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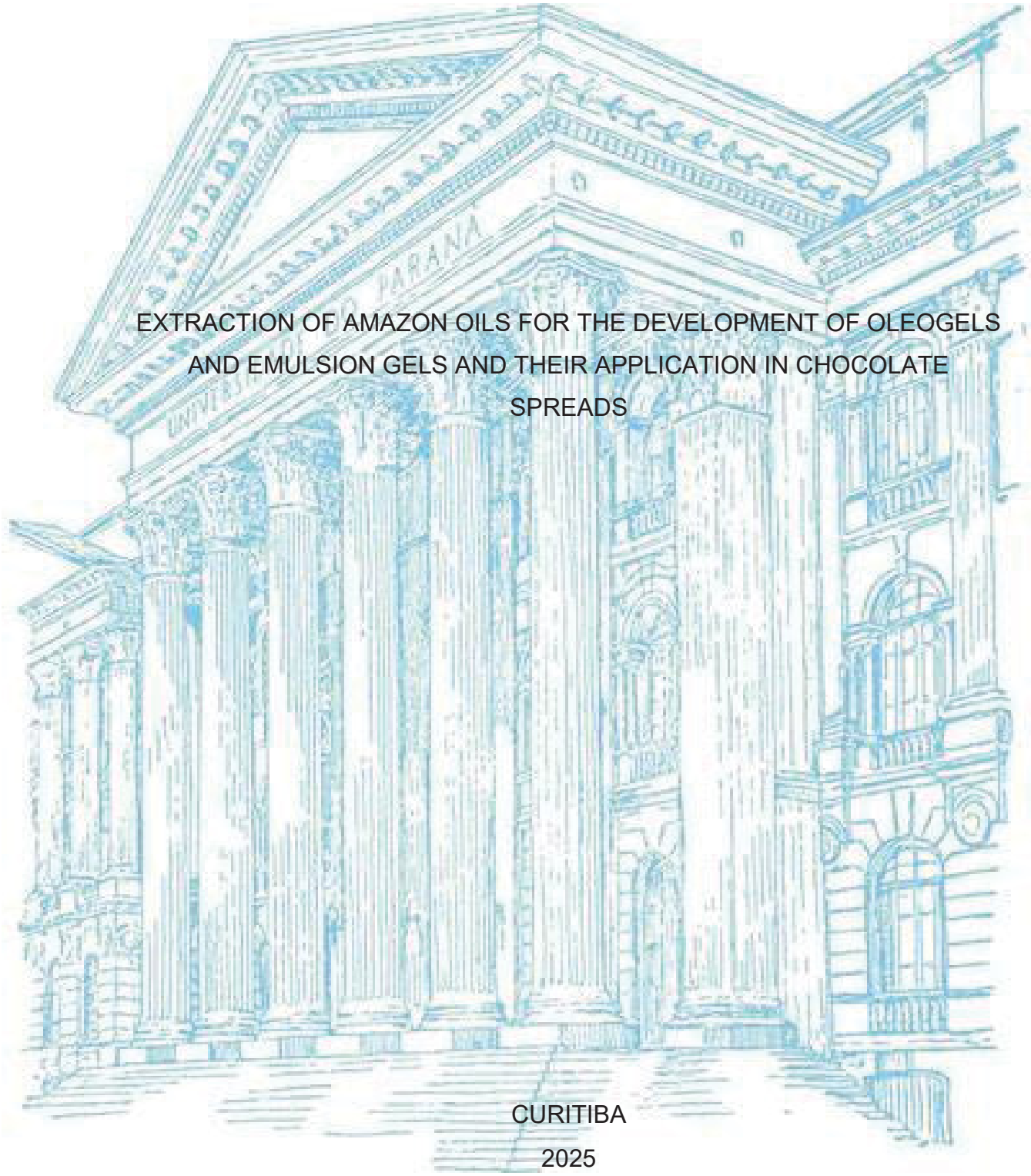

# EXTRACTION OF AMAZON OILS FOR THE DEVELOPMENT OF OLEOGELS AND EMULSION GELS AND THEIR APPLICATION IN CHOCOLATE SPREADS

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2025

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# EXTRACTION OF AMAZON OILS FOR THE DEVELOPMENT OF OLEOGELS AND EMULSION GELS AND THEIR APPLICATION IN CHOCOLATE SPREADS

Tese apresentada como requisito para obtenção do título de Doutor em Engenharia de Alimentos no Programa de Pós-Graduação em Engenharia de Alimentos, Setor de Tecnologia, da Universidade Federal do Paraná.

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação ENGENHARIA DE ALIMENTOS da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **ROGÉRIO WILLIAN SILVA DOS SANTOS**, intitulada: **EXTRACTION OF AMAZON OILS FOR THE DEVELOPMENT OF OLEOGELS AND EMULSION GELS AND THEIR APPLICATION IN CHOCOLATE SPREADS**, sob orientação da Profa. Dra. TIRZHÁ LINS PORTO DANTAS, que após terem inquirido o aluno e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.
A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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"The future begins in the

choices we make today, built on the hopes

we carried yesterday".

The Author

## RESUMO

Devido à crescente demanda por soluções sustentáveis que promovam a bioeconomia, protejam o meio ambiente e incentivem dietas mais saudáveis através do consumo de gorduras saturadas e trans, as oleaginosas amazônicas têm se destacado como matérias-primas promissoras. Entre elas, a Castanha-do-Brasil e o Tucumã-do-Amazonas se sobressaem pelo elevado teor de óleo, rico em ácidos graxos insaturados e nutrientes essenciais à saúde humana. No entanto, sua aplicação apresenta desafios, principalmente devido às limitações na extração, uma vez que processos convencionais, como prensagem mecânica, frequentemente apresentam baixos rendimentos, elevado consumo de energia e solventes, além da degradação de compostos sensíveis ao calor. Além disso, o uso desses óleos em alimentos como substitutos de gorduras saturadas e trans continua desafiador, pois essas gorduras sólidas são responsáveis por importantes características sensoriais. Nesse contexto, a extração assistida por Ultra-turrax foi investigada como uma alternativa sustentável e inovadora. O método proporcionou altos rendimentos de extração em tempos de processamento mais curtos, preservando a qualidade nutricional e físico-química dos óleos, quando comparado a métodos convencionais (Soxhlet) e não convencionais (propano comprimido). Utilizando esses óleos amazônicos, sistemas estruturados (oleogéis e géis de emulsão) foram desenvolvidos com sucesso em combinação com agentes gelificantes, apresentando comportamentos físicos e estruturais distintos. Notavelmente, os géis de emulsão à base de óleo de Castanha-do-Brasil mostraram grande potencial como fases contínuas capazes de substituir a fração lipídica em pastas de chocolate. Essa inovação possibilitou a formulação de produtos com concentrações significativamente reduzidas de ácidos graxos saturados e trans. De modo geral, esta pesquisa contribui para a bioeconomia amazônica e a preservação ambiental ao valorizar óleos regionais por meio da extração assistida por Ultra-turrax e explorar sua aplicação em sistemas estruturados, oferecendo alternativas mais saudáveis para os consumidores. Essa abordagem não apenas promove o uso sustentável dos recursos amazônicos, como também apoia o desenvolvimento de produtos alimentícios nutritivos e de menor impacto ambiental, beneficiando a saúde da população mundial.

**Palavras-chaves**: *Bertholletia excelsa*; *Astrocaryum aculeatum*; Extração assistida por Ultra-turrax; Lipídios estruturados; Substitutos de gordura; Bioeconomia.

## ABSTRACT

Due to the increasing demand for sustainable solutions that foster the bioeconomy, protect the environment, and promote healthier diets with reduced levels of saturated and trans fats, Amazon oilseeds have emerged as promising raw materials. Among them, Brazil nut and Tucumã-do-Amazonas stand out for their high oil content, rich in unsaturated fatty acids and nutrients essential to human health. However, their application poses challenges, primarily due to limitations in extraction, as conventional processes, such as mechanical pressing methods, often involve low yields, high energy and solvent consumption, and the degradation of heat-sensitive compounds. Furthermore, their use in foods as substitutes for saturated and trans fats remains challenging to achieve, since these solid fats are responsible for key sensory properties. In this context, Ultra turrax-assisted extraction was investigated as a novel and sustainable alternative. The method provided high extraction yields in shorter processing times, while preserving the nutritional and physicochemical quality of the oils, in comparison with conventional (Soxhlet) and unconventional (compressed propane) methods. Using these Amazon oils, structured systems (oleogels and emulsion gels) were successfully developed in combination with gelling agents, displaying distinct physical and structural behaviors. Notably, emulsion gels based on Brazil nut oil exhibited strong potential as continuous phases capable of replacing the lipid fraction in chocolate spreads. This innovation enabled the formulation of spreads with significantly reduced concentrations of saturated and trans fatty acids. Overall, this research contributes to the Amazon bioeconomy and environmental preservation by valorizing regional oils through Ultra-turrax extraction, and by exploring their application in structured systems, offering healthier alternatives for consumers. This approach not only promotes the sustainable use of Amazon resources but also supports the development of nutritious, lower-impact food products that benefit population health worldwide.



Keywords: *Bertholletia excelsa; Astrocaryum aculeatum*; Ultra turrax-assisted extraction; Structured lipids; Fat replacers; and Bioeconomy.

