

MCCLEMENTS, 2023), soy protein (TIAN et al., 2023), aquafaba from chickpea (RAIKOS; HAYES; NI, 2020), and apple pomace (MANGIAPELO et al., 2023). These alternative ingredients for mayonnaise formulation have demonstrated promising results compared to traditional mayonnaise.

According to Nikzade et al., (2012), pectin and proteins have already been evaluated and used to stabilize the emulsion and increase the viscosity of light mayonnaise. However, a significant gap was observed when reviewing alternative ingredients from fruit biomasses. For instance, peach co-products, such as peach seeds have yet to be explored as source to obtain a protein-rich fraction, for further use as ingredient for new food products, such as mayonnaise analogue. Therefore, the objective of this study was to develop a mayonnaise analogue, produced using an egg replacement derived from a protein-rich extract recovered from peach seeds, and also evaluate the properties and the quality parameters of the final product with peach protein, and compare with other vegan mayonnaise formulations and a commercial one.

4.2 MATERIALS AND METHODS

4.2.1 Mayonnaise analogue's preparation

Mayonnaise anallogues products were prepared following five different formulations: commercial vegan mayonnaise hellmann's brand (CM1) served as the control sample, Pea mayonnaise (PM2), Soya mayonnaise (SM3), Whey mayonnaise (WM4), for comparative purposes was choice isolated proteins (in powder) from the respective samples were purchased, as these proteins are well-established in the market and are widely used in vegan product formulations due to their functional properties. The Peach protein mayonnaise (PPM5) was made PSC was extracted from PLE, as described by Rudke et al., (2024) and also described chapter 3. The mayonnaise analog formulations (PM2, SM3, WM4, and PPM5) were prepared following the protocol described by Choi *et al*. (2023b) with modifications. Briefly, a protein solution containing 3 % protein was prepared by dissolving 0.6 g of protein in 20 mL of water. Then, salt (0.7 g) and sugar (1.3 g) were added. Subsequently, 50 mL of soy (Coamo®) oil was slowly added and

homogenized using an Ultra-Turrax® apparatus (model T 25 digital, IKA, São Paulo - Brazil) at 15000 rpm for approximately 8 minutes. Finally, 2.8 mL of vinegar (Heinig®) was added, and the mixture was homogenized for an additional 1 minute. The assays were performed in triplicate and stored under refrigeration until the next analyses.

4.2.2 Preparation of the peach protein-rich extract

Peach protein is obtained by the high pressure method, as described in item section 3.2.6.2 of the previous chapter 3 and as published in article Rudke et al., (2024). This protein was chosen because it presents a higher protein content obtained through the high-pressure process and for its functional properties. The protein fraction demonstrated excellent functional properties, including increased foaming, hydrophobic activity, and whiteness index.

4.2.3 Mayonnaise characterization

4.2.3.1 Color of the mayonnaise samples

The methodology for color determination followed as described by Armaforte et al., (2021). The color of the mayonnaise samples (CM1, PM2, SM3, WM4 and PPM5) was evaluated in a colorimeter (Delta Vista, model 450 GSN, São Leopoldo-RS, Brazil). The parameters L*, a*, and b* were used to calculate the chroma (C), the yellowness index, and the ΔE (color difference between two samples), which was use vegan commercial mayonnaise as control to calculated using the following equations below. The color parameters expresses the product color according to three values: L* for lightness, a* for red-green opponent colors, and b* for yellow-blue opponent colors.

$$Chroma\ (C) = \sqrt{(a^*)^2 + (b^*)^2} \qquad \text{Equation 4-1}$$

$$\text{Yellowness index} = \frac{142.88 \times b^*}{L^*} \qquad \text{Equation 4-2}$$

$$\Delta E = \sqrt{(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad \text{Equation 4-3}$$

4.2.3.2 Texture profile analysis (TPA)

The texture profile analysis (TPA) was evaluated using the TA.HD.plus Texture Analyzer equipment (Stable Micro Systems, Godalming, Surrey, England). The equipment has the following parameters established for analysis operation featured a 50 kg load cell, with a pre-test speed of 2 mm/s, a test speed of 1 mm/s, and a return speed of 1 mm/s. It had a trigger load of 10 g, a target mode distance of 20 mm, and a data acquisition rate of 10 points/s. Mayonnaise samples (20 g) were added to cylindrical aluminum container (50 mm diameter, 30 mm height) and the mesh probe compressed carried out twice the sample at a constant speed of 1 mm s^{-1} to a depth of 20 mm from initial height to room temperature. The parameters determined were hardness (firmness), adhesive strength (adhesion), resilience and cohesion (consistency). Hardness was calculated from the load detected at the highest peak during specification; adhesive strength was determined from the negative value of the peak; adhesiveness was obtained from the area under the negative peak; and cohesiveness from the ratio of the areas under the stroke during specification of the second and first compressions. Five readings were performed for each sample and analysis was performed in duplicate. The results of the texture parameters, for each mayonnaise sample, are expressed in $g.cm^{-2}$ (mean ± standard deviation).

4.2.3.3 Viscosity of mayonnaise

The viscosity of the mayonnaise samples was determined according to Sachko et al., (2023). The shear viscosity was measured with a rheometer (Anton Paar, Graz, Austria,model MCR302) over a range of shear rates (from 0.1 to 5 s^{-1}). Each shear rate was applied for 120 s and temperature of 20°C for each sample. The apparent viscosity

and the yield shear stress were performed on a rotational viscometer Visco QC 300R (Anton Paar, Graz, Austria) with a flate plate.

4.2.3.4 Microstructure observation

The microstructure of the mayonnaise samples was analyzed according to the procedure described by Raikos et al. (2020). The analysis used a confocal laser scanning microscope (Olympus®, BX41vertical, Japan). Nile red dye was used to stain the fat globules in the emulsions (samples), and observations were carried out at 543 nm using a 100× oil immersion objective. The mayonnaise samples were equally distributed on microscope glass slides, previously mixed with the dye, and quickly observed under a microscope. The images of the samples express the results.

4.2.4 Statistical analysis

All results were presented as mean followed by standard deviation. One-way analysis of variance (ANOVA) was carried out using the Software Statistica® (TIBCO, v. 13.0.5.017, Palo alto-CA, USA) was used to evaluate significant differences ($p < 0.05$) and the Tukey test was used to see the difference between the assay.

4.3 RESULTS AND DISCUSSION

4.3.1 Color parameters of the mayonnaise samples

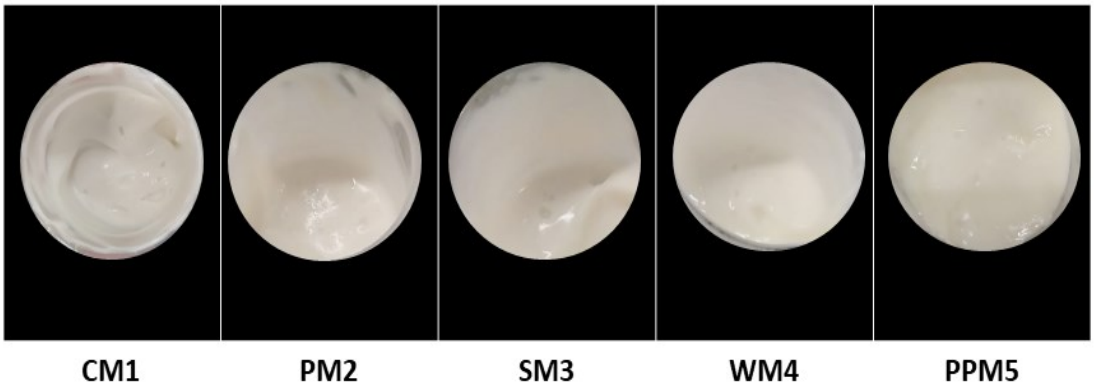
The produced mayonnaise samples (one control and four analogous products) are compared through pictures presented in Figure 4-1. The appearance of the samples was evaluated by color parameters, the results of which are shown in Table 4-1. Color parameters of different mayonnaise formulations (PM2, SM3, WM4, and PPM5) are for the five studied mayonnaise formulations. The formulation CM1, the control sample, obtained the highest luminosity value L* (95.57), statistically different from the other samples, while the lowest value was provided by PPM5 (84.75). The a* values ranged

from 3.40 to -1.47, with the highest value observed from control sample (CM1), and the lowest values, with no significant differences, for all other samples (egg replacement anallogue mayonnaise). This behavior reflects the results obtained by Choi et al., (2023b), for the color evaluation of mayonnaise made with a conjugate of isolated pea protein and xanthan gum. The sample presented lower values of L* (48.51), a* (-3.47) and b* (6.70) when compared to samples PM2 and PPM5. These differences may be related to light refraction, droplet size and the mixture of pea protein isolate and xanthan gum, since we have another polymer in the formulation.

Following the data from Table 4-1, the b* parameter ranged from 13.24 to 8.44, with CM1 presenting the highest value. Włodarczyk; Zienkiewicz; Szydłowska-Czerniak, (2022) evaluated aquafaba-based mayonnaise, obtaining parameter L*, a* and b* values of 48.7, 0.9 and 10.9, respectively. The same authors compared these values with those of commercial vegan mayonnaise, which presented values of 41.4 (L*), 2.6 (a*) and 11.3 (b*). It was observed similar a* and b values, which may relate the pigmentation characteristics of commercial vegan mayonnaise. On the other hand, the L* parameter for both the aquafaba-based mayonnaise sample and the commercial vegan mayonnaise were lower than the values obtained in our study. No significant differences in b* values were observed between the samples PPM5 and PM2, SM3 and WM4. For the Chroma values, all formulations were significantly different, ranging from 13.72 to 8.44, with highest for CM1 followed by PPM5 sample. The yellowness values ranged from 20.76 to 13.07, with the PPM5 and CM1 formulations with the highest values with no significant difference. Concerning the value of ΔE, the PPM5 sample had no significant difference with PM2, while the other samples were statistically equal. Color parameters are important in product formulation, as they represent a crucial visual aspect for the consumer. The appearance of the samples, represented by (Figure 4-1) of the products and by color parameters (Table 4-1) suggest that the analog formulation of egg replacement mayonnaise can be considered attractive, as they present color aspects very similar to commercial vegan mayonnaise. CM1 uses starch and xanthan gum in its formulation. This work has a significant impact and innovation, as there are still no protein-enriched mayonnaise formulations on the market and our findings verified

attributes similar to those of commercial vegan mayonnaise when compared with different proteins evaluated.

Figure 4-1. Images of the different mayonnaise formulations (PM2, SM3, WM4, and PPM5) and compared with commercial vegan mayonnaise CM1.



Source: Elaborated by the author.

Commercial vegan mayonnaise (CM1), Pea mayonnaise (PM2), soya mayonnaise (SM3), whey mayonnaise (WM4), and peach protein (PPM5).

Table 4-1. Color parameters of different mayonnaise formulations (PM2, SM3, WM4 and PPM5) and compared with commercial vegan mayonnaise CM1.

Parameters	CM1	PM2	SM3	WM4	PPM5
L* (whiteness)	95.57±0.11^{a}	87.92±0.25^{c}	91.16±1.32^{b}	92.20±0.94^{b}	84.75±0.42^{d}
a*(redness)	3.40±0.45^{a}	-0.22±0.07^{b}	-0.60±0.46^{b}	0.17±0.02^{b}	-1.47±0.75^{b}
b*(yellowne)	13.24±0.07^{a}	10.90±0.01^{b}	10.42±0.40^{b}	8.44±0.53^{c}	12.31±0.04^{a}
Chroma	13.72±0.1^{a}	10.90±0.0^{c}	10.44±0.3^{c}	8.44±0.53^{d}	12.41±0.1^{b}
Yellownesse index	19.87±0.18^{a}	17.71±0.0^{b}	16.33±0.8^{b}	13.07±0.6^{c}	20.76±0.1^{a}
ΔE	-	8.80±0.55^{ab}	6.67±0.80^{b}	6.75±1.17^{b}	11.91±0.3^{a}

* All analyses were expressed in triplicate and results are expressed as the mean ± standard deviation. Commercial vegan mayonnaise (CM1), Pea mayonnaise (PM2), soya mayonnaise (SM3), whey mayonnaise (WM4), and peach protein (PPM5).

4.3.2 Texture Profile of the mayonnaise analogous products

The texture parameters for the different mayonnaise formulations are presented at Table 4-2. Firstly, we can observe the hardness, which presented values ranging from 65.92 to 204.28 g , and all samples showed a significant difference, with the highest value for the control formulation CM1, and the lowest for PM2 sample. Hardness is a parameter that indicates the force required to compress a food (RAIKOS; HAYES; NI, 2020). The performance of the alternative formulation producer using the peach-protein substitutive (PPM5) presented the value of 110.08 g, and was similar to obtained by the sample produced using whey protein replacement. The hardness parameter is important in determining the desired texture of the mayonnaise formulation. High hardness values are desirable because they contribute to the product's resistance and significantly impact the stability of the emulsion and firmness (LEE et al., 2024). This ensures that the mayonnaise maintains its consistency and quality over time.

The second parameter evaluated was the adhesiveness, with values ranging from 136 to 812 g.s. The control sample obtained the highest adhesiveness, while the lowest value was for the PM2. Samples WM4 and PPM5 did not show a significant difference. Adhesiveness represents the force necessary to overcome the attractive forces between mayonnaise and the surface of another material (RAIKOS; HAYES; NI, 2020).

The resilience parameter, also presented at Table 4-2, ranged from 1.4 to 2.38 %. The control sample CM1 showed no significant difference with SM3, WM4 and PPM5. This parameter refers to the ability of mayonnaise to return to its original form after being spread or mixed with other ingredients. In mayonnaise formulation, high values of the resilience parameter are desired, as they indicate a good capacity to recover from mechanical stress, demonstrating a faster return to its original shape and texture.

The cohesion parameter, the forth texture atribute, varied between 0.81 and 0.91. The highest value was for the control sample, with no significant difference with WM4, followed by the SM3, and then to PM2 and PPM5, with similar values. This parameter is important as it indicates the cohesiveness refers to stickiness for mayonnaise samples (MUHIALDIN et al., 2021). This parameter is desired in the mayonnaise formulation which indicates the internal binding strength of a food matrix in the uniformity of the

formulation and directly influences the stability of the emulsion. High cohesion values ensure that the mayonnaise maintains a homogeneous texture and does not separate into distinct phases, resulting in a more stable (LEE et al., 2024).

Table 4-2. Texture analysis in terms of hardness, adhesiveness, resilence, and cohesion, determined for the different mayonnaise anallogues formulations (PM2, SM3, WM4 and PPM5) and compared with commercial vegan mayonnaise CM1.

	CM1	PM2	SM3	WM4	PPM5
Hardness (g)	204.28±3.96^{a}	65.92±1.50^{d}	123.32±4.4^{b}	111.43±1.22^{c}	110.08±5.36^{c}
Adhesiveness (g.s)	812.32±0.18^{a}	136.23±5.3^{d}	515.73±1.4^{b}	455.19±3.19^{c}	442.88±4.42^{c}
Resilence (%)	1.51±0.0^{b}	2.38±0.25^{a}	1.55±0.08^{b}	1.40±0.02^{b}	1.58±0.05^{b}
Cohesion (Pa/Pa)	0.89±0.0^{a}	0.81±0.00^{c}	0.85±0.02^{b}	0.91±0.01^{a}	0.82±0.01^{c}

***** All analyses were expressed in triplicate and results are expressed as the mean ± standard deviation. Commercial vegan mayonnaise (CM1), Pea mayonnaise (PM2), soya mayonnaise (SM3), whey mayonnaise (WM4), and peach protein (PPM5).

4.3.3 Viscosity performance of mayonnaise

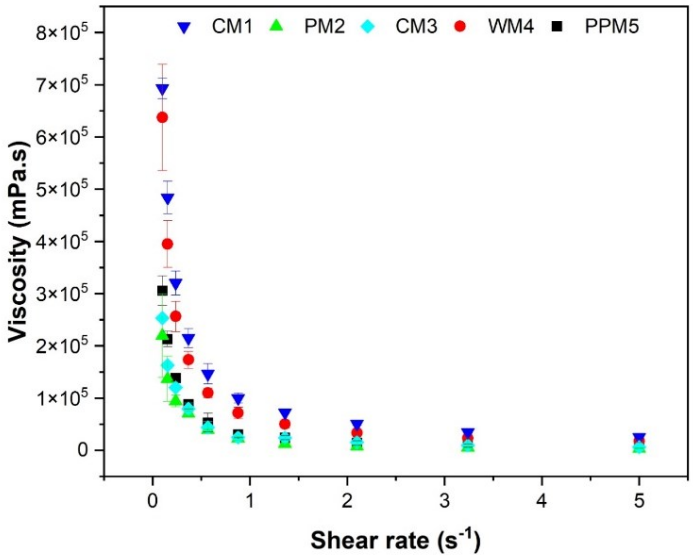
The rheological behavior, based on shear viscosity, apparent viscosity, and shear stress, were analyzed for different mayonnaise formulations, with a commercial vegan mayonnaise (CM1) used as a control. The results are presented in Figure 4-2, expressing the sample viscosity against the shear rate. It was possible to verify that the different formulations showed similar behavior of a decrease in viscosity with the shear rate increase, and all samples demonstrated thinning behavior, representing the most common type of non-Newtonian fluids behavior. According to Li & Mcclements, (2023), this shear thinning profile consists of the flocculation of droplets in the emulsions. As the shear rate increases, shear forces act on the flakes, causing them to deform, elongate, and rupture. According to Tian et al., (2023) high viscosity being related presence of smaller droplet sizes, when the shear strength of emulsions decreases, promotes a reduction in viscosity as can be seen in Figure 4-2. Formulations PM2, SM3, WM4 and PPM5 showed shear thinning, but with smaller viscosity values than the control formulation (CM1),

suggesting that these samples are less flocculated (droplets in the emulsions) than CM1. Based on the similarity of the viscosity behavior of formulated samples (PM2, SM3, WM4 and PPM5), it can conclude that the peach protein fraction, used to produce sample PPM5, presentes similar shear behavior than proteins from pea, soy and whey. These findings demonstrate that the protein fraction recovered from peach seeds can be an alternative source to use in formulations of emulsified products, such as mayonnaise.

Some of these ingredients are already being discreetly used in the formulation of innovative products, which makes it possible for the food industry to offer them for the development of new food products, such as vegetable-based mayonnaise and with modifications in nutritional composition and/or functional appeal (MENEZES et al., 2022). Therefore, it is important to highlight that different ingredients based on vegetable proteins, which have wide applications in the food industry and functional health aspects, simulate the sensorial and physicochemical attributes of traditional mayonnaise.

Therefore, it is important to highlight that further studies are needed to evaluate the rheological profile of mayonnaise and identify how the fluid behaves under different conditions. A more complete analysis provides information on the behavior of mayonnaise during industrial processing, in addition to corroborating texture analyses.

Figure 4-2. Viscosity performance of different mayonnaise formulations (PM2, SM3, WM4 and PPM5) and compared with commercial vegan mayonnaise (CM1).



Source: Elaborated by the author.

All analyses were expressed in triplicate and results are expressed as the mean ± standard deviation.
CM1: commercial mayonnaise, PM2: Pea mayonnaise, SM3: soya mayonnaise, WM4: whey mayonnaise, and PPM5: peach protein.

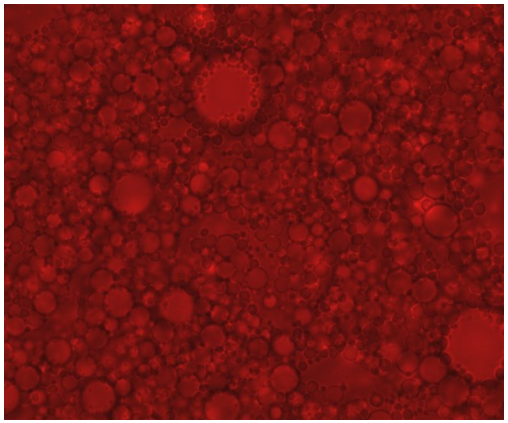
4.3.4 Microstructure observation

Figure 4-3 shows the microstructural images of the five mayonnaise analogous samples, with formulations from different protein sources. The red droplets shown at Figure 4-3 represent the oil droplets coated with the different proteins. It is possible to verify that the microstructure of the control sample (CM1) spherical oil droplets formed were trapped forming an interfacial layer (CHOI et al., 2023b), which may be related to the ingredients described in the formulation (starch and xanthan gum) adsorption and stabilization on the surface of the oil droplet, which is desired for emulsion preparation. The same behavior is observed in the other samples (PM2, SM3, WM4, and PPM5), where using different proteins may keep the droplets dispersed in the emulsion.

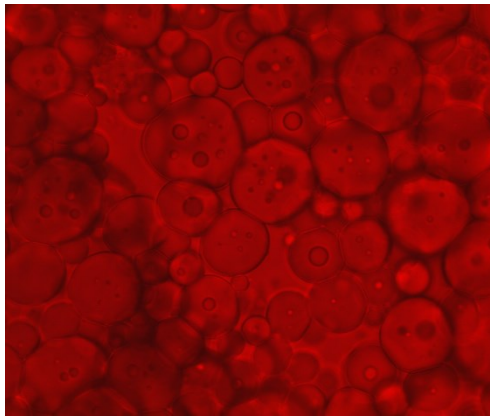
On the other hand, the formulations PM2, SM4, and WM5 presented regular structures of protein aggregates and oil droplets with different distributions and diameters. Then, PPM5 represents larger spheres and space between them, which could probably be related to homogenization time and the ratio between the protein and oil fractions used in the formulation. To avoid this, better process parameters must be evaluated for formulation by adjusting some parameters, including the protein concentration and the homogenization process, a long homogenization time can help the formation of small droplets, which may increase the stability of the formulation (HE et al., 2021).

These analyses were essential to verify the potential use of different proteins in the formulation of emulsions, which can be used in different areas: food, chemical, and cosmetic. Furthermore, other analyses, such as sensory analysis and assessment of peach protein toxicity, would be important for a more complete evaluation of the product prepared, making the study even more comprehensive

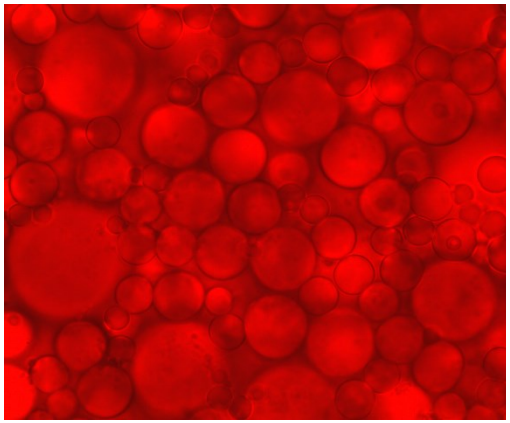
Figure 4-3 - The microstructure of different mayonnaise formulations (PM2, SM3, WM4, and PPM5) and compared with commercial vegan mayonnaise.