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## ABREVIATION

$\Delta H_m$ - Enthalpy

$A_w$ – Water activity

ANVISA - Agência Nacional de Vigilância Sanitária

ANOVA – Analysis of Variance

BN – Brazil Nut

BNO – Brazil Nut Oil

BEG – Brazil Nut Emulgel

BOG – Brazil Nut Oleogel

CE - Catechin Equivalent

CPBNO – Brazil Nut Oil Extracted by Compressed Propane

CPE – Compressed Propane Extraction

CPTAO – Tucumã-do-Amazonas Extracted by Compressed Propane

CCRD – Central Composite Rotatable Design

DSC – Differential Scanning Calorimetry

EG – Emulsion Gel

FAO – Food and Agriculture Organization

FDA - Food and Drug Administration

FTIR - Fourier Transform Infrared

G' – Storage Modulus

G" – Loss Modulus

GAE - Gallic Acid Equivalentes

GRAS – Generally Recognized as Safe

$H_{PM}$ – Enthalpy of the main peak

$H_{Total}$ – Total Enthalpy

LVR - Linear viscoelastic region

MUFAs - Monounsaturated Fatty Acids

OBC – Oil Binding Capacity

OG – Oleogel

PBD – Plackett-Burman Design

$PC_M$ – Temperature for the main peak

PLM - Polarized Light Microscopy

$P_{width}$ - Width of the peak

RDC - Resolução da Diretoria Colegiada

RFCS – Reduced-Fat Chocolat Spreads

SFA – Saturated Fatty Acid

SBNO – Brazil nut oil extracted by Soxhlet

STAO – Tucumã-do-Amazonas Oil Extracted by Soxhlet

SupFE – Supercritical Fluid Extraction

SubFE – Subcritical Fluid Extraction

PUFAs – Polyunsaturated Fatty Acids

TA – Tucumã-do-Amazonas

TAGs – Triglycerides

TAO – Tucumã-do-Amazonas Oil

$TC_{onset}$ – Onset temperature

$TC_{endset}$ – Endset temperature

TFC - Total Flavonoids Content

TGA – Thermogravimetry Analysis

TOG - Tucumã-do-Amazonas Oleogel

TEM - Tucumã-do-Amazonas Emulsion Gel

TPC - Total Phenolic Content

UFA – Unsaturated Fatty Acid

UTAE – Ultra Turrax-Assisted Extraction

UTBNO – Brazil nut oil extracted by Ultra-Turrax

UTTAO – Tucumã-do-Amazonas oil extracted by Ultra-Turrax

WHO – World Heath Organization

WI – Whiteness Index

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## GENERAL INTRODUCTION

In recent years, the search for healthy foods with lower saturated-fatty acid (SFA) and trans-fatty acids (TFA) content has intensified due to the population's growing concern for health and well-being. Scientific studies have consistently demonstrated that long-term intake of SFA and TFA contributes to diseases such as coronary heart disease, cardiovascular disease, cancer, atherosclerosis, type II diabetes and obesity (Liu et al., 2024; Suriaini et al., 2023). In this sense, awareness campaigns carried out by health institutions and regulatory changes in several countries, such as the restriction or prohibition of the use of industrial trans fats, have encouraged the reformulation of industrialized foods. For example, the Food and Agriculture Organization of the United Nations (FAO), in partnership with the World Health Organization (WHO), has promoted global guidelines and recommendations to reduce the consumption of TFA and SFA. Through the Codex Alimentarius, the FAO establishes international standards for food composition, encouraging governments to adopt policies that limit the content of trans fats in food products. These initiatives aim to ensure food safety and improve public health globally. In Brazil, the National Health Surveillance Agency (ANVISA) published RDC No. 332/2019, which determined limits for the presence of industrial trans fats in food.

This scenario has led consumers to adopt more balanced eating habits and demand products that promote health benefits without compromising flavor and quality. At the same time, the growing appreciation of natural and functional ingredients reflects the interest in a more sustainable diet aligned with practices that respect the environment (Durço et al., 2025; Erhard; Jahn; Boztug, 2024).

In addition to health-related issues, there has been a global increase in the prices of cocoa and its derivatives, such as cocoa butter and chocolate. This rise is mainly associated with growing consumer demand and prices volatility, which has raised concerns and driven the food industry to seek viable alternatives capable of supporting this production chain and meeting the increasing global demands for food production and nutrition (FAO, 2025; Sarpong, 2024).

In this context, the development of technological alternatives, such as oleogels (OGs) and emulsion gels (EGs), makes it possible to replace TFA and SFA with oils rich in unsaturated fatty acids (UFAs), offering healthy and

innovative options (Ferdaus; Jones; Silva, 2025; Zare et al., 2024). This movement not only meets consumer expectations, but also contributes to the reduction of public health problems associated with inadequate fat consumption. OGs have gained significant attention in food science and technology due to their efficient production methods, improved fatty acid profiles, and their role in creating healthier food options (Mahmud; Ferdaus; Silva, 2024). Distinguished by their adaptable physical and chemical properties, OGs present a dynamic alternative for replacing traditional solid fats in a wide range of food products (Silva et al., 2023). These structured materials allow the incorporation of oils rich in UFAs into a solid or semi-solid matrix without the need for chemicals modification, reducing significantly the negative impacts of trans fats. Their applicability is not limited to simple fat replacement, but also allows for enrichment with bioactive compounds, such as fat-soluble vitamins, omega-3 fatty acids, and antioxidants, promoting additional health benefits (antibacterial and antioxidant) (Ferdaus; Jones; Silva, 2025).

At the same time, another oil structuring method known as EG has also attracted the attention of the scientific community in order to increase the polyunsaturated fatty acids (PUFAs), essential amino acids, minerals, and other nutrients while lowering the saturated fatty acid levels. EGs are a semi-solid material with unique functional properties and mechanical strength, making it a desirable ingredient in various food products (Taktak et al., 2021). EGs typically consist of three-dimensional network structures created by a biopolymer (polysaccharide or protein), with encapsulated or aggregated oil droplets and solid particles irreversibly adsorbed at the oil-water interface, distinguishing these gels from simple oil droplet emulsions. In addition, EG presents amphiphilic properties and controlled release of bioactive compounds, such as vitamins, antioxidants, and essential fatty acids, present in vegetable oils, adding functional value to the products (Keramat; Golmakani, 2024; Zare et al., 2024).

In this sense, the use of unconventional vegetable oils, such as Amazon oils, can play a crucial role in promoting a healthier diet (Dos Santos et al., 2017; Machado et al., 2022; Oliveira et al., 2022; Pereira et al., 2019). Among the oilseeds typical of the region, the Brazil nut (*Bertholletia excelsa*, BN) and the Tucumã-do-Amazonas (*Astrocaryum aculeatum*, TA) are rich in unsaturated fatty

acids, such as omega-3, omega-6 and omega-9, in addition to containing bioactive compounds, such as tocopherols, phytosterols and natural antioxidants (Cardoso et al., 2017; Carvalho, 2022; Machado et al., 2022; Maranhão et al., 2011; Oliveira et al., 2022). Considering the scenario of socioeconomic fragility, increasing deforestation and loss of Amazon biodiversity, the use of Amazon oils from the oilseed pulps has potential to promote forest conservation, income generation for local communities and the valorization of regional natural resources. Thus, they stand out as strategic alternatives for combining health, sustainability, and technological innovation in food production.

These oils are traditionally extracted using conventional methods, but these processes face challenges such as low yields, excessive solvent use, long processing times, and degradation of thermally sensitive bioactive compounds (Freitas et al., 2024; Santos et al., 2025; Thilakarathna et al., 2023). More stringent regulations, such as the European Directives and the Registration, Evaluation, Authorization, and Restriction of Chemicals framework, have increased interest in sustainable solvents and advanced assisted extraction techniques, including ultrasound-assisted, enzyme-assisted, and supercritical fluid extraction. Ultra Turrax-Assisted Extraction (UTAE) stands out as a novel promising alternative, which can enhance the solid-liquid interface to intensify mass transfer between the oil matrix and the solvent by shear forces. This method has the potential to increase yields, reduce processing times, and minimize reliance on harmful solvents. In addition, UTAE can help preserve oil quality by protecting bioactive compounds and improving the unsaturated fatty acid profile, meeting the industry's needs for efficiency and sustainability (Siqueira et al., 2024; Sturm et al., 2018; Vázquez-Vázquez et al., 2024; Xu et al., 2016).

Thus, this project aimed to develop OGs and EGs for the production of chocolate spreads with a reduced percentage of SFAs and TFAs, using Amazon vegetable oils. The first part of the research consisted of a literature review on the main topics involved in the project and an approach to what has already been addressed in the literature (Chapter I). In the second part, the focus is on the development and optimization of the extraction of Brazil nut oil (BNO) and Tucumã-do-Amazonas oil (TAO) through UTAE (Chapter II). For the third part, OGs and EGs were developed using BNO and compared based on their

physicochemical properties (Chapter III). For the fourth stage, OGs and EGs were developed using TAO and compared based on their physicochemical properties (Chapter IV). Finally, the last stage was the application of EG for the production of chocolate spread (Chapter V).

## OBJECTIVES

General objective

The general objective of this project was to use Amazon vegetable oils for the development of oleogels and emulsion gels and apply them to the production of chocolate spreads.

Specific objectives

**Chapter I** – Valorization of Amazon oils: extraction technologies and its application as trans and saturated fat replacers.

- To contextualize the problem related to the consumption of trans and saturated fats;
- To identify and deepen information related to new strategies used to reduce the consumption of trans and saturated fats;
- To contextualize the importance and potential of oilseeds from the Amazon region for the industry;
- To contextualize the problems and challenges of extracting and enhancing the production chains of Amazon oilseeds;
- To identify and compare new strategies used for extracting vegetable oils with conventional methods;
- To propose a new approach for intensifying vegetable oil extraction;
- To provide support for the discussion and interpretation of results related to vegetable oil extraction, the development of structured systems, and the development of chocolate spreads.

**Chapter II** – Ultra turrax-assisted extraction of Amazon oils: Optimization and comparison with Soxhlet and compressed propane extraction.

- To apply high shear disperser (Ultra-turrax) develop to extract of Brazil nut and Tucumã-do-Amazonas oils from the pulp;
- To apply the Plackett-Burman methodology and identify the important variables in the extraction process (extraction temperature,

extraction time, solvent/solid ratio, type of solvent, sample, and Ultra-turrax rotation);

- To optimize the extraction process by applying the Response Surface Methodology;
- To compare the oils obtained under the optimized conditions with Soxhlet and compressed propane extraction in relation to yield, fatty acid composition, thermal stability properties, bioactive compounds, and antioxidant activity.

**Chapter III** – Tucumã-do-Amazonas oil loaded with protein to develop a novel emulsion gel: A comparative study with oleogels.

- To develop oleogels using Tucumã-do-Amazonas oil and carnauba wax as structuring agent;
- To develop emulsion gels using Tucumã-do-Amazonas oil and gelatin as structuring agent;
- To characterize the oleogels and emulsion gels in terms of macroscopic appearance, polarized light microscopic, oil binding capacity, rheological, and thermal properties.

**Chapter IV** - Development and physical properties of Brazil Nut oil-based oleogel and emulsion gel.

- To develop oleogels using Brazil nut oil and carnauba wax as structuring agent;
- To develop emulsion gels using Brazil nut oil and gelatin as structuring agent;
- To characterize the oleogels and emulsion gels in terms of macroscopic appearance, polarized light microscopic, oil binding capacity, rheological, and thermal properties.

**Chapter V** – Brazil nut emulsion gel as an innovative strategy to develop reduced-saturated fat chocolate spreads: physicochemical and sensory evaluation.

- To apply the Brazil nut oil emulsion gel for the production of chocolate spreads;
- To characterize chocolate spreads in terms of macroscopic appearance, polarized light microscopic, oil loss, rheological, textural, and thermal properties.

**Chapter VI** – Contributions and suggestions for future research

- This chapter aims to highlight the main contributions of the thesis and propose future research directions;
- It serves to demonstrate the originality and relevance of the study;
- To connect its findings to broader scientific and societal challenges;
- To identify knowledge gaps that can be explored by future investigations;
- Additionally, it reflects the author's critical understanding of the subject and offers guidance for the continuity and application of the research in academic and industrial contexts.