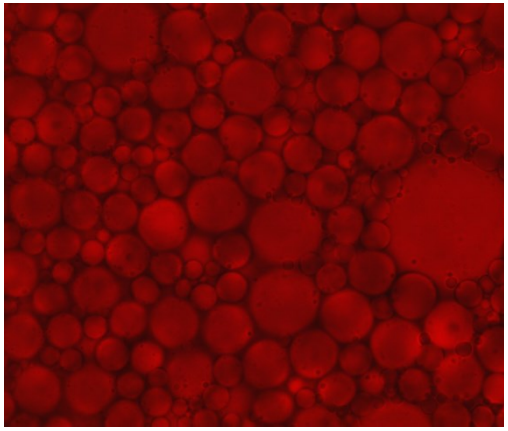
CM1



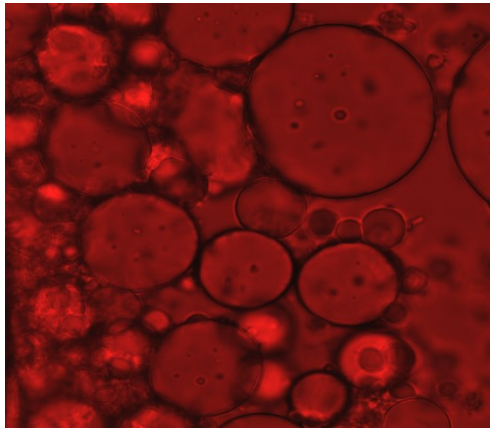
PM2



SM3



WM4



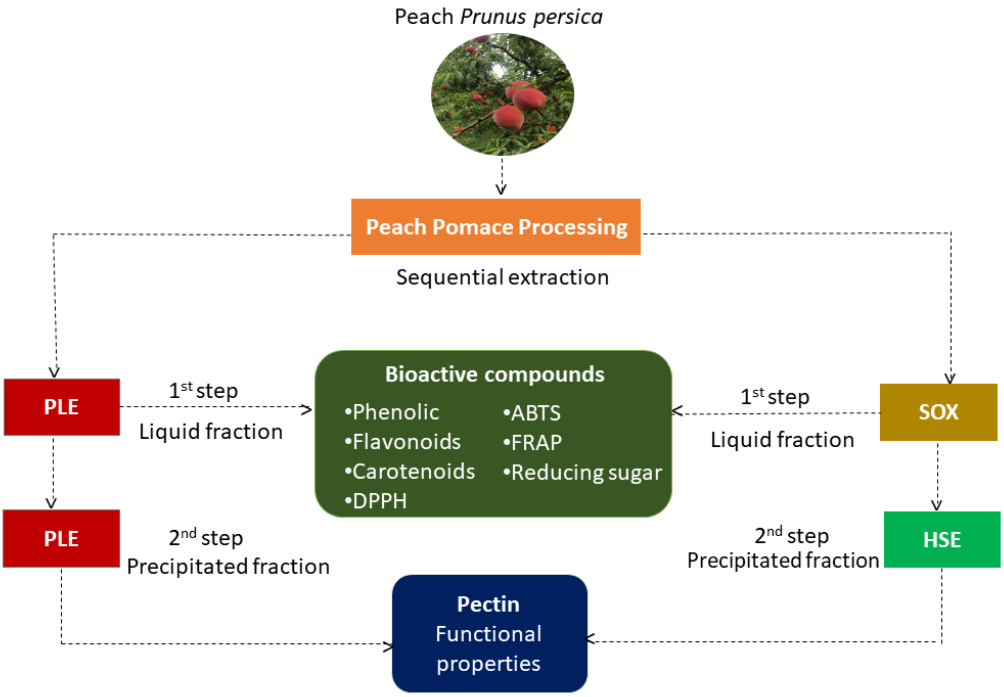
PPM5

CM1: commercial mayonnaise, PM2: Pea mayonnaise, SM3: soya mayonnaise, WM4: whey mayonnaise, and PPM5: peach protein.

4.4 CONCLUSIONS

Formulating a mayonnaise analogue presents several considerable challenges due to the complexity of sellecting the egg replacement, with equivalent techno-functional properties, such as foaming, whiteness index and hydrophobicity. The results of this study highlighted that the peach isolate protein (PPM5) matched closelly the color profile compared to the control sample (CM1), and also to traditional mayonnaise, in a visual observation. Texture analysis was crucial to evaluate the hardness, adhesiveness, resilience, and cohesion behavior of the samples, comparing different protein sources. These results demonstrated that the PPM5 sample present the properties of hardness, adhesiveness, and resilience similar to the samples produced with soy and whey proteins (SM3 and WM4), otherwise, cohesion behavior was comparable to sample produced with pea protein (PM2). Furthermore, the peach alternative source provided rheological profile close to the control sample (CM1), produced using a protein source of starch and xanthan gum described list of ingredients on the label. Additionally, microstructural analysis revealed the presence of lipid droplets entrapped in the aqueous phase of all formulation. This characteristic is essencial for emulsions of mayonnaise preparation, even the analogous products using non-animal protein sources. These findings suggest that peach isolate protein, obtained from green extraction method from the peach seeds (RUDKE et al., (2024)), and could serve as a viable alternative for producing mayonnaise analogues, offering characteristics similar to conventional vegan-based products. Nevertheless, a further sensorial analysis should be conducted to confirm the quality attributs of the proposed new product. Moreover, this chapter suggests the upcycling concept applied to peach processing, demonstrating that an agro-industrial co-product (peach seeds) could enhance the value of the peach processing chain, resulting in a design closely resembling the commercial vegan mayonnaise one. Understanding the strategy for applying the upcycling concept is relevant to design a high-quality product that aligns the sustainability concepts proposed by the Sustainable Development Goals (SDGs) from United Nations.

CHAPTER 5 – Phenolic compounds and pectin-rich extracts recovered from peach pomace by sequential pressurized liquid extractions



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5.1 INTRODUCTION

Peach fruit is globally recognized as a significant specialty crop, ranking as the 6th most important tree fruit crop in the world market (ANTHONY; MINAS, 2022). Peach pomace, a co-product obtained during peach juice processing, comprising the fruit peel and pulp. According to FAOSTAT, (2022) the Brazilian peach and nectarine production reached 208,823 tons in 2022. The pomace from the juice processing is estimated to account for approximately 10 % of the initial fruit weight, which is equivalent to more than 20 thousand tons of pomace generated in Brazil each year.

The valorization of agro-industrial wastes or co-products has been receiving significant attention since the establishment of the Sustainable Development Goals (SDGs) from United Nations. These biomasses present valuable compounds, such as phenolics and pectin, with potential applications in cosmetic, pharmaceutical and food industries. Phenolics and pectin are widely distributed in plants and play a significant role in the human diet (STEIGERWALD et al., 2022). Phenolics interest is high due to their antioxidant properties that provide health benefits (MAATALLAH et al., 2020). On the other hand, pectin is a polysaccharide present in fruit cell walls. It is an additive intentionally added (INS 440), and widely applied in food industry (DIAS et al., 2020), as gelling agent in various food formulations, such as yogurts, juices, and jellies, among other products (BEZUS et al., 2022).

The growing demand for natural products and greener extraction technologies, which aim to reduce toxic solvents, reflects the public interest in safer and more sustainable products. The search for green methods for extracting bioactive compounds drives the development of sequential processes, integrating different technologies for more efficient and environmentally friendly extraction, producing different products until the sample is exhausted (REBELATTO et al., 2020).

According to Pereira et al., (2024), the high molecular weight phenolics can complex with the protein, forming aggregates that precipitate during pectin extraction. To overcome this challenge, one potential strategy is the removal of the phenolic compounds from the peach pomace, before the recovery of a pectin-rich fraction. This strategy suggests the use sequential extractions for the separation of the phenolics- and the pectin-rich fractions from peach pomace. The recovery of valuable components from fruit co-products, such as phenolic compounds and pectin, has been gaining significant attention with several studies documented in the literature. Notably, *Passiflora edulis* sp rinds, apple pomace, and banana peel (COSTA; FORSTER-CARNEIRO, 2023; PEREIRA et al., 2024, 2021) have been explored as promising sources for these bioactive compounds. However, there are still a lack of studies exploring phenolic and pectin extraction from peach pomace (FARAVASH; ASHTIANI, 2008; PAGÁN; IBARZ, 1999; PLAZZOTTA et al., 2020).

The use of pressurized liquid extraction (PLE) for the recovery of the phenolic-rich and pectin-rich fraction from peach pomace, using ethanol and citric acid solution as solvents, remains unexplored. Considering the rising production of agro-industrial co-products, and aligning with the principles of a circular economy and green techniques, this research primarily aims to fractionate the peach pomace by a sequential recovery of two valuable fractions (phenolics and pectin) using high-pressure green solvents.

5.2 MATERIALS AND METHODS

5.2.1 Raw material

The peach pomace samples were kindly provided by Villa Puree Company, located in Santo Antônio do Paraíso, PR, Brazil (coordinates 23°30'42''S and 50°38'43"W). The peach pomace results from a blend of the peach varieties “aurora,

rubimel and tropbeltt". First, the peach pomace was dried in an oven with air circulation (De Leo, Porto Alegre/RS, Brazil) at 40°C for 48 h. Finally, the dried pomace was ground in a knife mill (De Leo, model EDB-5, Porto Alegre/RS, Brazil), and the mean particle diameter obtained was 0.49±0.02 mm.

5.2.2 Proximate composition

The dried pomace was characterized according to AOAC methods (AOAC, 2012), as follows: the moisture content was determined by gravimetric method (925.09), the lipid content (920.39), the protein content by Kjeldahl method (954.01), ash (923.03), and crude fiber (962.09). The carbohydrate content was determined by difference. The carbohydrate content was determined by difference from other macronutrients (moisture, lipid, protein, ash, and crude fiber). The assays were performed in triplicate, and the standard deviation was presented.

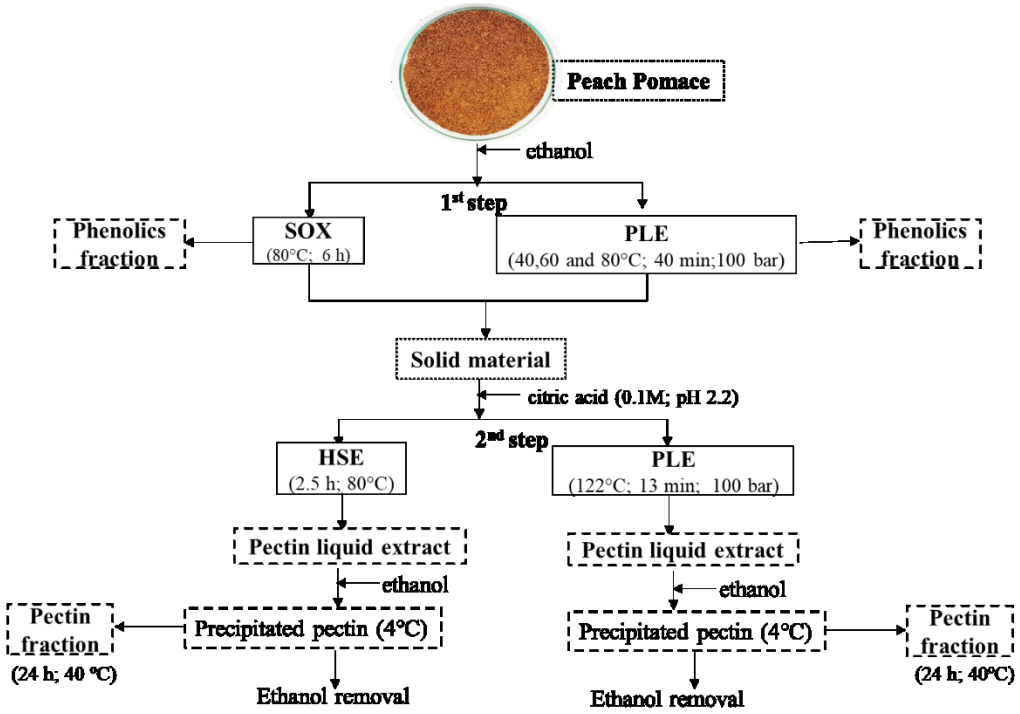
5.3 EXTRACTION METHODS

5.3.1 Soxhlet (SOX) and Heat Stirred Extraction (HSE)

The Soxhlet (SOX) procedure, used as conventional extraction, was conducted by method 920.39 from AOAC (2012). Shortly, pomace (5 grams dry mass) was weighed and inserted into the filter paper cartridge. Pure ethanol (150 mL) was used as a solvent to recover the phenolics liquid fraction from peach pomace for 6 h with reflux. Then, the solvent from the liquid fraction was evaporated in a rotary evaporator (Fisatom, model 801, São Paulo, Brazil), the yield was determined, and the extract was stored at -18 ºC in an amber flask for further analysis. The SOX assays were conducted in triplicate and results were expressed as mean and standard deviation.

Then, the remaining solid fraction (after phenolics extraction using ethanol) was used to recover the pectin-rich fraction from peach pomace by the Heat Stirred Extraction (HSE) method conducted as described by BENVENUTTI; ZIELINSKI; FERREIRA, (2022). The HSE solvent was 0.1M citric acid solution with pH 2.2 at 80°C. The solid/solvent ratio was 1:30 (g/mL), conducted during 2.5 h. The recovered pectin-rich fraction was precipitated using 99% ethanol at twice the extract volume, and then kept at 4 ºC. A vacuum pump separated the precipitated pectin from the solvent. Then, the material was dried in an oven with air circulation (De Leo, Porto Alegre/RS, Brazil) for 24 h at 40 ºC, the yield was determined, and the samples stored at -18 ºC for further analysis. The extraction steps are summarized in the flowchart from Figure *5-1*. The extraction of pectin was conducted in triplicate, and results were expressed as mean and standard deviation.

Figure 5-1. The flowchart demonstrates the steps for extracting the phenolic fraction using PLE and SOX and the pectin fraction using HSE and PLE.



Source: Elaborated by the author.

5.3.2 Pressurized liquid extraction (PLE)

Pressurized liquid extraction (PLE) was used as an alternative method for the recovery of the phenolics-rich and pectin-rich fractions from peach pomace. The extractions were performed in bath continuous mode using the apparatus described by GONÇALVEZ -RODRIGUES et al., (2019). The PLE procedure included the formation of a fixed bed with 5 g of dried peach pomace completed with 90 g of glass beads to avoid preferred paths, packed inside a jacketed stainless-steel reactor (AISI 316 stainless) with an internal volume of 90 mL. The reactor was kept at constant temperature by a

thermostatic Dubnoff-type bath (Ethik technology, 304 TPA model, SP, Brazil). The solvent flow rate of 3 $mL \cdot min^{-1}$ was maintained at 100 bar (± 2 bar) by an HPLC pump (Costech SSI Series III). The extraction time was defined by a kinetics assay performed with ethanol at 60ºC and 100 bar, with extract samples recovered at fixed time intervals: 3 min from (0 to 15 min), 5 min from (15 to 30 min), 10 min from (30 to 60 min), and 20 min from (60 to 120 min). The samples were collected in amber vials. A kinetics study was conducted for the PLE assays in order to define the adequate extraction time and performed with ethanol as solvent at 60ºC and 100 bar, with results expressed as accumulated mass (g) versus time (min).

After the kinetics study, the PLE was evaluated through assays conducted at 40, 60, and 80°C, in a dynamic mode and maintaining the flow rate at 3 $mL \cdot min^{-1}$ and pressure at 100 bar. The samples were collected at a pre-defined time (by kinetics study), placed in amber flasks, then evaporated at in a rotary evaporator (Fisatom, model 801, São Paulo, Brazil) to remove the solvent, weighed for yield calculation, and stored at -18 ºC for further analysis. All extractions were triplicated, and results were expressed as mean and standard deviation.

The solid material resulted after the phenolics extraction (ethanol as solvent) was used to recover the pectin-rich fraction, in sequential PLE with citric acid solution (0.1M) as solvent. The conditions used were solid/solvent ratio of 1:30 ($g.mL^{-1}$), 122°C, 13 min, and 100 bar, in static mode as based previously described by BENVENUTTI; ZIELINSKI; FERREIRA, (2022). The recovered solution (pectin-rich fraction) was precipitated with ethanol 99 % using twice the sample volume, and maintained at 4 ºC overnight. The precipitated pectin was separated from the solvent by vacuum pump, and the material dried in an oven with air circulation (De Leo, Porto Alegre/RS, Brazil) for 24 h at 40 ºC for yield determination. The pectin extraction was conducted in triplicate and the results are expressed mean and standard deviation. The samples were stored at -18 ºC for further analysis. The combination of PLE-ethanol, followed by PLE-citric acid

solution (0.1 M) represents the high-pressure route (route 2), which was compared with the low-pressure extractions: SOX followed by HSE (route 1).

5.4 THE CHARACTERIZATION OF THE BIOACTIVE COMPOUNDS

5.4.1 Phenolic compounds

The phenolics recovered by SOX and PLE with ethanol were evaluated in terms of the total phenolics content (TPC), by Folin Ciocalteu method described by SINGLETON; ROSSI, (1965). Briefly, a solution of distilled water (480 µL), sample (30 µL) and Folin's reagent (30 µL), reacted for 3 minutes, then 60 µL of sodium carbonate 20 % ($w.v^{-1}$) was added and react for 1 h in the dark. The absorbance was read at 765 nm on spectrophotometer (Agilent BiotTek, Epoch microplate, San Francisco-USA). The results were expressed as gallic acid equivalent (mg $EAG.g^{-1}$ extract) and the results expressed as mean value and standard deviation of triplicate assays. The curve was y = 0.9119x + 0.031, where y is the absorbance, and x is the GAE equivalent calculated according to equation (1).

$$TPC = \frac{mg\ GAE}{D\ extract} \times 100 \qquad \text{Equation 5-1}$$

TPC is the total phenolic content expressed in mg $GAE \cdot g^{-1}$ of extract; D is the dilution of the extract in $mg \cdot mL^{-1}$, and GAE is gallic acid equivalent, which was obtained by the standard curve (mg $GAE \cdot mL^{-1}$).

5.4.2 Total Flavonoids Content (TFC)

The total flavonoids content (TFC) was determined according to the methodology described by WOISKY; SALATINO, (1998). First, 10 mg of the extract sample obtained from the first extraction step (by SOX and by PLE) was resuspended in 1 mL of ethanol.

For TFC determination, an aliquot of 150 μL of the extract solution was added to 150 μL of $AlCl_3$ solution (2 %) and followed by 700 μL of ethanol. The time for the reaction was 30 min of rest, after the readings were done, on the UV-Vis spectrophotometer (Agilent BiotTek, Epoch microplate, San Francisco-USA) at 415 nm. The linear regression curve was y = 0.0078x + 0.0342, where y is the absorbance, and x is the TFC concentration. The results were expressed as mean value and standard deviation of triplicate assays.

5.4.3 Total carotenoids content (TCC)

The total carotenoids content (TCC) was determined by the method by KUHNEN et al., (2009). Briefly, 5 mg of extract sample (SOX and PLE from the first extraction step) was diluted in a 2:1:1 (v/v/v) mixture of methanol, hexane, and acetone. The solution was placed in an ultrasound bath (Ultrasonic cleaner Unique, USC-4880, Brazil) until the extract was solubilized and centrifuged (Quimis, Q22TM, Diadema-SP, Brazil) for 10 min and 3400 rpm. The supernatant was collected, and the absorbance read at 450 nm on the spectrophotometer (Agilent BiotTek, Epoch microplate, San Francisco-USA). The total carotenoid content (TCC) of the extracts was calculated from a β-carotene standard curve (≥97 % UV, Sigma Aldrich, USA). The linear regression curve was y = 11.826x + 0.0557, where y is the absorbance, and x is the TCC concentration. The results were expressed as mean value and standard deviation of triplicate assays.

5.4.4 Antioxidant Potential (DPPH, ABTS and FRAP assay)

The antioxidant potential of the extract samples obtained in the first step of extraction using SOX and PLE was determined by the methods 2,2 diphenyl-1-picrylhydrazyl hydrate (DPPH) and 2-2'-azino-di-(3-ethylbenzthiazoline sulfonic acid) acid (ABTS), following the procedures described by BRAND-WILLIAMS; CUVELIER; BERSET, (1995) and RE et al. (1999), respectively. The absorbance was read at 517 and 734 nm on the spectrophotometer (Agilent BiotTek, Epoch microplate, San Francisco-

USA). The results were expressed as equivalent antioxidant capacity in Trolox (μmol E trolox. g-1 extract). The linear regression curve standard was yDPPH=0.1726x+0.1637 and yABTS= -0.0007x+0.7505. The results were calculated by the equation below. AbsE and AbsC are the absorbances of the extract and the negative control, respectively. Which were obtained by the standard curve (μmol. L^{-1}) and the results were expressed as mean value and standard deviation of triplicate assays.

$$AA\ (\%)=[1-\ Abse/Absc]\ \times 100 \qquad \text{Equation 5-2}$$

Besides the above-mentioned methods, the antioxidant potential was also determined by Ferric Reducing Antioxidant Power (FRAP) method according to BENZIE; STRAIN, (1996). The FRAP reagent was prepared using a 0.3 M sodium acetate buffer (pH 3.6), a 20 mM ferric chloride solution, and 2,4,6-tripidylstriazine (TPTZ) diluted in a 40 mM hydrochloric acid solution. The extract sample was added to the reagent and incubated in dark at 37°C for 30 minutes. Then, the absorbance was read at a wavelength of 593 nm at a spectrophotometer (Agilent BiotTek, Epoch microplate, San Francisco-USA). Trolox was used as the standard curve, with y = 0.002x + 0.1564, where y is the absorbance, and x is the Trolox equivalent. The results were expressed as mean value and standard deviation of triplicate assays.

5.4.5 Reducing Sugar Analysis

Reducing sugars was determined from the samples obtained in the first extraction step by SOX and by PLE following the protocol outlined by MONTEIRO et al., (2021) with some modifications. Initially, 500 μL of the liquid extract was transferred to an Eppendorf tube, and 500 μL of acid 3,5 dinitrosalicylic (DNS) was added. The tube was then shaken and heated in a Dubnoff-type bath (Ethik technology, 304 TPA model,

SP, Brazil) at 100°C for 5 minutes, then cooled in an ice bath for 5 minutes. Subsequently, 300 µL of the solution was transferred to a microplate reader the absorbance readings were taken at 540 nm on the spectrophotometer (Agilent BiotTek, Epoch microplate, San Francisco-USA).

The total reducing sugar content (TRS) was determined from samples obtained in the first extraction step by SOX and by PLE, following the methodology described by MONTEIRO et al., (2021) was determined using the following steps: 500 µL of H2SO4 (2M) was added to the liquid extract (500 µL), and the Eppendorf tube were placed in a bath at 100°C for 5 minutes. After heating, the tubes were cooled in an ice bath. Then, 500 µL of the sample that reacted with the acid was added to 500 µL of NaOH (2M) and 500 µL of DNS. Again, the tubes were placed in a bath at 100°C for 5 minutes, then cooled in an ice bath. Finally, 300 µL of the solution was transferred to a microplate reader. The absorbance readings were taken at 540 nm (Agilent BiotTek, Epoch microplate, San Francisco-USA). The concentration of RS e TRS was calculated using a glucose calibration curve spanning various concentrations (mg/mL), employing the equation: y = 2.8728x - 0.114 The results for each sample were expressed in mg equivalent glucose. g^{-1} of extract. The results are expressed in percentage as mean value and standard deviation of triplicate assays.

5.4.6 Phenolics profile by LC-MS/MS

In this study, the phenolic compounds were identified and quantified from the extract samples recovered from peach pomace by SOX and PLE methods using ethanol as solvent, and obtained from the first step of the sequential extraction. This analysis was performed using a high-performance liquid chromatography system (Series 1200, Agilent Technologies, Waldbronn-BW, Germany), following the methodology described by SCHULZ et al. (2015). The compounds were separated using a Synergi column (4.0µm,

2.0×150mm i.d.; Phenomenex, Torrance-CA, USA), employing a gradient elution composed of methanol:water (95:5, v/v) and aqueous solution of formic acid 0.1 % (v/v). The liquid chromatography system was coupled to a hybrid triple quadrupole/linear ion trap mass spectrometer (Q Trap 3200 Applied Biosystems/MDS Sciex, Concord-ON, Canada). Analysis took place in negative electrospray ionization mode (TurboIonSpray Applied Biosystems/MDS Sciex, Concord-ON, Canada), with MS/MS parameters configured according to SCHULTZ et al., (2015) capillary needle at −4500V; curtain gas at 10psi; temperature at 400°C; gas 1 and gas 2 at 45psi; CAD gas in medium mode. Chromatographic conditions and mass spectrometry parameters followed previously established guidelines. System control and data analysis were conducted using Analyst software (1.5.1). This improved analytical approach provided a detailed characterization of the phenolic compounds in peach pomace extracts, contributing substantially to the understanding of the chemical composition of these samples.

5.5 PHYSICO-CHEMICAL AND FUNCTIONAL PROPERTIES OF THE PECTIN-RICH FRACTION

5.5.1 Galacturonic acid content

The galacturonic acid (GalA) content was determined for the pectin-rich extract samples, obtained from the second step of the sequential extractions (by HSE and by PLE-citric acid). The GalA content was determined following the spectrophotometric method described by BLUMENKRANTZ; ASBOE-HANSEN, (1973). First, 200 µL of an aqueous pectin-rich extract solution (15 mg. mL^{-1}) was combined with 6 mL of 0.0125 M sodium tetraborate in concentrated H_2SO_4 in tubes. The mixture was then heated and cooled in a thermostatic water bath (TECNAL, model TE-2005, SP, Brazil) at 95°C for 5 min, then cooled (room temperature). Finally, 20 µL of 0.15 % (w/v) 3-phenyl phenol in 0.5% NaOH was added and thoroughly mixed. The absorbance of the pectin fraction was

read at 525 nm. A standard curve was prepared with galacturonic acid and the straight-line equation obtained was y= 0.0825x+0.1314. The absorbance of the samples was read on the plate reader spectrophotometer (Agilent BiotTek, Epoch microplate, San Francisco-USA). The results were expressed in percentage as mean value and standard deviation of triplicate assays.