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# CHAPTER I

## Valorization of Amazon oils: extraction technologies and its application as trans and saturated fat replacers

This chapter is **an UNPUBLISHED review manuscript** and aimed to address nutritional and regulatory aspects of oils and fats in foods, contextualize the health problems caused by the high consumption of TFAs and SFAs, and the opportunity in the structural modification of lipids by new approaches using Amazon vegetable oils. Among lipid modification, OGs and EGs have emerged as promising alternatives for replacing SFAs and TFAs in the food industry. Furthermore, this review explores the potential of healthy Amazon oils, with a particular focus on BNO and TAO, which are rich in bioactive compounds and high-value lipids. Traditional and non-conventional vegetable oil extraction methods are also analyzed, assessing their efficiency and impact on the quality of the extracted products. Moreover, a state-of-the-art analysis is provided, synthesizing recent research on the intensification of oil extraction processes, as well as the production of OGs and EGs using carnauba wax and gelatin as gelling agents, respectively. This comprehensive bibliographic synthesis seeks to contextualize the research problem, justify the study, establish a theoretical and conceptual foundation, support result interpretation and discussion, offer references for future studies, and contribute to the development of healthier and more sustainable alternatives for the food industry.

## 1. Introduction

Fats and oils play essential roles in the human diet, as they are a concentrated source of fat-soluble vitamins and energy (1 gram of lipids generates approximately 38.7 kJ/g or 9.1 kcal), vital for human regulatory processes (López-Pedrouso et al., 2021; Spajić, 2020). In the food industry, fats play a crucial function due to their functional properties. Thus, they are responsible for imparting texture for dairy products, and satisfying the processing requirements of dairy products for plasticity and strong stability (Zhang et al., 2024b). Moreover, solid fats influence the melting profile of foods, which affects the sensory experience. For instance, fats that melt at body temperature, such as butter and cocoa, create a smooth and luxurious mouthfeel.

On the other hand, in the context of ultra-processed foods – often rich in SFAs derived from sources such as palm oil and partial hydrogenated vegetable fats – growing awareness of the associated health risks has driven global initiatives aimed at reformulating food products toward healthier lipid profiles. For instance, WHO has issued guidelines on SFA and TFA intake, which recommend to reduce the intake of SFA to less than 10 % of total energy consumption and replace them with PUFAs and monounsaturated fats (MUFAs) to minimize the incidence of cardiovascular diseases (WHO, 2023). Those actions have improved the food environment for 3.7 billion people, or 46 % of the world's population, as compared to 6 % just 5 years ago, expecting to save approximately 183,000 lives a year (WHO, 2024). In parallel, there is an increasing demand for clean-label and sustainable ingredients, prompting the food industry and scientific community to seek alternative fat-structuring strategies that align nutritional, technological, and environmental goals.

Structured lipid systems, such as OGs and EGs, have emerged as innovative and promising approaches for replacing conventional solid fats without compromising the textural, sensory, or stability attributes of food matrices (Silva et al., 2023; Xu et al., 2024). OGs are semi-solid systems formed by trapping liquid oils within a three-dimensional network of gelling agent, while EGs combine oil-in-water emulsions with gelling agents to create stable, viscoelastic structures (Blount; Ferdaus; Silva, 2025; Zhao et al., 2022). These technologies offer the

potential to incorporate healthier oils rich in UFAs, reduce SFA and TFA content, and deliver functional and bioactive compounds (Ferdaus et al., 2024).

In this context, Amazon vegetable oils represent an underexplored yet highly valuable source of lipids with exceptional nutritional and functional properties (Freitas et al., 2024a). Research efforts have been directed toward estimating the valuable potential of one of the world's largest reserves of food and medicinal plants (Berto et al., 2015; Faria et al., 2021; Serra et al., 2019). Pesce (2009) documented over 84 oilseed species, derived from these native oilseeds, whose oils are rich in MUFAs and PUFAs, bioactive compounds (such as vitamins A and E, and carotenoids), and minerals, composition attractive for food, cosmetics, and pharmaceutical industries (Barboza et al., 2022; Cardoso et al., 2017; Machado et al., 2022).

Despite their promising potential, there is limited information in the literature regarding the extraction of Amazon vegetable oils using intensified extraction techniques and lack of studies on the structuring of these lipids for the development of reduced SFA and TFA in foods. Therefore, this review aimed to provide a comprehensive overview of the current advances in extraction techniques for Amazon vegetable oils and its subsequent use in the development of structured lipid systems. Special emphasis is placed on the application as fat replacers in food products, with the goal of reducing SFA and TFA content while enhancing the nutritional quality, and sustainability of the final formulations. Furthermore, the valorization of Amazon oilseeds can contribute to regional bioeconomy development, promoting the conservation of biodiversity and the generation of socioeconomic benefits for local communities.

## 1.1. Nutritional and regulatory aspects of oils and fats in food

Not only as an energy source, lipids also play essential roles in maintaining the cellular activities of living organisms. As structural components of cell membranes, lipids are important signal transduction factors in biological systems and are widely used in food formulations due to their nutritional values, sensory quality, and texture effects (López-Pedrouso et al., 2021). Moreover, solid fats influence the melting profile of foods, which affects the sensory experience. For instance, fats that melt at body temperature, such as cocoa butter, create a

smooth and special mouthfeel. Solid fats are also essential for aeration, which is important in products like whipped creams and certain frostings, giving them their characteristic volume and texture (Campbell; Mougeot, 1999).

In terms of molecular structure, fats and oils are made up of triglycerides (TAGs) molecules structured from three fatty acids linked to glycerol. Thus, the characteristics of the resulting fat depend on the type of fatty acids (saturated, cis and trans MUFA and cis PUFAs). A high level of saturation produces more solid materials, which group together in crystalline structures of TAGs (Pernetti et al., 2007). From a biochemical point of view, SFA are molecules that do not have double bonds between carbon molecules since they are saturated with hydrogen molecules (Ghotra; Dyal; Narine, 2002). SFA are predominantly found in products of animal origin – cheese, meat products, butter, ice cream and others – but can be found in some plant sources. SFA tend to have higher melting points and, at room temperature, are mainly solid.

The use of fats and oils in food and non-food products has been reported since the early 20th century and is common in all cultures. On the other hand, in the 1970s and 1980s, countries such as the United States and Canada expressed concerns about the impact of these raw materials on human nutrition through the “Dietary Goals for the United States” and “A New Perspective on the Health of Canadians” (Spajić, 2020). These publications, mainly government-related, raised awareness among consumers to reduce their consumption of TFA and SFA fats, since high blood cholesterol levels have been correlated with cardiovascular disease, cancer, obesity, smoking, and others.

Over the years, several government agencies have taken various actions to eliminate the consumption of industrially produced TFAs. In 2005, Canada became the first country to regulate mandatory nutritional labeling of TFAs in processed foods. In January 2006, Denmark limited the presence of TFAs in all foods on the market, including imported foods and restaurant services. In the United States, a detailed cost-benefit analysis that considered the health benefits of reducing TFA-fat consumption and the additional costs of including this component in nutritional labeling determined the mandatory inclusion of TFA fats on all food labels and the recommendation that individuals reduce their consumption of TFA fats to a minimum.

In Brazil, the National Food and Nutrition Policy of the Ministry of Health follow international debates and the results of monitoring the nutritional transition of the Brazilian population, establishing clear guidelines for promoting healthy eating practices and preventing and controlling nutritional disorders. In this context, the publication of the Food Guide for the Brazilian Population, published in 2006, stands out, defining the dietary guidelines for this population. With regard to fats, especially trans fats, the recommended consumption is less than 1 % of the total daily energy value (maximum 2g/day for a 2,000-kcal diet).

In March 2022, ANVISA released Board Resolution (RDC) 632/2022, which proposes restricting the use of industrial TFA fats in food (ANVISA, 2022). This Resolution applies to all foods, including beverages, ingredients, food additives, and technological adjuvants, including those intended exclusively for industrial processing and those intended for food services. In relation to Art. 6, since January 2023, the amount of industrial TFAs may not exceed 2 grams per 100 grams of total fat in foods intended for the final consumer and in foods intended for food services.

Nowadays, it is known that a healthy diet is not only essential for good health and nutrition, but also protects against many diseases such as heart disease, diabetes and cancer (WHO, 2019). Therefore, in order to preserve our health, there are recommendations to reduce the consumption of salt, sugars, SFA fats and industrially produced TFA fats. According to the WHO, to avoid unhealthy weight gain, total fat should not exceed 30 % of total energy intake (WHO, 2019). In addition, the intake of SFAs should be less than 10 % of total energy intake and the intake of TFAs should be less than 1 % of total energy intake, with a change in consumption of SFA and TFA to UFAs, with the aim of eliminating harmful industrially produced fats.

According to the FAO, daily energy intake from fat should be at least 15 % and 20 % for adults, men and women respectively (FAO, 2010). For maximum intake, it should be 30 % and not more than 35 % for healthy adults, where daily energy intake from SFA should be 5 % to 10 % of the total daily calorie intake of an adult or child.

Excessive fat consumption is not healthy and it is essential to distinguish between different fats in adequate daily nutrition. WHO highlighted the risks of

heart disease, cellular dysfunction, metabolism, gallstones, endometriosis, diabetes and some types of cancer and Alzheimer's disease have been associated with excessive consumption of SFA and TFA (Frías et al., 2023; Langley et al., 2020; López-Pedrouso et al., 2021; Spajić, 2020).

New strategies are needed to replace and structure lipids in foods. However, the food industry has faced major challenges in trying to develop products that are free of SFA and TFAs, but that also preserve and improve the properties and functionality of processed foods. In this context, companies have sought partnerships to meet regulations and consumer needs. McDonald's® was one of the first to respond and worked with Cargill to reach zero grams of TFA fat per serving of its French fries, without sacrificing flavor, texture, and quality. Based on the partnership's studies, it was identified that a mixture of canola oil with a high oleic acid content allowed McDonald's to increase the nutritional value of its French fries, without losing their golden color and flavor, its exclusive trademarks (CARGILL, 2015).

On the other hand, the replacement of SFA and TFA in processed products is not easily achieved. These changes affect sensory characteristics, since these types of fats play a fundamental role in the development of appearance, palatability and texture characteristics such as mouthfeel, bite, tenderness and juiciness. Thus, possible fat substitutes must present good performance in terms of nutritional value, in addition to presenting good structure and sensory attributes (LÓPEZ-PEDROUSO et al., 2021).

## 1.2. Structural modification of lipids

Natural fats and oils are composed mainly of TAGs and the composition and molecular structure of TAGs determine the physicochemical, functional and nutritional properties of lipids (Sivakanthan; Madhujith, 2020). In order to optimize the structures of natural oils and fats to expand their industrial use, methods such as fractionation, interesterification, or hydrogenation (Mensink et al., 2016). The combination of these methods is also applied, but it is more common as a pre-process for the development of structured lipids (Ribeiro et al., 2017).

Until recently, one of the most widely used oil and fat modification processes in the food industry was partial hydrogenation, which reduces the concentration of PUFAs and leads to the formation of TFAs (Adu-Mensah et al., 2019; Viriato et al., 2018). Among its objectives, this process aims to transform oils rich in UFAs into semi-solid fats with greater oxidative stability, as an alternative to animal fats (Sivakanthan; Madhujith, 2020).

The interesterification process there is an exchange of fatty acid molecules in the same TAG or in another TAG (Sivakanthan; Madhujith, 2020). Thus, during its reaction, the ester bonds linked to the fatty acids in the glycerol skeleton are broken, placed in different positions than the original and, in this way, intermediate properties are obtained in relation to the initial oils due to the random distribution of fatty acids that form a new triacylglycerol (Gómez et al., 2022). As mentioned by Jimenez-Colmenero et al. (2015), interesterification process shows the drawback of deterioration of oxidative quality in most cases, especially when susceptible oil sources are used.

Furthermore, this process can be performed in two ways: chemically and enzymatically. The advantages of the last are based on the fact that the enzymatic process uses lipases that act with high selectivity, mild reaction conditions, less waste, ease of product recovery and elimination of the use of toxic chemicals (Sivakanthan; Madhujith, 2020).

Fractional crystallization or fractionation is a physical method that is also used to alter the structure of lipids. The process is basically divided into two stages: crystallization and separation. In this sense, the crystallization process can be dry (without solvent) or wet (with solvent). However, the separation of the solid phase (crystals) from the liquid phase is easier in the presence of a solvent, which dilutes the oil and reduces viscosity (Timms, 2005).

While partial hydrogenation is considered to be the main process that leads to the formation of artificial TFAs, other methods can bring a high content of SFAs in the final food product composition, increasing the risk of developing cardiovascular diseases, leading to obesity and diabetes (Puscas et al., 2020). Thus, due to the increase in human health, the food industry has been motivated to find alternatives (Sivakanthan; Madhujith, 2020). One alternative currently explored is the production of OGs and EGs from vegetable oils, allowing the

development of products with a high content of UFA, without chemical modification, and low level of SFA and TFA (Ghorghi et al., 2023; Godoi et al., 2019; Huang et al., 2023; Jing et al., 2022; Wolfer et al., 2018).

### 1.2.1. Oleogels (OGs)

Currently, the food industry is interested in vegetable oils to partially or fully replace partial hydrogenated fat, animal fats, and oils with a high composition of SFA, since their consumption is related to cardiovascular diseases (Suriaini et al., 2023). This action intensified after government agencies around the world, such as the Food and Drug Administration (FDA) decided to restrict the use of trans fats in processed foods (ANVISA, 2022; FDA, 2018).

Based on industrial demands for replacing SFA and TFA, as well as overcoming emulsion instability (such as flocculation, coalescence, and Ostwald ripening that occur during storage), controlled release of hydrophobic bioactive molecules, and others, some strategies are being developed. Among the solution routes, the creation of a structured network from the incorporation of a gelling agent into an oil phase presents itself as an alternative (Ghorghi et al., 2023). In this sense, a structuring approach known as organogelation was recently developed to produce OG, systems similar to solids fats (Marangoni, 2012). This product obtained through organogelation is defined as a continuous two-phase system: liquid oil encapsulated within the self-assembled structure of the gelling agent molecule (Toro-Vazquez et al., 2013).

OGs are a thermoreversible viscoelastic semi-solid systems that can contain more than 90 % of healthy oils. OG forms a three-dimensional gel network that traps healthy fats with high UFA content whereby π-π stacking, hydrogen bonding, electrostatic and van der Walls interactions (Zhang et al., 2024b). The OG provides a new idea to replace the traditional solid fats. At the same time, the introduced liquid oils can include flaxseed oil, soybean oil, and camellia oil rich in PUFAs and natural antioxidants, for example.

The first report on organogelation of edible oil was published by Gandolfo et al. (2004), followed by the work of Bot & Agterof (2006) on phytosterol-oryzanol and Wright & Marangoni (2006). According to Moghtadaei et al. (2018), the

physical and mechanical state of OGs are influenced by the type, concentration and molecular weight of the organogelator (gelling agent), type and polarity of the oil, crystallization temperature, duration and intensity of the shear force and the presence of other additives such as surfactants.

The organogelation process is defined as the production of a three-dimensional, self-sustaining and thermo-reversible gel network from oil that has viscoelastic properties. In fact, in this approach, a mass of liquid oil is trapped in a low concentration of gelling agent by forming an organogelator network (Pernetti et al., 2007). Organogelation changes the physical state of edible oils to a gelled, crystalline and solid state, without any change in the fatty acid composition of the oil (Marangoni, 2012).

#### 1.2.1.1. Gelling agent

The gelling agent molecules facilitate the oleogelation of the oil by forming a stable gelled network, which is induced mainly through covalent interactions, such as hydrogen bonds, Van der Waals forces, ionic interactions, and π-π stacking (Demirkesen; Mert, 2019; Guo; Cui; Meng, 2023). Thus, edible OGs provide new methods for obtaining vegetable oils similar to solid fats and healthier fatty acid profiles to be applied in the food industry, such as in the production of chocolates, confectionery products, ice cream, cottage cheese, sausages, emulsions, and others.

Gelling agents are classified into crystalline particles, low-molecular-weight particles, polymers, and inorganic particles (Guo; Cui; Meng, 2023). Depending on the type of gelling agent, the OG can present a myriad of structures, such as crystal particle networks, entangled 3D networks, self-assembled networks, particle aggregation networks, and others.

Among the gelling agents, waxes of plant origin have enormous potential due to their high availability, good structuring properties, high oil binding capacity and lower cost (Thakur et al., 2022). Among them, carnauba wax has high potential for the development of OGs (Aliasl khiabani et al., 2020; Qiu et al., 2023).

carnauba wax is extracted from the leaves of the Brazilian palm tree *Copernicia prunifera* and is composed of a mixture of aliphatic acids, esters, hydrocarbons (paraffins), free ω-hydroxycarboxylic acids and triterpene diols, aromatic acids, and free alcohols (Thakur et al., 2022). The waxy material prior to extraction from the leaves is called carnauba powder, which is the raw material for the production of wax. Its melting range is about 81–86 °C, which is much higher than other waxes, such as beeswax (Ferdaus; Blount; Silva, 2022; Ferdaus; Jones; Silva, 2025).

carnauba wax, unlike other natural waxes such as beeswax, contains many more branched methyl groups and a higher percentage of carbon atoms with double and triple bonds (Basson; Reynhardt, 1988; Doan et al., 2018). Furthermore, no chronic toxicity results are available for natural waxes such as carnauba wax, whose maximum permitted levels for food coating are in the range of 200 to 1500 mg/kg of food (Lim et al., 2017). Because of these advantages, carnauba wax finds several applications in food, including lubricant, formulation aid, anti-caking agent, mold release agent, surface finishing agent in baked foods and mixes, confectionery, and others.

#### 1.2.1.2. Oleogel structuring methods

The OG formulation is made using base raw materials such as oil, water, emulsifiers, and gelling agents. Thus, OGs are generally produced by dissolving the gelling agent (in low concentration) in vegetable oils, followed by heating (above the melting point) and cooling for gelation (Toro-Vazquez et al., 2013). Some researchers usually divide the structuring into four distinct processes: the direct dispersion method and indirect methods, such as emulsion modeling, foam modeling and solvent exchange.

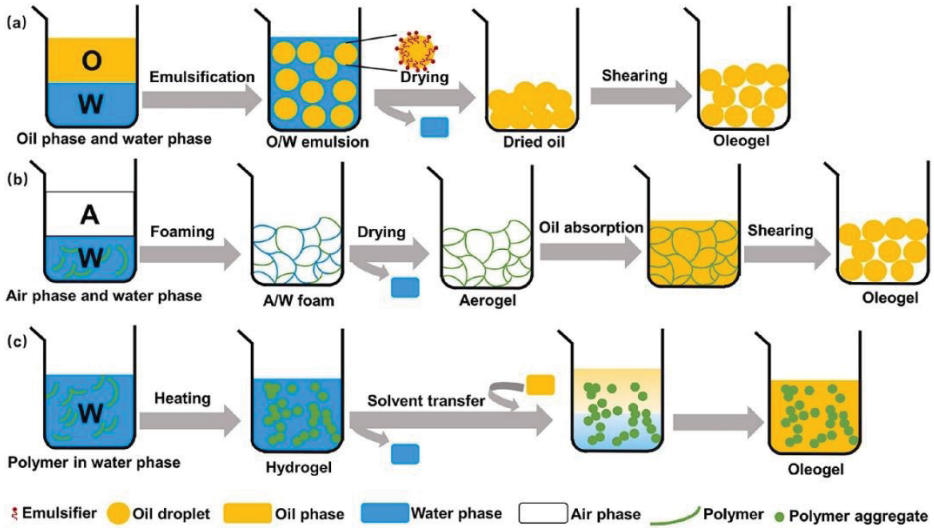
According to Guo et al. (2023), in the direct method, the gelling agents are dispersed directly in the oil phase at temperatures above their melting points, followed by cooling to lower temperatures under shear conditions, transforming liquid oil into OGs through crystallization or self-assembly. In the cooling process, crystal nucleation and an increase in storage modulus (G′) and viscosity after passing the gel point (G′ = G″) can be attributed to a three-dimensional network

formation resulting from random aggregation and mutual entanglement of wax crystals as well as interaction with the solvent (Doan et al., 2018). In the indirect method, polymers are used to gel vegetable oil to form structural networks in aqueous solvents or continuous water emulsions to trap a large amount of liquid oil, which requires careful removal of aqueous solvents to maintain the gel networks (Guo; Cui; Meng, 2023; Huang et al., 2023).

The emulsion modeling method (FIGURE 1.1a) first forms an oil-in-water emulsion, followed by water removal and subsequent shearing into OGs. These types of OGs stabilize oil droplets by forming a protective film with strong steric resistance and charge repulsion at the phase interface through intermolecular interaction. The oil/water emulsion is formed by emulsification, and then the moisture in the emulsions is removed by drying at atmospheric pressure or freeze-drying to form dehydrated soft solid oil (Guo; Cui; Meng, 2023; Wang et al., 2020).

In the indirect foam modeling method (FIGURE 1.1b), the aqueous phase removal process (freeze-drying, for example) is also used to create pores in the water/polymer solution. Then, oil is added until oil absorption is saturated, or the foam is inserted into the oil to incorporate into its pores (Jiang et al., 2021). Similar to the emulsion molding method, the oil-sorbed foams need to be sheared to obtain OGs. Unlike the other processes, this method does not require heating and chemical crosslinkers, and the dry aerogel-type foams can be prepared and transported separately instead of being mixed with oil beforehand, thus increasing the flexibility and controllability of this method (Abdollahi; Goli; Soltanizadeh, 2020; Li et al., 2022b; Mohanan; Nickerson; Ghosh, 2020). In the indirect solvent exchange method (FIGURE 1.1c), organogelation is done by using polymers. Polymers develop networks to form hydrogels first, acting as architectural systems, then a step-by-step procedure using organic solvents to remove water, resulting in continuous solvent exchange through sequential immersions in vegetable oils, to incorporate oils into polymeric structures (Guo; Cui; Meng, 2023). Li et al. (2023) used this process to produce OG by exchanging water for methanol, ethanol or 1-propanol, where the final properties of the OG (solubility, mechanical properties and structural stability) were strongly influenced by the type of solvent used.