5.5.2 Degree of esterification (DE) and methylation content (MC)

Pectin-rich fractions, recovered by sequential methods (SOX-HSE and PLE-PLE), were analyzed to determine the degree of esterification (DE) and the methylation content (MC) by titration procedure described by TRAN et al., (2023). First, the pectin-rich fractions (dry) extracted by PLE and by HSE (50 mg) was dissolved in 2 mL of ethanol, and 20 mL of distilled water was added. Then, three drops of phenolphthalein were added, and the solution was titrated with 0.1 M NaOH (V1), and 10 mL of 0.1 M NaOH was added, and submits to agitation magnetic stirrer for 2 hours. The sample rest for 20 minutes, and add 10 mL of 0.1 HCl and stir until the pink color disappears. Finally, 5 drops of phenolphthalein were added again, and the titration placed by adding 0.1 M of NaOH until a light pink color persists (V2). The degree of esterification (DE) and the methylation content (MC) of the pectin-rich fractions were calculated using the following equations:

$$DE(\%) = \frac{V2}{V1 + V2} \times 100$$ Equation 5-3

$$MC(\%) = \frac{V2 \times normality \times 3.1}{weight\ of\ sample\ (g)} \times 100$$ Equation 5-4

5.5.3 Viscosity of the pectin-rich fractions

Pectin-rich extracts were obtained from the second extraction step of the sequential routes (SOX-HSE and PLE-PLE). The samples were analysed in terms of viscosity profile and compared with the results from a commercial pectin (citric pectin), used as control. The samples were dissolved in distilled water in 15 mL of distilled water for a concentration of 0.5%. A temperature ramp analysis ranged from 25 to 90°C with a constant shear rate 50 s^{-1}. Rheological parameters were assessed using a Rheometer (Anton Paar® model MCR 72, concentric cylinder-CC 27, Austria) following the procedure described by TRAN et al., (2023).The outcomes were presented graphically, depicting viscosity as a function of temperature. The results were expressed in percentage as mean value and standard deviation of triplicate assays.

5.5.4 Water solubility

The water solubility of pectin-rich fractions, obtained by SOX-HSE and PLE-PLE was determined as described by TRAN et al. (2023). First, 50 mg of pectin-rich fractions were weighed and dissolved in distilled water (40 mL) with agitation (magnetic stir plate) for 20 minutes. Subsequently, the sample was centrifuged (Quimis, Q22TM, Diadema-SP, Brazil) for 15 minutes at 5000 x *g*, and then the supernatant was removed. The following equation was used for water solubility determination. The results were expressed in percentage as mean value and standard deviation of triplicate assays.

$$Solubility\ (\%) = \frac{(initial\ \ dry\ weight - final\ dry\ weight}{initial\ \ dry\ weight} \times 100$$

Equation 5-5

5.5.5 Water holding capacity and oil holding capacity

The water holding capacity (WHC) and oil holding capacity (OHC) of each pectin-rich fraction, obtained by SOX-HSE and PLE-PLE were determined in accordance by DU et al., (2014). The pectin-rich fraction was dissolved into water and oil at a concentration of 0.12 g.mL^{-1}. The solutions were agitated magnetic stir plate for 30 minutes. Subsequently, the samples were centrifuged (Quimis, Q22TM, Diadema-SP, Brazil) for 15 minutes at 5000 g, and the supernatant was carefully removed and weighed. The WHC and OHC were determined using the following equation, with results expressed as mean value and standard deviation of triplicate assays.

$$WHC\ and\ OHC\ (g/g) = \frac{final\ weight\ (g)}{initial\ \ weight\ (g)} \qquad \text{Equation 5-6}$$

5.5.6 Emulsion activity and stability

The emulsion activity (EA) and the emulsion stability (ES) of the pectin-rich fraction, obtained by SOX-HSE and PLE-PLE were determined according to HOSSEINI; KHODAIYAN; YARMAND, (2016) with some modifications. Firstly, the pectin dissolved in distilled water at 0.5 %. After that, 5 mL of pectin solution and 5 mL soybean oil were homogenized using apparatus (Ultra-Turrax®, model T25 digital, IKA, Campinas-SP, Brazil) for 1 min at 14000 rpm. Emulsion Ability (EA) was calculated as the (initial emulsion volume/total suspension volume) × 100, and the emulsion Stability (ES) was calculated after 4 weeks of sample incubation at 4°C with results expressed as emulsion volume after 4 weeks/total suspension volume) × 100. The results were expressed in percentage as mean value and standard deviation of triplicate as assays.

5.5.7 Foaming ability (FA) and foaming stability (FS)

The forming properties of the pectin-rich fraction, obtained by SOX-HSE and PLE-PLE were determined as described by EZZATI et al., (2020). The pectin-rich fraction was dissolved in water were prepared at a concentration of 0.5 % w.v^{-1} and agitated for 1 minute and 9000 rpm using a homogenized using apparatus (Ultra-Turrax®, model T25 digital, IKA, Campinas-SP, Brazil). Changes in foam volume were recorded immediately after preparation, and Foam Ability (FA) was calculated as the (initial foam volume/total suspension volume) × 100. Foam Stability (FS) was calculated after 30 minutes of incubation at room temperature. The results were expressed in percentage as mean value and standard deviation of triplicate as assays.

5.5.8 Color

The color parameters of pectin- rich fraction, recovered by SOX-HSE and PLE-PLE, were evaluated in a colorimeter (Delta Vista, model 450 G SN 7012003357, São Leopoldo-RS, Brazil). The parameters L*, a*, and b* were used to calculate values of hue angles (H*), and chroma (C) as described by SPINEI; OROIAN, (2023) and following the equations and the results were expressed in percentage as mean value and standard deviation of triplicate as assays.

$$Hue\ angle\ (H^*) = Tan^{-1}\left(\frac{a^*}{b^*}\right)$$ Equation 5-7

$$Chroma\ (C) = \sqrt{(a^*)^2 + (b^*)^2}$$ Equation 5-8

Where the letters mean L* represent lightness; + a* is redness and - a* the greenness, while +b* is the yellowish and -b* the blueness.

5.6 STATISTICAL ANALYSIS

All results were presented as mean followed by standard deviation. One-way analysis of variance (ANOVA) the significant differences ($p < 0.05$) was carried out using the Software Statistica® (TIBCO, v. 13.0.5.017, Palo alto-CA, USA) and the Tukey test was used for average determination, as mentioned previously.

5.7 RESULTS AND DISCUSSION

5.7.1 Proximate composition of peach pomace

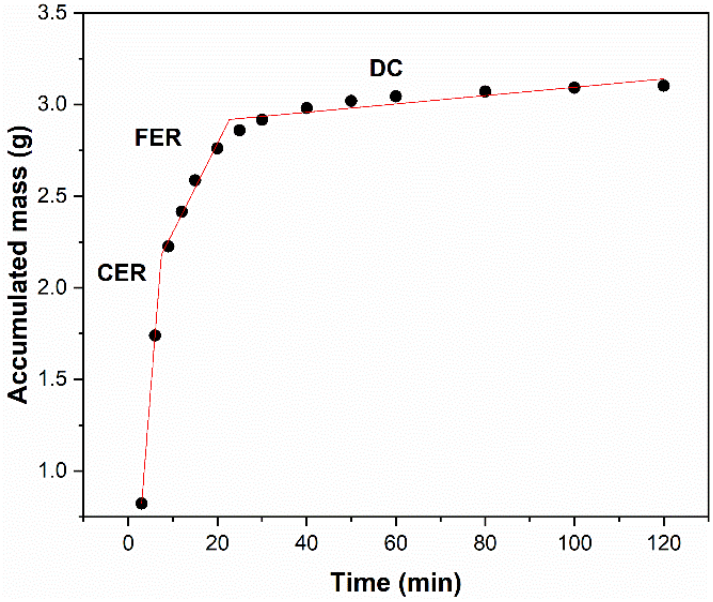
The results of the proximate composition analysis for peach pomace indicate that the pomace fraction is particularly rich in carbohydrates (58.71 ± 0.46 %), followed by crude fiber (20.93 ± 0.09 %), protein (6.69 ± 0.38 %), lipids (1.27 ± 0.03 %), and moisture (9.77 ± 0.23 %). These values are in accordance with reports by PAGÁN; IBARZ, (1999) for peach pomace, where protein and lipid contents were 7.5 % and less than 3 %, respectively. GARCÍA-APARICIO; CASTRO-RUBIO; MARINA, (2024) reported a fiber content of 14.65 % for peach pomace, less than reported in this study (20.93 %). These differences can be attributed to variations in peach maturity, climate and geographic and cultivation conditions (BENTO et al., 2020).

5.7.2 Kinetics curve for PLE with ethanol

The extraction time for PLE assays was defined based on the kinetics extraction curve (Figure 5-2), expresses as accumulated mass of extract with time, for a curve obtained at 60°C, 100 bar, and solvent flow rate of 3 $mL.min^{-1}$. The kinetics curve represent three mass transfer regions: the Constant Extraction Rate (CER), characterized by convection-driven mass transfer of a readily accessible solute; the Falling Extraction

Rate (FER), with significant decreasing extraction rate due to the combination of convection and diffusion mass transfer mechanisms; and finally the Diffusion Controlled phase (DC), where the mass transfer rate is governed by the diffusion of the innermost solute (MESQUITA et al., 2022; SOVOVÁ, 1994). Then, based on the kinetics curve, 40 min was defined as the adequate extraction time for PLE-ethanol assays, due to reaching the DC period approaching the complete exhaustion of the material.

Figure 5-2. Kinetics curve from PLE - ethanol



Source: Elaborated by the author.

5.7.3 Ethanolic fractions by PLE and SOX (first extraction step)

The ethanolic fractions from peach pomace were obtained by low- and high-pressure methods (SOX and PLE, respectively), where PLE was conducted in 40 min for each temperature (40, 60, and 80°C) (section 5.3.2). The yield results are compared in Table 5-1, which also provides the quality parameters of the recovered extracts.

The SOX method provided the highest yield (48.39 %), a value statistically similar to obtained by PLE at 80ºC (43.76 %), where the yield for the high-pressure method increased with temperature (Table 5-1). GARCÍA-APARICIO; CASTRO-RUBIO; MARINA, (2024) also used PLE method, but at the conditions of 100°C, 5 min, and ethanol 50 %, for the recovery of bioactive compounds from peach pomace, and the yield result was 23 %, much lower than provided from the present work. The yield differences are probably due to variation in PLE conditions (time, temperature), the solvent type used and raw material characteristics. Also, for the high-pressure assays, the PLE yield values increased with increasing temperature (Table 5-1), probably due to the growth in the solubility of the target components in the solvent, such as ethanol, favouring the process yield (RUDKE et al., 2019).

The quality of the ethanolic fractions from the first extraction step (by SOX and PLE) was evaluated through the aspects as TPC, TFC, TCC and by antioxidant potential (by DPPH, ABTS and FRAP methods).

5.7.4 TPC, TFC and TCC from the ethanolic fractions by SOX and PLE

The ethanolic fractions from peach pomace recovered by SOX and PLE at first extraction step were characterized concerning the total phenolics content (TPC), the total flavonoids content (TFC) and the total carotenoids content (TCC), as indications for the antioxidant potential of the peach pomace extracts.