FIGURE 1.1 – DIFFERENT INDIRECT FORMATION METHOS OF OLEOGELS, EMULSION-TEMPLATED METHOD (a), FOAM-TEMPLATED METHOD (b), AND SOLVENT EXCHANGE METHOD (c), RESPECTIVELY.




Source: Adapted from Guo et al. (2023).

### 1.2.2. Emulsion gels (EGs)

EGs, also known as emulgels or gelled emulsions (Lin; Kelly; Miao, 2020), are structured semi-solid systems formed by stabilizing emulsions within a gel matrix, where dispersed oil droplets are embedded in a continuous hydrogel network. They combine the properties of emulsions and hydrogels, resulting in materials with unique mechanical, structural, and functional characteristics. Typically, EGs include two steps: first preparing emulsions and then turning emulsions into gels matrix (biopolymers such as proteins – e.g., gelatin, whey protein – or polysaccharides – e.g., alginate, carrageenan).

This structural organization leads to improved mechanical properties, where the dispersed oil droplets act as active fillers, reinforcing the gel network and contributing to its firmness and elasticity (Xu et al., 2024). The mechanical behavior of EGs is primarily influenced by the type and concentration of the gelling agent, the oil phase composition, and the droplet size distribution. Rheological properties, including storage modulus (G') and loss modulus (G''), provide insights into the gel's viscoelastic behavior, reflecting its balance between solid-like and liquid-like characteristics. A higher G' compared to G'' indicates a predominantly elastic structure, essential for maintaining shape and texture under stress. Moreover, EGs exhibit high oil-binding capacity (OBC), thermal stability,

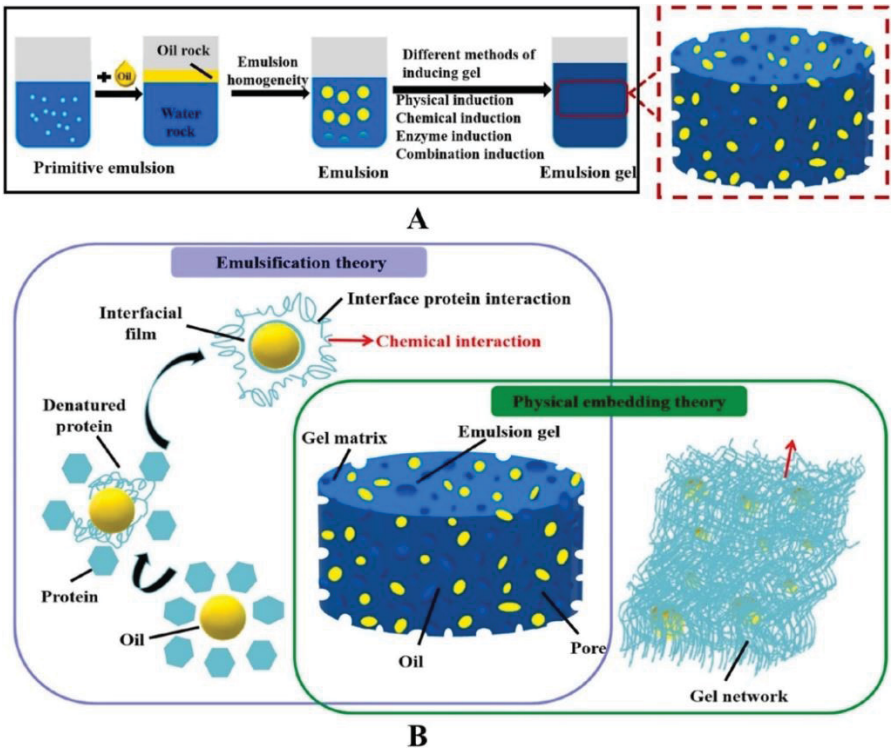
and resistance to phase separation, which are critical for their stability during processing and storage (Patel et al., 2013; Zhao et al., 2022).

From a functional perspective, EGs offer versatility in food applications by enabling the reduction or replacement of SFAs and TFAs while preserving desirable textural and sensory properties (Alejandre et al., 2016; Guo; Cui; Meng, 2023). Their ability to encapsulate and protect bioactive compounds, such as vitamins, antioxidants, and essential fatty acids, enhances the nutritional value of food products and improves the stability and controlled release of these compounds (Li et al., 2023b; Muñoz-González et al., 2021). Additionally, the tunable texture and spreadability of EGs make them suitable for various food products, including spreads, dressings, meat analogs, and bakery items (Li et al., 2024b; Tirgarian et al., 2023; Xu et al., 2024). Beyond food applications, EGs are also utilized in pharmaceuticals and cosmetics for controlled drug delivery and as carriers for active ingredients (Fardous et al., 2021). Overall, the development of EGs represents a promising strategy for designing healthier, functional foods and innovative materials, offering a balance between improved nutritional profiles and desirable mechanical and sensory attributes.

#### 1.2.2.1. Formation and mechanism of emulsion gel

The formation of EGs is a complex process that involves the integration of emulsification and gelation mechanisms to create a structured, semi-solid system, which are typically prepared by selecting induction methods according to the characteristics of food products during production. This process begins with the preparation of a stable emulsion by homogenization after adding oil droplets to an initial solution or via physical induction (ionic interactions, or pH adjustments), chemical induction (covalent crosslinking), enzyme induction, or a combination of methods (FIGURE 1.2A). Once a stable emulsion is achieved, the system undergoes gelation, where the continuous aqueous phase transitions into a three-dimensional network, entrapping the dispersed oil droplets (Xu et al., 2024).

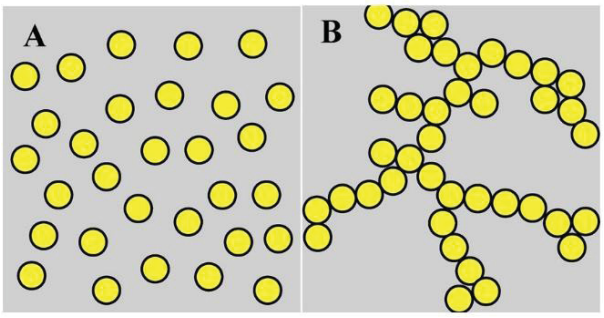
FIGURE 1.2 – THE EMULSION GEL PREPARATION METHOD (A). A SCHEMATIC REPRESENTATION OF TWO THEORIES FOR THE LATEX FORMATION MECHANISM (B).




Source: Adapted from Xu et al. (2024).

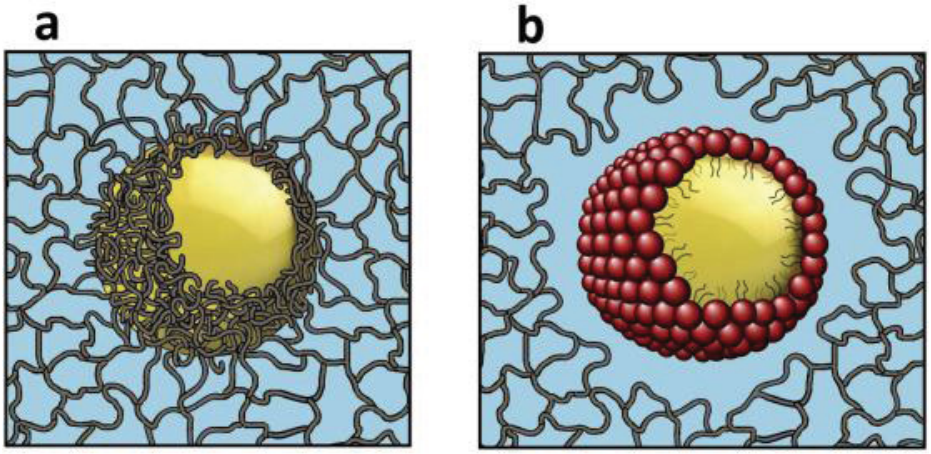
According to Lin et al. (2020), EGs are generally classified into two distinct structural types: emulsion droplet-filled gels and emulsion droplet-aggregated gels (FIGURE 1.3). In emulsion droplet-filled gels, the continuous phase – commonly composed of protein – or polysaccharide-based gels – forms a cohesive gel matrix that serves as the structural framework. Within this matrix, emulsified oil droplets are uniformly dispersed and physically entrapped, contributing to the gel's stability and mechanical integrity. The droplets can be classified as either active or inactive fillers depending on their effect on gel properties. Active fillers (so-called bound fillers) are connected to the gel network and contribute to gel strength whereas inactive (unbound) fillers show low chemical affinity to gel matrix, and do not interact or interact limitedly with gel matrix (FIGURE 1.4) (Farjami; Madadlou, 2019).

FIGURE 1.3 – STRUCTURES OF TWO IDEALIZED MODELS OF EMULSION GELS: (A) EMULSION DROPLET-FILLED GELS, AND (B) EMULSION DROPLET-AGGREGATED GELS.




Source: Adapted from Dickinson (2012).

FIGURE 1.4 – SCHEMATIC REPRESENTATION OF TWO EMULSION-FILLED GELS. a) OIL DROPLETS BEHAVE AS ACTIVE FILLER PARTICLES, b) OIL DROPLETS BEHAVE AS INACTIVE FILLER PARTICLES.




Source: Adapted from Farjami & Madadlou (2019).

Conversely, emulsion droplet-aggregated gels are characterized by the aggregation of emulsion droplets, which interact and organize into a network structure. This aggregation can disrupt the continuity of the gel matrix, leading to a more heterogeneous structure. In many practical formulations, the internal structure of EGs often represents a hybrid of these two models. This mixed structure typically results from the non-uniform distribution of emulsion droplets within the gel, leading to regions where droplets are either well-embedded in the gel matrix or clustered into aggregates. This structural variability significantly influences the mechanical properties, stability, and functional performance of the EGs, affecting their applications in food, pharmaceutical, and cosmetic industries (Dickinson, 2012; Lin; Kelly; Miao, 2020; Xu et al., 2024).

The formation mechanism also depends on the molecular interactions within the system. Proteins can form networks through hydrogen bonding, hydrophobic interactions, and disulfide bonds, while polysaccharides may gel via ionic crosslinking or hydrogen bonding (Li et al., 2023b). The size, distribution, and concentration of oil droplets, as well as the type and concentration of the gelling agent, significantly influence the final texture, mechanical strength, and stability of the EG. These gels exhibit unique viscoelastic properties, combining solid-like and liquid-like behaviors and understanding the formation mechanisms and structural dynamics of EGs is crucial for designing products with tailored textures, improved stability, and enhanced delivery of bioactive compounds, enabling the development of innovative and functional products across various industries (Farjami; Madadlou, 2019).

#### 1.2.2.2. Protein-based emulsion gels

Several studies focus on protein emulsion-based gels instead of the conventional emulsions to avoid protein aggregation issues, coalescence, and thermal denaturation spawning a weak stability of protein emulsion (Taktak et al., 2021). Proteins such as gelatin, whey protein, soy protein, and casein are commonly used due to their amphiphilic nature, enabling adsorption at the oil–water interface and stabilization of emulsions. During the formation of protein emulsion gels, proteins or other types of emulsifiers move close to the lipids and attach to the lipid surface by reducing the surface tension (FIGURE 1.2B). Eventually, the molecular conformation of the emulsifier changes and an interfacial film is formed around the lipid. The hydrophobic region of the partially denatured protein binds to the dispersed lipid, forming an interface termed the interfacial protein film. The interfacial film can stabilize lipid particles and prevent particle aggregation. Thus, lipid particles can be filled as fillers in the gaps of the gel network, wrapped in films and participate in the extension of the gel network as copolymers (Li et al., 2023b).

Gelatin, non-globular and a natural amphiphilic protein derived from collagen, exhibits excellent gelling, emulsifying, and film-forming capabilities (Yan et al., 2023). It’s a kind of promising raw materials for commercial emulsion production without pretreatment, owing to its characteristics of gelation,

biocompatibility, wide source, low cost and nontoxicity (Mao et al., 2022). Its amphiphilic nature allows it to adsorb effectively at the oil–water interface, stabilizing emulsions by reducing interfacial tension and preventing droplet coalescence. One-step cold-set or cold-set after heat treatment is normally used for preparing gelatin-based EG. The gelation mechanism of gelatin is that, when the gelatin solution is cooled below 30 °C, a self-assembly process of gelatin occurs and helices are created forming gels with active-fillers (bound droplets, FIGURE 1.2B). Heat treatment (above 40 °C) is normally used to increase the solubility of gelatin before cold-set treatment (Lin; Kelly; Miao, 2020; Nicolai, 2019).

This gelatin structure imparts desirable functional properties, including improved water and oil retention, controlled release of bioactive compounds, and resistance to phase separation. Protein-based emulsion gels can be engineered to exhibit specific rheological and textural characteristics, making them suitable for fat replacement, texture modification, and nutrient delivery in food products (Taktak et al., 2021).

#### 1.2.2.3. Oils

Oil plays a crucial role in the structure, functionality, and performance of EGs. In these systems, oil serves as the dispersed phase, contributing significantly to the gel's mechanical strength, texture, and stability. The oil droplets, stabilized within the protein or polysaccharide-based continuous phase, act as "active fillers", reinforcing the gel network and influencing its rheological and textural properties. The type and composition of the oil critically impact on the formation, stability, and functionality of EGs (Farjami; Madadlou, 2019). For example, replacement of liquid oil by solid fat in an emulsion leads to an increased stiffness of the emulsion-filled gel (Oliver et al., 2015)

The fatty acid profile, particularly the ratio of SFA to UFA, directly affects the gel’s physical properties. Oils rich in UFAs (e.g., oleic and linoleic acids) tend to have lower melting points and can result in softer gels with greater flexibility but lower structural rigidity (Li et al., 2022a). In contrast, oils with higher SFA content contribute to more rigid and stable gel networks due to their higher

crystallization tendency (Oliver; Scholten; Van Aken, 2015). Additionally, the chain length of TAGs (short-, medium-, or long-chain) influences droplet size, distribution, and the gelation process, affecting mechanical strength and oil binding capacity (Dickinson, 2012; Li et al., 2024a).

Moreover, bioactive compounds naturally present in oils, such as antioxidants, vitamins, and phytosterols, can enhance the nutritional value and functional properties of EGs (Chen et al., 2020; Li et al., 2023b). Thus, selecting appropriate oils based on their composition allows for the design of tailored EGs with desired mechanical properties, improved stability, and enhanced health benefits, making them suitable for functional and reduced-fat food applications (Muñoz-González et al., 2021).

#### 1.2.2.4. Emulsifier

An emulsifier is a kind of surfactant that can simultaneously stabilize the oil–water phase interface to form the emulsion. Emulsifiers stabilize the interface between the two phases by forming micellar structures or an oil–water emulsion mixture. Usually, the hydrophilic part of the emulsifier molecule interacts with the water phase, and the lipophilic part interacts with the oil phase so that the oil droplets are dispersed in the water or the water droplets are dispersed in the oil, forming a stable emulsion system (Cen; Li; Meng, 2024). Surfactants are usually applied in EGs because, as the polymer function, can also contribute to emulsion stability and rheological properties. Thus, the effects of polymer-surfactant interactions depend on the type of added surfactant (non-ionic, cationic or anionic), concentration of surfactant and the solution conditions such as pH, temperature and ionic strength (Farjami; Madadlou, 2019).

At the emulsion interface, surfactants can influence the adsorption of polymers through binding of both (altering solubility) and through competitive adsorption between the adsorbed polymer and the surfactant (Chen et al., 2020). If there is a strong attraction between the surfactant and the polymer, adsorption of the polymer can be enhanced as a result of decreased solvency. This can be explained by the fact that association of an ionic surfactant to oppositely charged polymer reduces the polymer charge, which leads to compaction of the polymer

chain and a diminished solubility. Thereby, an enhanced adsorption of the surfactant–polymer complex can be attained due to charge neutralization. In addition, polymers can also be desorbed from an O/W interface due to competitive adsorption between the adsorbed polymer and surfactant. When there is a repulsive interaction or a weak attractive interaction between the surfactant and the polymer, i.e. the surfactant and polymer have the same charge or surfactant is non-ionic, then the surfactant can displace the polymer from the interface to reduce the surface energy. Under such conditions and at below the critical micelle concentration of the surfactant, both the polymer and the surfactant are adsorbed to interface. Above the critical micelle concentration, almost all polymer is desorbed and only the surfactant is present at the interface (Farjami; Madadlou, 2019).

Polysorbate 20, commonly known as Tween 20, is a nonionic surfactant composed of a polyoxyethylene sorbitan backbone esterified with lauric acid, resulting in a molecular structure with hydrophilic polyether chains and a hydrophobic fatty acid moiety. This amphiphilic nature enables it to reduce surface tension and stabilize emulsions in aqueous systems, making it widely used in pharmaceuticals, cosmetics, and food products (Kerwin, 2008). Tweens/Polysorbates were approved by the Directives for Food Additive (1995) and are used as synthetic flavoring substances or auxiliary substances. It was confirmed that following oral administration in rats, the ester bond sites of polysorbates are hydrolyzed, within the digestive tract, by pancreatic lipase. Free fatty acids are then absorbed from the digestive tract and oxidized and excreted, mainly as carbon dioxide in exhaled breath with same as in ordinary fatty acid metabolism. The rates of hydrolysis of the polysorbate (Tween 20) within the digestive tract is 87 % (Kaur; Mehta, 2017). Tween 20 has replaced the protein from the oil-water interface during heat treatment. That is, the system has changed from a protein-stabilized emulsion to a protein- and surfactant-stabilized emulsion (Cen; Li; Meng, 2024).

## 1.3. Amazon biodiversity

### 1.3.1. Oilseed resources

The Amazon rainforest, recognized as the largest tropical forest globally, harbors one of the most diverse ecosystems on Earth. Covering approximately 49 % of Brazil's territory and 40 % of South America, this region is home to over 13,000 tree species (Araujo et al., 2021). Berto et al. (2015) emphasize that about 4 % of Brazil's native fruit diversity, comprising approximately 220 species, originates from the Amazon, presenting significant potential for local circular economies and agribusiness development.

Research efforts have been directed toward estimating the valuable potential of one of the world's largest reserves of food and medicinal plants (Berto et al., 2015; Faria et al., 2021; Serra et al., 2019). A pioneering contributor to the identification and study of these resources was Pesce (2009), who, during the 1940s, focused on uncovering the potential of oilseeds in the Amazon region. Pesce (2009) documented over 84 oilseed species, including those from the genera *Astrocaryum* (e.g., Tucumã and Murumuru), *Lecythidaceae* (e.g., Brazil nut), *Attalea* (e.g., Babaçu and Inajá), *Oenocarpus* (e.g., Bacaba and Patauá), *Mauritia* (e.g., Buriti), *Bactris* (e.g., Pupunha and Marajá), *Euterpe* (e.g., Açaí), *Meliaceae* (e.g., Andiroba), *Clusiaceae* (e.g., Bacuri), *Sterculiaceae* (e.g., Cupuaçu), *Icacinaceae* (e.g., Umari), *Humiriaceae* (e.g., Uxi), and other.

In the 21$^{th}$ century, Amazon oilseeds have gained attention for its applications in the food, pharmaceutical, and cosmetic industries (Freitas et al., 2024a). Despite the extensive cultivation and industrial utilization of certain oilseeds in the food industry, such as açaí (*Euterpe oleracea*) and dendê (*Elaeis guineensis*), and in the chemical sector, including murumuru (*Astrocaryum murumuru*), pataua (*Oenocarpus bataua*), and tucumã (*Astrocaryum vulgare*) – the latter being primarily exploited by companies such Natura for body care products – there remains a significant gap in research and investment for exploring other oilseeds. Most of the species mentioned above are currently limited to local markets (FIGURE 1.5) and traditionally used in the production of jellies, pulps, and juices, as well as in folk medicine for treating conditions such as diabetes, hepatitis, malaria, parasitic infections, hypertension, and for relieving stomach and ear pain (Araujo et al., 2021).

FIGURE 1.5 – SALE OF SOME OILSEEDS AT THE MUNICIPAL MARKET OF CAMETÁ, PARÁ. 1) TUCUMÃ-DO-AMAZONAS; 2) TUCUMÃ-DO-PARÁ; 3) BACABA; 4) BRAZIL NUT; AND 5) INAJÁ.


Source: The Author (2024).

Many of these species are rich in oil content with essential fatty acids, dietary fibers, bioactive compounds (such as vitamins A and E, and carotenoids), as well as other micro- and macronutrients beneficial to human health (Jaramillo-Vivanco et al., 2022; Santos; Rodrigues; Silva, 2022). Among the UFAs and PUFAs of interest found in most Amazon oilseeds, oleic acid (C18:1) and linoleic acid (C18:2) are the most prevalent. These acids possibly can be present in higher concentrations compared to traditionally known oilseeds (Serra et al., 2019).

### 1.3.2. Amazon bioeconomy versus deforestation

As aforementioned, certain Amazon oilseeds have recently garnered attention from researchers and industry due to their unique compositions and nutraceutical properties, which distinguish them from conventional oilseeds, particularly in terms of their UFA profiles (Pereira et al., 2019). However,

significant under-exploration persists, especially in relation to their application and development in food products.