The TPC result from SOX sample provided the highest value (28.68 mg GAE.g^{-1} of extract), with statistical differences with PLE samples, which varied from 6.85 to 10.31 mg GAE.g^{-1} of extract, with highest value for the PLE sample recovered at 40ºC. The best TPC performance from SOX sample may be associated with conditions of solvent reflux, and higher time and temperature of SOX method compared to PLE, which contribute to the solubilization of phenolic compounds (MAZZUTTI et al., 2018; MUSTAFA;

TURNER, 2011). However, the PLE at 40°C provided a sample with high TPC value, compared to PLE at higher temperatures. This behaviour may be related to the combined effect of temperature and pressure, contributing to degradation of some phenolic compounds at higher temperatures. The TPC results for PLE samples agree with data presented by PLAZZOTTA et al. (2020) for peach pomace extracts (up to 6.39 mg GAE.g^{-1} extract) obtained by ultrasound and microwave methods with ethanol and water mixtures as solvents (59 ºC and up to 120 seconds). The TPC results for PLE samples are close to reported by GARCÍA-APARICIO; CASTRO-RUBIO; MARINA, (2024) for peach pomace, with 4.9 mg GAE.g^{-1} extract. A lower TPC value was provided by the PLE sample at 60ºC (with significant difference) compared to that obtained at 40 and 80ºC.

The values of total flavonoid content (TFC) from the ethanolic extracts (by SOX and PLE) are also presented in Table 5-1, ranging from 98 to 649 µg QE.g^{-1} extract (with statistical differences). The highest value was provided by SOX sample (649 µg QE. g^{-1} extract). For the PLE samples, the effect of temperature on TFC was the same as presented by TPC (Table 5-1). Lowest TFC was obtained at 60°C, probably due to degradation of flavonoid compounds with temperature increase from 40°C to 60°C. Otherwise, TFC increased from 60°C to 80°C may be associated with higher flavonoids extraction with increasing temperature (note the yield increase), or to the solubilization of different flavonoid group at 80ºC, contributing to TFC value (GAO et al., 2022). The TFC value for the SOX sample is close to reported by Plazzotta et al. (2020), from 750-900 µg QE.g^{-1} extract, for peach pomace extracts obtained by ultrasound and microwave methods up to 120 seconds, probably because the very short time prevented the flavonoids degradation (SORITA; LEIMANN; FERREIRA, 2023). 2022).

Total carotenoids content (TCC) ranged from 3.82 to 7.05 (mg BCE.g^{-1}) for the ethanolic extracts by SOX and PLE (Table 5-1). The highest value was provided by PLE at 60°C, with no difference than PLE-80ºC. Otherwise, the lowest value was observed at

PLE-40°C, with no significant difference compared to SOX and PLE-80°C. The present results were higher than report by VARGAS et al. (2017) for peach pomace ethanolic extracts obtained under stirring (25°C), which provided 0.811 mg BCE.g^{-1}. This difference may be due to variations in extraction method, in fruit variety and maturity, and also in climatic and post-harvest conditions (ZAGHDOUDI et al., 2015).

5.7.5 Antioxidant activity (DPPH, ABTS and FRAP) from ethanolic extracts

The antioxidant potential from the ethanolic extracts (by SOX and PLE) was evaluated by DPPH, ABTS and FRAP methods, with results presented at Table 5-1. The antioxidant potential by all methods was better for the SOX sample, compared to PLE.

The DPPH results from Table 5-1 indicate the highest value for SOX sample (49.73 μmol TE. g^{-1}) while PLE samples ranged from 9.05 to 20.01 μmol TE. g^{-1}, with the lowest result associated with the highest temperature (80°C). DPPH results were higher than reported by (PLAZZOTTA et al., 2020) for ultrasound extracts (0.09 μmol TE/g extract).

The ABTS results from the PLE method ranged from 37 to 43 μmol TE. g^{-1} (Table 1), which were higher than reported by TSIAKA et al. (2023) for peach by-product extract obtained by ultrasound and microwave (22.8 and 24.21 μmol TE. g^{-1}), respectively). Opposite to DPPH behaviour, the sample obtained at PLE-80 ºC provided higher ABTS value (43.57 μmol TE. g^{-1}) compared to other PLE samples. Similar ABTS behaviour was described by Mesquita et al., (2022) for acerola pomace extracted by subcritical water (100 bar) observing that increasing the temperature from 70 to 90°C increased the ABTS values.

The highest FRAP result was provided by SOX sample, followed by PLE-40 ºC, with a value of 155.15 μmol TE.g^{-1} (Table 5-1), while the lowest values, with no significant difference, were from samples at 60 and 80 ºC. The FRAP reduction with

increase in PLE temperature may be due to degradation of thermolabile compounds (MUSTAFA; TURNER, 2011).

5.7.6 Sugar content

Table 5-1 also shows the values for total reducing sugar (TRS) from the phenolic-rich fractions by SOX and PLE. The TRS content ranges from 481 to 708 mg EG. g^{-1} extract, with highest value obtained by PLE-60°C (708 mg EG. g^{-1}), followed by SOX sample, with no significant difference than PLE-80ºC, and the lowest TRS was recorded by PLE-40°C (482 mg EG. g^{-1}). The TRS from peach pomace by-product was reported by GARCÍA-APARICIO; CASTRO-RUBIO; MARINA, 2024) as 55 mg EG. mL^{-1} extract, a value much lower than the results presented at Table 5-1, probably due to the high-pressure process (present work), which may have altered the properties of the fibers in the plant matrices, making them more accessible to the detection by the TRS assay, and contributing to the sugar fraction (MONTEIRO et al., 2022).

The content of reducing sugars (RS) (Table 5-1) was the highest for the samples provided by PLE at 60 and 80ºC, with no significant difference, while the lowest value was obtained by PLE-40°C (339 mg EG.g^{-1} extract), with the SOX sample providing the intermediate value. The lowest non-reducing sugar (NRS) value (Table 5-1) was provided by PLE-80°C (57 mg EG.g^{-1}), while the highest result was achieved by PLE-60°C in PLE (228 mg EG.g^{-1} extract). The samples by SOX and PLE-40 ºC had no significant difference and presented intermediate values compared to other samples.

From the results obtained, it is clear that high-pressure influenced the extraction of reducing sugars. The positive effects of high pressure are associated with improving hemicellulose depolymerization and saccharification of pretreated materials Monteiro et al., (2022). According to Shpigelman et al., (2015) high-pressure depolymerization has a significant influence on RS caused by the increase in sugars release. Then, the pectin depolymerization is related to the presence of reducing sugar side chains (SHPIGELMAN

et al., 2015). Therefore, the PLE 40°C was the selected method for sequential pectin extraction (second step of extraction) since RS had low value at 40°C (Table 5-1).

Table 5-1 . First extraction step (PLE and SOX with ethanol as solvent): Extraction yield and quality parameters of the recovered extracts (TPC TFC, TCC, DPPH, ABTS, FRAP and TRS, RS and NRS).

Analysis	**SOX**	**PLE** 40°C	60°C	80°C
Yield (liquid fraction, %)	48.39±0.21^{a}	28.00±2.02^{c}	38.8±2.48^{b}	43.76±0.10^{ab}
TPC (mg GAE.g $^{-1}$)	28.68±0.08^{a}	10.31±0.16^{bA}	6.85±0.08^{cB}	9.49±0.39^{dA}
TFC (µg QE.g $^{-1}$)	649.10±4.53^{a}	270.90±0.91^{bA}	98.46±0.00^{cC}	111.28±1.81^{dB}
TCC (mg BCE. mg.g $^{-1}$)	4.50±0.24^{b}	3.82±0.51^{bC}	7.05±0.73^{aA}	5.26±0.30^{abAB}
DPPH (µmol.TE.g $^{-1}$)	49.73±1.29^{a}	20.01±0.81^{b}	14.05±0.97^{c}	9.05±1.13^{d}
ABTS (µmol TE.g $^{-1}$)	59.64 ±0.40^{a}	37.57±1.11^{cB}	39.79±1.01^{cB}	43.57±0.10^{bA}
FRAP (µmol TE.g $^{-1}$)	194.28±6.89^{a}	155.15±4.24^{bA}	137.15±0.36^{cB}	137.65±0.35^{cB}
TRS (mg EG.g $^{-1}$)	541.07±0.0^{b}	481.76±0.0^{cC}	708.99±0.0^{aA}	521.58±0.0^{bB}
RS (mg EG.g $^{-1}$)	404.72±0.0^{b}	339.00±0.1^{cB}	481.20±0.0^{aA}	464.87±0.0^{aA}
NRS (mg EG.g $^{-1}$)	136.36±0.0^{b}	143±0.07^{bB}	228±0.09^{aA}	57±0.05^{cC}

Total Phenolic Content (TPC), Total Flavonoids content (TFC), Total Carotenoids Content (TCC), antioxidant activity (DPPH, ABTS, and FRAP), total reducing sugar (TRS), reducing sugar (RS) and non-reducing sugar (NRS); All analyses were expressed in triplicate and results are expressed as the mean ± standard deviation; Different letters on the same line indicate a significant difference between the means ($p < 0.05$); Lower case letters compare all means (SOX and PLE at all temperatures). Upper case letters compare only the different temperatures in the PLE; g-1 refers to gram of dry extract); GAE: Gallic acid equivalent; QE: quercetin equivalent; BCE: beta-carotene equivalent; TE: Trolox equivalent; EG: equivalent glucose.

5.7.7 Phenolic profile by LC-MS analysis from peach pomace

Table 5-2 presents the phenolic compounds detected from peach pomace ethanolic extracts obtained by SOX and by PLE-40 ºC. The high-pressure sample was selected base on the *in vitro* TPC results that demonstrated better activity at 40°C. The LC-MS analysis of the samples provided the identification and quantification of 31 phenolic compounds, which are listed in Table 5-2 divided by the categories of phenolic compounds such as phenolic acids and flavonoids. Then, also in high concentration the components detected for both samples were protocatechuic acid, mandelic acid, p-coumaric acid, and vanillin. These results agree with BALTACIOĞLU et al., (2024), which identified several phenolic compounds in peach pomace, with the main component represented by protocatechuic acid, p-coumaric acid, and caffeic acid. Liu; Cao; Jiang, (2015) detected chlorogenic acid as the major compound (2944. $\mu g.g^{-1}$ fresh weigh) from peach peel methanolic extract. These composts were also identified by GARCÍA-APARICIO; CASTRO-RUBIO; MARINA, (2024) from peach pomace ethanolic extract.

The components identified and quantified from SOX and PLE samples (Table 5-2) are relevant substances, especially protocatechuic acid, p-coumaric acid, mandelic acid and vanillin, which present valuable use in pharmaceutical and food products (GONÇALVES RODRIGUES et al., 2019), The composition of the phenolics fraction from peach pomace suggest high possibility to improve its use, adding value to the peach juice processing chain by applying the upcycling concept.

Table 5-2 Phenolics components from peach pomace extracts obtained by SOX and PLE with ethanol as the first extraction step, in $\mu g.g^{-1}$ of dry extract.

	Phenolic Compounds	SOX	PLE (40 ºC)
	Phenolic acids		
1	Caffeic acid	12.73 ±0.03	11.43±0.18
2	Chlorogenic acid	0.46±0.00	0.50±0.00
3	Ellagic acid	23.36±0.99	17.28±0.60
4	Gallic acid	4.66 ±0.03	<LOQ
5	Protocatechuic acid	148.30±0.16	116.20±0.34
6	Sinapic acid	0.92±0.03	1.27±0.04
7	Syringic acid	15.09±0.03	15.46±0.00
8	p-coumaric acid	56.17±0.22	76.82±0.49
9	4-aminobenzoic acid	13.19±0.54	17.84±0.30
10	Mandelic acid	112.34±0.23	66.67±0.17
11	4-hydroxymethylbenzoic acid	2.37±0.02	3.50±0.01
12	rosmarinic acid	3.8±0.22	2.7±0.11
13	Abscisic Acid	13.56±0.03	8.06±0.09
	Flavonoids		
14	Hispidulin	<LOQ	<LOQ
15	Isoquercetin	<LOQ	36.36±0.60
16	Kaempferol	nd	<LOQ
17	Myricetin	5.70±0.12	3.19±0.02
18	Quercetin	8.18±0.34	5.75±0.16
19	Naringenin	30.32±0.06	9.89±0.05
20	Rutin	<LOQ	<LOQ
21	Taxifolin	5.54±0.01	<LOQ
22	Pinocembrin	<LOQ	<LOQ
23	Apeginin	<LOQ	<LOQ
24	Galangina	nd	<LOQ
25	Chrysin	<LOQ	<LOQ
26	Eriodictyol	2.87±0.03	1.27±0.02
	Phenolic aldehydes		
27	Vanillin	97.74±0.00	45.03±0.00
28	Syringaldehyde	18.21±0.00	nd
29	Coniferaldehyde	nd	7.11±0.26
	Coumarin		
30	Umbelliferone	5.06±0.04	3.34±0.03
31	Scopoletin	2.35±0.01	<LOQ

All analyses results are expressed as the mean ± standard deviation; different letters on the same line indicate a significant difference between the means ($p < 0.05$) nd: not detected and < LOQ: not quantifiable.

5.8 PECTIN-RICH FRACTION: SECOND EXTRACTION STEP

5.8.1 Pectin fraction yield

The pectin-rich fractions were obtained at the second extraction step, by two routes (section 5.3.2), SOX-HSE and PLE-PLE using different temperatures, and with the solvent citric acid, and the extraction yield values are presented in Table 5-3. The conventional technique (SOX) provided the highest yield (21.08 %), showing a significant difference compared to PLE. The PLE yield values presented no statistical difference between the extraction temperatures used. The pectin yield values are better than reported by FARAVASH; ASHTIANI, (2008). The authors recover pectin from peach pomace using a liquid-solid extraction method (acidified HCl solution), with solid/solvent ratio of 1:5, and 120 minutes, 60°C extraction, obtaining a maximum pectin yield of 9.94 %. The pectin yield values are comparable to obtained from guava pomace by KAMAL et al., (2023). The authors recovered pectin by a maceration method with hydrochloric acid as solvent, and at 85°C, pH 2, and 70 minutes, resulting in pectin yield of 16.59 %. Therefore, the pectin yield values (Table 5-3) suggest that peach pomace is as a relevant industrial co-product for pectin recovery, contributing to valorise the peach processing chain. The use of an eco-friendly solvent such as citric acid at PLE method, and short extraction times (13 min), compared to HSE method (2.5 hours), allow the recovery of high pectin yields, compared to literature data, showing the potential of the emerging PLE technology. Considering the no significant difference in yield values by PLE at different temperatures, the sample recovered by PLE-40 ºC was selected for further pectin characterization analyses. This choice was based on minimize energy consumption, at lower temperature, and also considering the lower impact on reducing sugar content from the PLE-40 ºC sample, as presented in Table 5-3.

Table 5-3 Yield, physical-chemical and functional properties of the pectin-rich fraction as the second extraction step

Yield of pectin (%)			
HSE-acid	**PLE-acid**		
	40°C	**60°C**	**80°C**
21.08 ± 1.57[a]	11.85±1.08[b]	14.06±1.19[b]	12.14±1.23[b]
Pectin Physico-chemical and functional properties			
Analysis		**HSE-acid** (80°C/2.5 h)	**PLE-acid** (122°C/13 min)
Galacturonic acid content (%)		10.90±0.03[b]	47.43±0.07[a]
Degree of esterification-DE (%)		22.15±1.16[b]	31.86±1.06[a]
Degree methylation content-DM (%)		4.23±0.0[b]	7.95±0.00[a]
Water Solubility (%)		94.65±1.93[a]	96.47±1.10[a]
Water Holding Capacity-WHC (g water. g^{-1})		0.04±0.00[a]	0.03±0.00[a]
Oil Holding Capacity-OHC (g oil.g^{-1})		1.68±0.09[a]	1.73±0.03[a]
Emulsion activity (%)		90.00±2.93[a]	60.42±2.95[b]
Emulsion stability 4 weeks (%)		70.00±2.83a	47.92±2.95 b
Foaming ability (%)		97.50±1.21[a]	82.39±6.55[a]
Foaming stability (%)		34.19±0.41[a]	28.33±2.26[a]
L*		14.16±0.29[a]	12.82±0.08[b]
a*		4.42±0.08[b]	7.86±0.32[a]
b*		7.09±0.11[b]	12.07±0.11[a]
Chroma (C)*		14.40±0.08[a]	8.35±0.05[b]
Hue angle (H)*		0.56±0.02[a]	0.58±0.02[a]

All analyses were expressed in triplicate and results are expressed as the mean ± standard deviation. Different letters on the same line indicate a significant difference between the means ($p < 0.05$).

5.8.2 Galacturonic acid content (Gal) from the pectin fractions

Galacturonic acid (GalA) is the main component from the pectin structure, therefore, the quality of pectin is typically based on its GalA content. Then, the pectin-rich fractions, recovered from the second step of routes SOX-HSE and PLE-PLE, were evaluated in terms of galacturonic acid content (GalA), with values at Table 5-3.

The results for the galacturonic acid content show 47.43 and 10.90 % for the PLE and HSE samples respectively. The decrease in GalA content from HSE sample can be attributed to thermal degradation during the first extraction step (SOX) for phenolics recovery, and also to the extended period of HSE method (2.5 hours), compared to PLE (13 min). These results agree with reported by ZHOU et al., (2022b), which compared different solvents for the recovery of peach pomace pectin using the instantaneous controlled pressure drop (DIC) method. The authors used Na_2CO_3 soluble pectin (NSP) method at 105°C and 90°C, with GalA content of 44.83 % and 45.68 %, respectively, while the chelator-soluble pectin (CSP) method at 105°C and 90°C provided GalA of 8.55 % and 9.99 % respectively. Therefore, the GalA results found in the present study suggest that the high-pressure sample (PLE) presents better quality than HSE sample. Nevertheless, the PLE sample seems very promising, if compared with a high-quality pectin which, according to EZZATI et al., (2020), should contain at least 65 % GalA.

5.8.3 Degree of esterification (DE) and degree of methylation content (DM)

The degree of esterification (DE) and methylation content (DM) were determined from a pectin-rich fraction recovered by the second step of routes SOX-HSE and PLE-PLE, and the results are presented at Table 5-3. These parameters are relevant for pectin characterization, aiding to define its application (CHANDEL et al., 2022). Significant differences were observed for DE and DM between HSE and PLE samples, with higher values for the high-pressure sample for both parameters.

Degree of esterification (DE) is defined as the amount of galacturonic acid units that are methyl-esterified at C6. DE values of less than 50 % are classified as low methoxyl pectin, while DE values higher than 50 % are considered high methoxyl pectin (EZZATI et al., 2020; MONICA; PRABHA; KAPOOR, 2022).

In the present study, the pectin samples (HSE and PLE) exhibited DE of 22.15 % and 31.86 %, respectively, characterizing both samples as pectin with low methoxyl content. These results are close to reported by MONICA; PRABHA; KAPOOR, (2022), for the pectin extraction from pomegranate peel using a microwave and 50 mM sodium phosphate buffer (pH 6; 1:20) as solvent under conditions of 70 W and 3 minutes. The recovered pectin fraction had low DE (<50 %). This pectin attribute (low DE value) can contribute to its use in formulations of low-calorie, low-fat foods, especially for diabetic products (MONICA; PRABHA; KAPOOR, 2022).

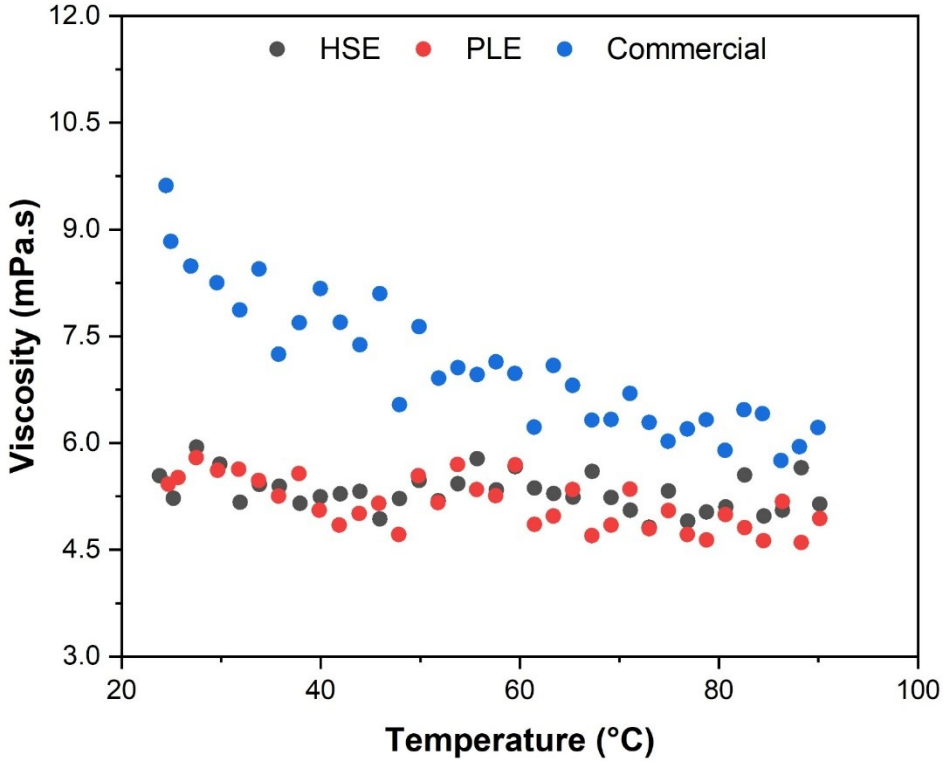
5.8.4 Viscosity of the pectin-rich fractions

Viscosity measurements were performed for the pectin-rich fractions recovered by the second step of routes SOX-HSE and PLE-PLE, and the results were compared with a commercial pectin and presented at Figure 5-3. Viscosity data showed the same behavior with increasing temperature for both samples, ranging from 4.7 to 5.8 mPa.s. The viscosity of the commercial pectin showed a different pattern, ranging from 5.8 to 9.6 mPa.s, and with a more perceptible decrease in viscosity for temperatures between 20 and 50 ºC. The data are in agreement with reported by NASCIMENTO OLIVEIRA et al., 2018; PASANDIDE et al., (2017) for pectin fractions recovered from sources such as Ubá mango peel (5.81 mPa.s) and citrus medica peel (around 5 mPa.s). Therefore, pectin solution from HSE and PLE presented similar viscosity profile of decreasing with increasing temperature, which can be an advantage for its use in food formulations.

The determination of the viscosity parameter, as well as other factors such as the pectin intrinsic characteristics (GalA, DE, DM, and molecular mass of the biopolymer),

and environmental conditions (pH, temperature, ionic strength) are essential to evaluated the proper application for a pectin-rich fraction and it use in food formulations (PASANDIDE et al., 2017; PICOT-ALLAIN; RAMASAWMY; EMMAMBUX, 2022).

Figure 5-3. Viscosity of pectin-rich fractions obtained from peach pomace by HSE and PLE as second extraction steps, and compared with the viscosity of a commercial sample.



Source: Elaborated by the author.

5.9 FUNCTIONAL PROPERTIES OF THE PECTIN-RICH FRACTIONS

5.9.1 Water Solubility

The water solubility values for the pectin-rich fractions recovered by the second step of routes SOX-HSE and PLE-PLE are compared at Table 5-3. No statistical difference between samples was detected, with values of 94.65 % (SOX-HSE) and 96.47 % (PLE-PLE). The water solubility of the commercial pectin sample was 95.40±1.37, with no statistical difference compared to the samples provided by SOX-HSE and PLE-PLE. These results show higher solubility compared to pectin fraction recovered from sunflower (81.36 %), apple pomace (90.8 %), and papaya peel (39 %) (EZZATI et al., 2020; KOUBALA et al., 2014; NAQASH et al., 2021).

The water solubility is related to the crystalline and amorphous structure of the pectin. High solubility is associated with amorphous solids characterized by a high degree of free energy. In such structures, molecular segments are disordered, providing numerous hydrogen bond positions for hydration upon water dissolution. This leads to a rapid dissolution rate and enhanced solubility (NAQASH et al., 2021).

5.9.2 Water holding capacity (WHC) and oil holding capacity (OHC)

WHC and OHC values for the pectin-rich fractions recovered by the second step of routes SOX-HSE and PLE-PLE are presented at Table 5-3. The WHC results show no significant difference between samples, with 0.04 g water.g^{-1} (HSE) and 0.03 g water.g^{-1} (PLE). These results are lower than reported by PAGÁN; IBARZ, (1999) for peach pomace pectin (3.5-4.3 g water.g^{-1}). The OHC values were 1.68 g oil.g^{-1} (HSE) and 1.73 g oil.g^{-1} (PLE), also with no significant difference, and higher than reported by BENVENUTTI; ZIELINSKI; FERREIRA, (2022) for pectin fraction from jaboticaba peel, recovered by HSE and by PLE with citric acid as solvent, and values of 1.01 g.g^{-1}

(HSE) and 1.19 $g.g^{-1}$ (PLE). The pectin-rich fractions recovered from peach pomace have higher OHC performance, compared to WHC, indicating a promising potential for use as stabilizer and emulsifier in various food systems.