Beyond their nutritional and functional appeal, these oilseeds hold significant socioeconomic value (Costa et al., 2021). The harvesting of Amazon oils represents vital income sources for the indigenous and riverine communities, coordinated through cooperatives that handle the processing and marketing of the oilseeds (Araujo et al., 2024). By integrating these native resources into value chains, it is possible to foster sustainable livelihoods, enhance regional development, and promote forest conservation and bioeconomy.

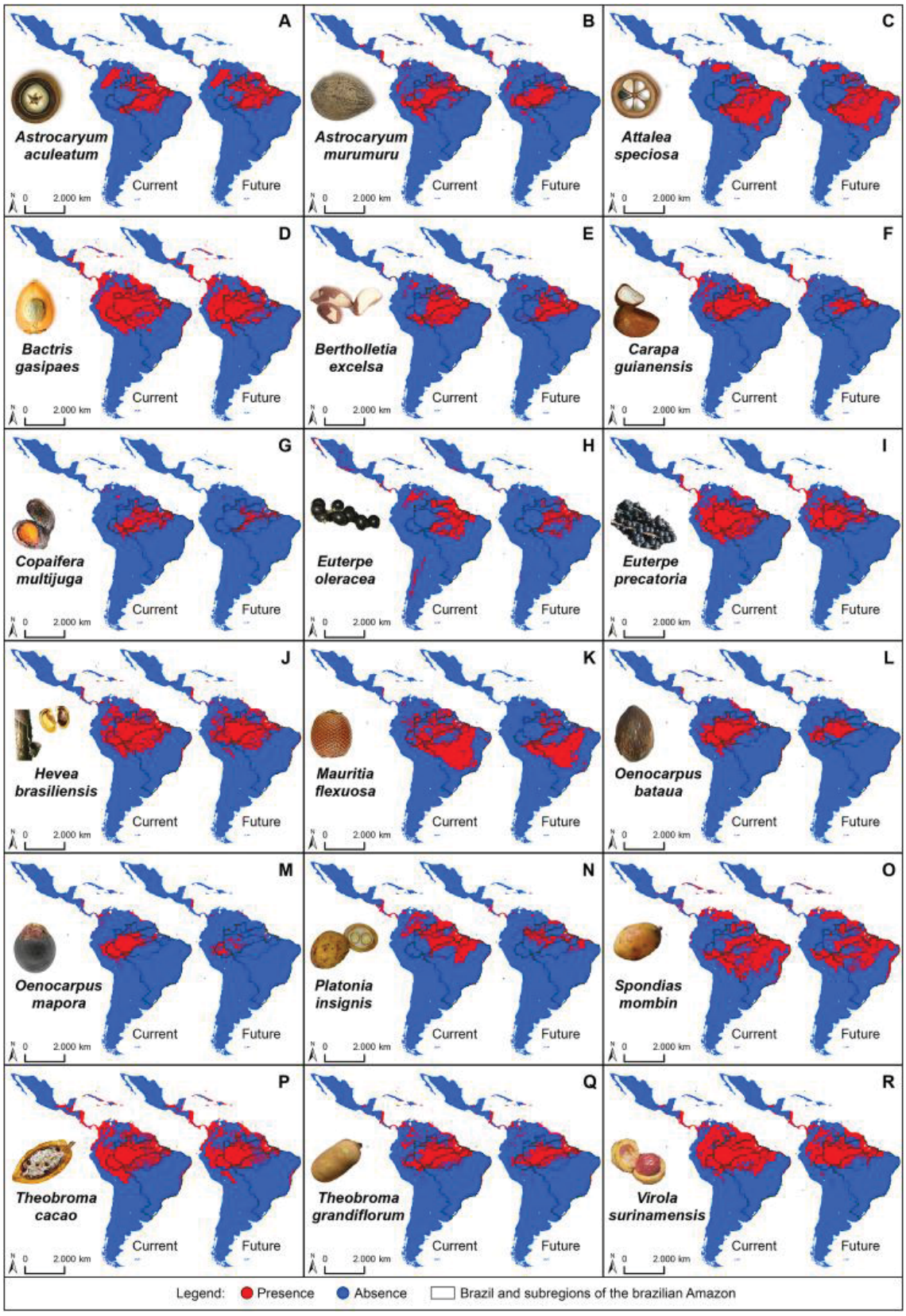
This is the objective of the “Amazon 4.0” program, which focuses on promoting the sustainable development of the region based on new technologies emerging from the 4th industrial revolution, transforming the region’s raw materials into high value-added products (AMAZÔNIA 4.0, 2025). According to projections by the Organization for Economic Cooperation and Development, the bioeconomy is expected to represent 2.7 % of the gross domestic product of the richest countries within a decade (OCDE, 2009). Regarding actions involving only the state of Pará, the estimative are that the advancement of the state’s bioeconomy could add more than USS 35 billion in revenue by 2040 (Costa et al., 2021).

On the other hand, to reach this level and avoid the advance of monocultures and livestock (Ollinaho; Kröger, 2023), there is a need for major changes in public and economic policies and paradigms linked to plant extraction for the verticalization of the Amazon bioeconomy (Bergamo et al., 2022). Worryingly, some lesser-known species are at risk of disappearing due to deforestation and a lack of understanding of their chemical properties, which limits their potential even within local commerce. The progressive loss of native vegetation due to illegal logging, agricultural expansion (monocultures) and infrastructure development (Flores et al., 2024; Nobre et al., 2016) threatens habitats essential for the survival of many oilseed species (Silva, 2024). This changes not only impact biodiversity, but also involve irreversible loss of people’s livelihoods, human well-being, health, and cultural values (Artaxo et al., 2021). According Evangelista-Vale et al. (2021), until 2050, the deforestation may affect the geographic distribution of at least 18 species of palms and threes essential

for traditional populations (FIGURE 1.6). For instance, according to the estimate, there is the projection to reduce the Patauá extractive area by up to 47 % in the Amazon, reaching 100 % in southern Amazon.

Additionally, the decline of Amazon animal populations, particularly seed-dispersing rodents, can significantly impact the regeneration and survival of key oilseed species. For instance, the Brazil nut tree (*Bertholletia excelsa*, FIGURE 1.6E) produce large seeds encased in hard shells that cannot be dispersed by wind or water. Instead, its propagation relies heavily on scatter-hoarding rodents (Agoutis or Cutia, *Dasyprocta punctata and Dasyprocta leporine*), which cache and bury the seeds for later consumption (Peres; Schiesari; Dias-Leme, 1997). Some of these buried seeds are forgotten or not retrieved, allowing them to germinate and establish new trees (Scoles; Gribel, 2012). This mutualistic interaction is crucial for the natural regeneration and genetic dispersal of BN populations across the forest. A reduction in rodent populations – caused by habitat loss, hunting pressure, or ecological imbalance due to deforestation – disrupts this process, potentially leading to decreased seedling recruitment and long-term decline of these oilseed species.

FIGURE 1.6 – AREAS OF ENVIRONMENTAL SUITABILITY UNDER CURRENT CLIMATE AND PROJECTIONS FOR 2050.




Source: Adapted from Evangelista-Vale et al. (2021).

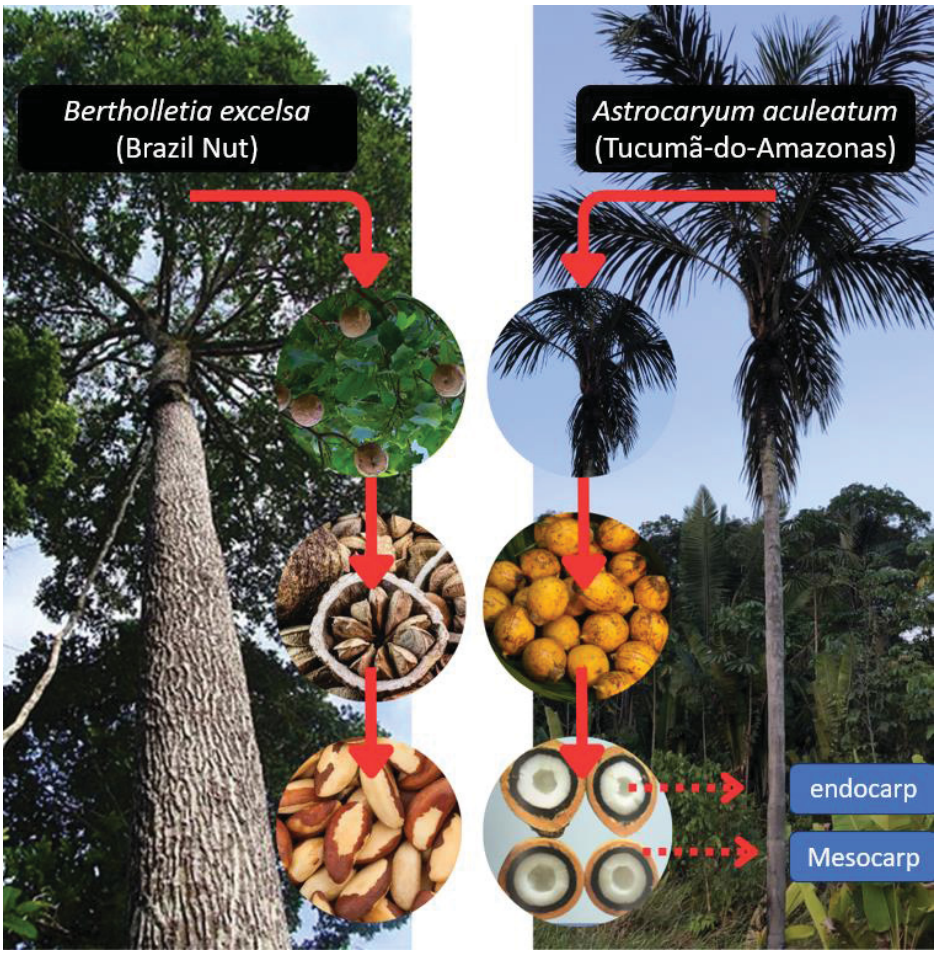
Amid the current Amazon socioeconomic challenges, restrictions and reduction of SFA and TFA in food products, increasing public awareness of healthy eating, and shortages of raw materials, Amazon oilseeds present a promising opportunity to meet the growing global demand for alternative sources of vegetable oils. Beyond satisfying commercial needs, the strategic utilization of these oilseeds is crucial for fostering sustainable development in the Amazon bioeconomy, offering an economic pathway that aligns environmental conservation with income generation. Species such as BN and TA oils with potential applications in food, cosmetics, and pharmaceutical industries, promoting biodiversity preservation, strengthening local production chains, and encouraging sustainable management practices that integrate economic growth with ecosystem conservation.