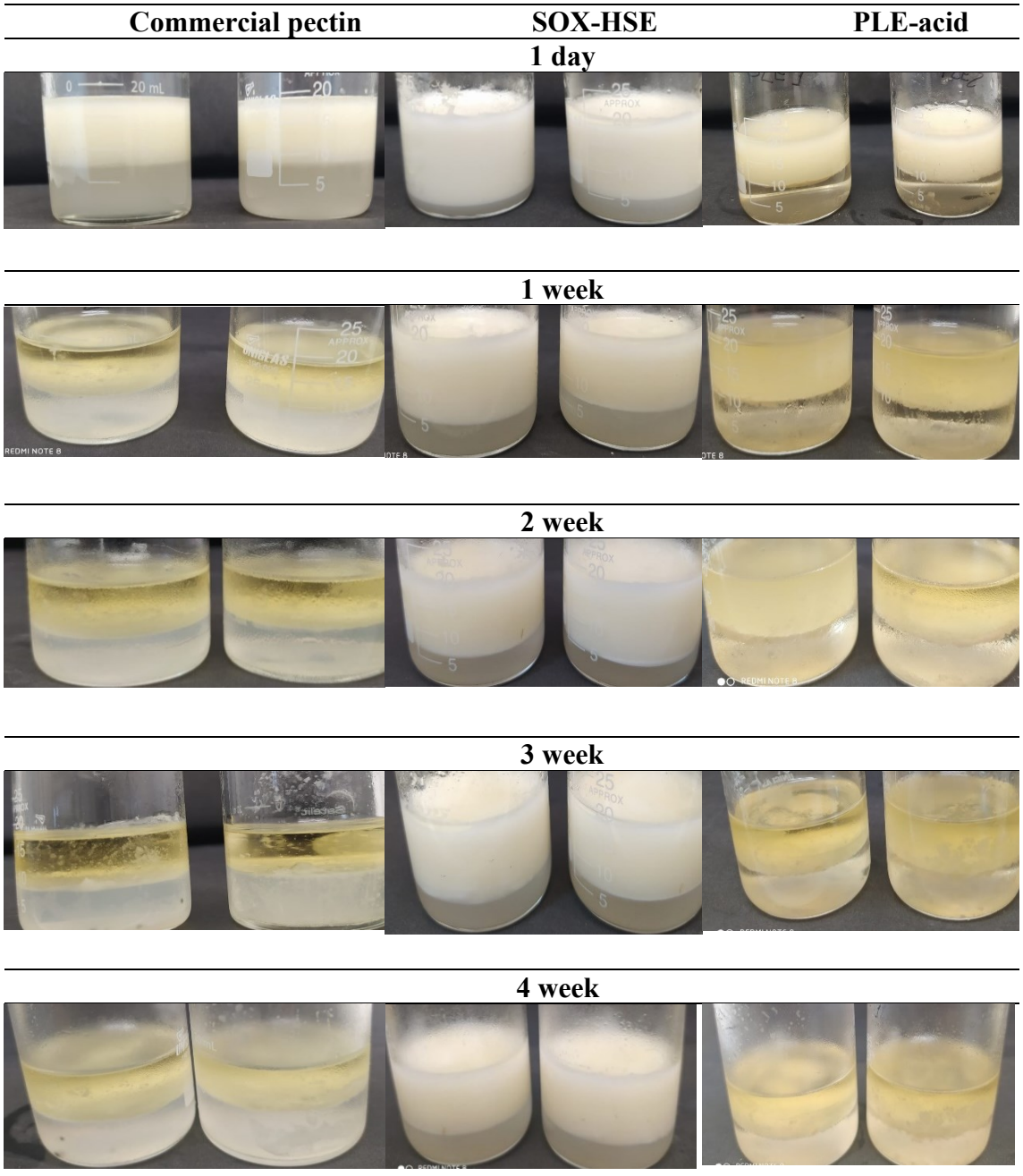
5.9.3 Emulsion Activity (EA) and Emulsion Stability (ES)

The EA and ES values for the pectin-rich fractions recovered by the second step of routes SOX-HSE and PLE-PLE are also presented at Table 5-3. The EA for the HSE sample was notably higher (90 %) than PLE sample (60.42 % indicating the HSE samples with emulsion capacity 1.5 times higher than PLE sample. The PLE value is similar with reported by VAKILIAN et al., (2023) for commercial pectin samples from apple and citrus, with EA of 63.33 % and 72.1 3 %, respectively, while the HSE sample provides a better EA performance.

The emulsions stability (ES) was recorded visually and photographed (Figure 5-4) for a period between the first day of emulsion preparation up to four weeks storage at 4°C. The ES values were 70 % (HSE) and 47.92 % (PLE), with statistical difference. These results are similar to reported by Benvenutti; Zielinski; Ferreira, (2022), of 60.4 % (HSE) and 52.6 % (PLE), for pectin fraction from jaboticaba peel. The lower ES for PLE sample may be attributed to the high-pressure, which potentially influenced the lower molecular weight of the pectin. According to Zhang et al., (2023), the emulsification property of pectin can be influenced by the extraction method used, neutral sugar side chain, the presence of hydrophobic groups and the structural property. Besides, polysaccharides of pectin with a molecular weight ranging between 100×10^3 $g.mol^{-1}$ and 200×10^3 $g.mol^{-1}$ demonstrate enhanced emulsification properties and have the capacity to impart prolonged stability. The EA and ES parameters are of relevant importance to

suggest applications for the pectin-rich fraction, as gelling agent or stabilizer for food, pharmaceutical, or cosmetic industries (MORALES-CONTRERAS et al., 2020).

Figure 5-4. Emulsification stability of pectin-rich fraction as the second extraction step from SOX and PLE and compared to commercial pectin

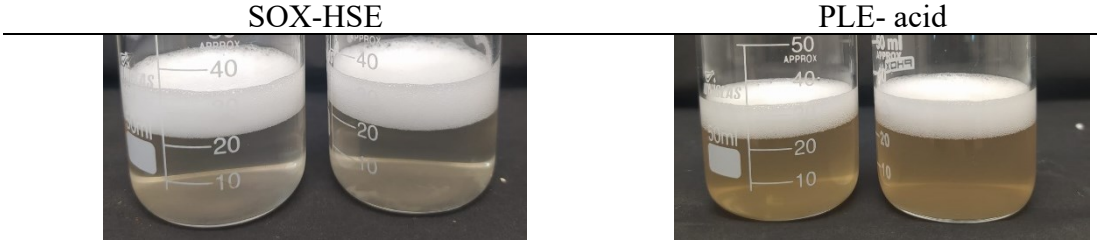


Source: Elaborated by the author

5.9.4 Foaming ability and foaming stability

The foaming ability (FA) and foaming stability (FS) for the pectin-rich fractions, recovered by the second step of routes SOX-HSE and PLE-PLE, are also presented at Table 5-3, with details presented at Figure 5-5. The results indicate similarity between HSE and PLE samples in terms of FA and FS results, with no significant difference. The FA performance was higher than reported by GOLBARGI et al. (2021) for pectin from melon peel, recovered by microwave-assisted extraction (12 min, 75°C, water as solvent and 414 W), with a value around 90 %. Otherwise, FS was higher 30 % for melon pectin. The FA and FS results for the pectin-rich fractions from peach pomace are promising to suggest its application in food formulations where foam-forming attributes are desirable.

Figure 5-5. Foaming stability of pectin-rich fraction as the second extraction step from SOX and PLE.



Source: Elaborated by the author

5.9.5 Color

Completing the quality attributes, the colour parameters (L*, a*, and b*) were evaluated for the pectin-rich fractions recovered by the second step of routes SOX-HSE and PLE-PLE, and are at Table 5-3.

The L* value was higher for HSE sample (14.16), with significant difference compared to the PLE one (12.82). The a* values, with significant difference were 7.86

(PLE) and 4.42 (HSE), while b* values were 12.07 (PLE) and 7.09 (HSE), also with significant difference. These parameters, compared with pectin from grape pomace (*Feteasca neagra*) (SPINEI; OROIAN, 2023) recovered by a conventional extraction method, presented L* values (64.99) higher than reported in this study, while the chroma (C*) (14.28) was similar reported by the SOX-HSE technique (14.40). While angle hue (H*) values had no significant difference between the sample HSE and PLE. Therefore, determining the color parameters of pectin is important for its application in a given product, and these parameters will depend on the structure of the pectin (SPINEI; OROIAN, 2023).

5.10 CONCLUSIONS

This work demonstrated the benefits of sequential extractions for the fractionation of bioactive and functional extracts from peach pomace, with relevant industrial importance, the phenolic compounds and the pectin fractions. The most abundant phenolic compounds identified from the by-product from the peach juice processing (peach pomace) were protocatechuic acid, mandelic acid, and vanillin, for the samples recovered by SOX and PLE methods with ethanol as solvent (first step of the sequential extraction). Then, from the solid material after phenolics recovery, the pectin-rich extracts were obtained by the second step of the sequential extractions (by HSE and by PLE-citric acid as solvent). The results from pectin recovery by PLE showed promising due to reasonable yield values 14 % for PLE-PLE, while SOX-HSE provided 21.8 % of pectin from the second extraction. Besides, the functional properties of the pectin fractions show good foam and emulsion attributes, proving the functionality of this fraction as additive for the food industry. These findings can be explored by adding phenolic compounds and pectin in cosmetic, pharmaceutical products and food formulations. Therefore, the results

from the present study enhances the potential of peach pomace as source of valuable components, providing upcycling and valorization of biomass from food industries.

CHAPTER 6 – **Conclusion and perspectives**

6.1 CONCLUSIONS

Peach processing by-products, such as pomace and seeds, are valuable renewable biomass due to their significant content of lipids, proteins, phenolic compounds and pectin. The recovery of these phytochemicals using emerging green technologies, such as supercritical fluid extraction, microwaves and ultrasound, or pressurized liquid extraction can boost the development of new products in the plant-based food industry.

The valorization of peach by-products through biorefinery and design thinking approaches can optimize the processing chain, transforming underutilized by-products into promising sources of high-value molecules. Peach seed cake, rich in oil and protein, demonstrated significant extraction yields by methods such as Soxhlet (SOX) and supercritical fluid extraction (SFE), with the oily fraction being particularly rich in unsaturated fatty acids beneficial for human metabolism.

The application of emerging extraction techniques, such as microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE) and pressurized liquid extraction (PLE), showed promising results in the recovery and quality of the protein fraction. Peach protein isolate showed desirable functional properties, being comparable in many aspects to conventional proteins.

Furthermore, the use of isolated peach protein obtained from the green extraction method of peach seeds in the formulation of mayonnaise analogs proved to be viable, offering texture properties and structural characteristics similar to conventional vegan products. These advances exemplify the concept of upcycling agro-industrial waste to create new valuable products, such as mayonnaise analogs, with potential for application in various industries.

Sequential extraction of bioactive and functional compounds from peach pomace, including phenolic compounds and pectin, has also revealed significant benefits for industrial applications. The identified phenolic compounds, such as protocatechuic acid

and vanillin, together with the pectin fractions obtained, demonstrated functional properties suitable for use in the food, cosmetic and pharmaceutical industries.

These findings highlight the potential of peach by-products, such as seeds and pomace, as valuable sources of lipids, proteins, pectin, and bioactive compounds. Due to their functional properties, these molecules have demonstrated important attributes for the development of products such as mayonnaise. Furthermore, the use PLE method is aligned with the concepts of sustainability and innovation in the food industry and can be further explored in this segment.

6.2 PERSPECTIVES

- Evaluate sensory analysis and physical-chemical parameters such as titratable acidity and peroxide index to guarantee the quality and safety of the mayonnaise analog product with progressing storage time.
- Evaluate the shelf life of the developed mayonnaise analog, including stability studies under different storage conditions, and explore other concentrations and formulations using isolated peach proteins (PPM5).
- Expand the rheological analysis of mayonnaise.
- Develop food or cosmetic products using pectin extracted from peach pomace, exploring its functional properties as a thickening and stabilizing agent.

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