### 1.3.3. *Bertholletia excelsa* (Brazil nut)

The BN, or Pará nut, is derived from *Bertholletia excelsa*, a towering tropical tree in the *Lecythidaceae* family that can exceed 45 meters in height (FIGURE 1.7). As one of the largest trees in South America, it primarily grows and bears fruit in dense, low-density forest areas (Baldoni et al., 2020) . The tree produces woody capsules, commonly referred to as “ouriço”. Each capsule weighs around 2 kg, measures about 15 cm in diameter, and features a hard shell nearly 1.27 cm thick (FIGURE 1.7) . Additionally, each capsule typically contains about 20 individual nuts.

Its significance stems from both extractives and export, which provide a substantial source of income for the local population, including indigenous communities (Baldoni et al., 2020). According to data on the extraction of natural plant resources (IBGE, 2020), the production value of BN increased by 3.7 % in 2019, reaching a total of R$ 135.8 million. The State of Amazonas continues to lead national production, contributing 12.2 thousand tons, with the municipality of Humaitá accounting for 13.7 % of the total volume recorded in the country.

FIGURE 1.7 – TREE, WOODY CAPSULE AND NUTS OF BRAZIL NUT AND TUCUMÃ-DO-AMAZONAS FRUITS.




Source: The Author (2025).

BNs are a significant native product of the Amazon region, traditionally used in indigenous and riverine medicine to treat various diseases (treating liver diseases and as an antimalarial remedy) due to their notable nutritional constituents (Fontoura et al., 2023). BN offers several health benefits, including the reduction of cardiovascular risk factors in obese individuals by positively influencing lipid profiles and microvascular nutrient reactivity, as well as lowering TAG and cholesterol levels (Cominetti et al., 2012; Hou et al., 2021; Maranhão et al., 2011). Additionally, BN has been associated with reduced oxidative stress (Stockler-Pinto et al., 2010), cancer prevention (Ip; Lisk, 1994; Yang, 2009), and improved cognitive function (Cardoso et al., 2016). This is attributed to its high selenium content, nutritional value, and antioxidant and anti-inflammatory properties, due to its rich composition of UFAs, proteins, dietary fiber, minerals, phenolic compounds, and tocopherols (Hou et al., 2021; Vasquez et al., 2021a).

According to Yang (2009), a single BN provides 160 % of the recommended daily intake of selenium, making it one of the richest plant-based sources of this essential mineral.

In New Zealand, BN has been recommended for dietary addition to improve *glutathione peroxidase* activity (Hou et al., 2021). *Glutathione peroxidase* is a class of antioxidant enzymes with the ability to scavenge free radicals. This in turn helps prevent lipid peroxidation and maintain intracellular homeostasis and redox balance (Ihsan et al., 2018).

Furthermore, the oil and protein content of BN is also remarkable, with 60-70 % and 15-20 % protein per fresh mass, respectively (De Oliveira et al., 2025; Mattos et al., 2025; Pereira et al., 2019). For instance, Pereira et al. (2019) highlight that oleic acid is the main UFA in the composition of BNO (41.63 %), followed by the high content of linoleic acid (31.73 %). As mentioned by Mattos et al. (2025), BNs have a high concentration of phenolic compounds, phytosterols, and selenium, with high levels of MUFAs (29 - 36 %) and PUFAs (36 %), and low SFA (26 %). Besides, these researchers highlighted that the most representative fatty acids are linoleic (36–40 %), oleic (25–36 %), palmitic (14–16 %), and stearic (9–11 %) (Mattos et al., 2025).

Regarding to squalene compound, related to certain beneficial effects linked to the reduction of cholesterol and triglyceride serum levels and to the protection from a variety of cancers (one of its most relevant quality markers), the cold-pressed extra virgin olive and Brazil nut oils resulted among the investigated oils characterized by the highest quantities of squalene (1046.44 µg/g and 591.04 µg/g, $p < 0.05$) (Cicero et al., 2018). On the other hand, Ryan et al. (2006) not only reported high levels of squalene (1377,8 mg/g oil), but also identified levels of tocopherols and phytosterols.

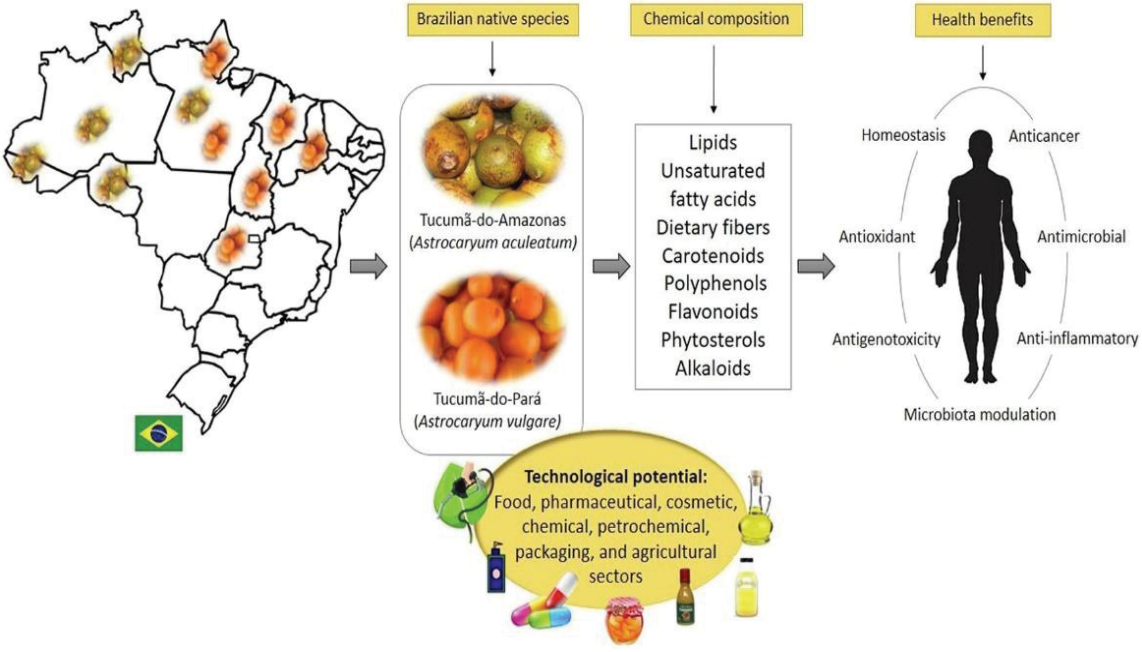
### 1.3.4. *Astrocaryum aculeatum* (Tucumã-do-Amazônas or Jabarana)

Palm trees (*Arecaceae, Palmae*) are a highly diverse pantropical family of woody monocotyledons that grow abundantly in the Amazon basin, where they are represented by more than 150 species (Henderson; Galeano; Bernal, 1995). Among the species, the fruits of Tucumã are little known, being limited mainly to

northern Brazil and parts of Central America. The varieties *Astrocaryum aculeatum* and *Astrocaryum vulgare* are widely spread in this region and predominate in different areas of the Amazon (FIGURE 1.7 and FIGURE 1.8), the first being found more in the western region and the second predominant in the eastern region.

On average, this palm tree is covered in thorns up to 15 cm long and can reach a height of 10 - 25 m (Araujo et al., 2021). After reaching 7 years of maturity, TA can have a productivity ranging from 12 to 50 kg/year and, unlike *Astrocaryum vulgare*, it has a longer harvest period between February and August, while the oriental variety is harvested between January and April (Amorim et al., 2024). Regarding the fruit, its color varies from green to yellow, and it can weigh between 20 and 100 g, with a length and diameter of 4.5 to 6.0 cm and 3.5 to 4.5 cm, respectively (FIGURE 1.7). The fruit pulp is yellow to orange (FIGURE 1.8).

FIGURE 1.8 – TUCUMÃ SPECIES AND THEIR CHEMICAL COMPOSITION, HEALTH BENEFITS, AND TECHNOLOGICAL POTENTIAL.




Source: Machado et al. (2022).

Regional commercialization of TA primarily involves the sale of fresh fruit for direct consumption or the extraction of its pulp for the production of ice cream, sweets, liqueurs, and creams. Thus, TA fruits are of great importance to small

rural producers, most of whom are organized in Brazilian cooperatives in the Amazon region following agroforestry management practices. According to Mota et al. (2022), the TA production chain is an important strategy for the sustainable development of traditional communities in the Amazon region, as the benefits include reduced fires and reduced deforestation for the growth of non-native species, livestock production, land alienation, and illegal economic activities (e.g., timber and minerals). However, fruit consumption generates a residue primarily consisting of the endocarp (almonds), which contains oil- and fat-rich seeds. These almonds (byproduct) are currently discarded into the environment (Carvalho et al., 2024a).

Like other oilseeds in the region, the economically viable potential of its exploitation in various industrial sectors is also considered. This is mainly due to its composition of dietary fibers, high presence of lipids (40-70 % and 40 % for pulp and almonds, respectively) and attractive sensory and nutraceutical characteristics (Mota et al., 2022; Silva et al., 2018).

According to Menezes et al. (2023), TA can stand out in the national and international market mainly due to its mesocarp. According to the authors, the pulp contains bioactive compounds such as PUFAs and other high value-added bioactive compounds, such as oleic acid (C18:1), palmitic acid (C16:0) and stearic acid (18:0), whose percentage composition can vary from 64.14 % to 73.81 %, 22.60 % to 26.49 % and 0.85 % to 5.16 %, respectively. In addition, there are other minor constituents with nutraceutical and pharmacological properties, including β-carotene (20.97 mg.100 g-1), flavonoids (26.06 mg.100 $g^{-1}$) and rutin (14.51 mg.100 $g^{-1}$) (Silva et al., 2018).

In recent decades, studies have reported in vitro and in vivo pro-health effects associated with TA fruits or their products. Evidence from the literature suggests the following established effects: antioxidant, anti-inflammatory, antidyslipidemic, antihyperglycemic, antimicrobial, antifungal, cytoprotective, genoprotective, antiproliferative, anticarcinogenic and neuroprotective. Especially, studies associated with inflammation, oxidative stress and cancer seem to be more frequent (Machado et al., 2022).

## 1.4. Vegetable oil extraction

Vegetable oils are products consisting mainly of glycerides of fatty acids from a plant species, and their physical state at room temperature is liquid. They are insoluble in water, but soluble in organic solvents such as petroleum ether, hexane and others. These oils can be extracted from seeds (Liu et al., 2023a; Sangeetha et al., 2023), peels (Cuco et al., 2019; Wei et al., 2023), plant stems (Desta; Molla; Yusuf, 2020), and fruit pulp (Santos; Rodrigues; Silva, 2022).

The extraction methods for these oils vary from traditional to unconventional methods, and the choice of processes is influenced by the type of oil matrix, extraction temperature, type of solvent, yield, energy costs, and others. The type of process is extremely important, as it directly impacts the extraction efficiency, nutritional quality – as is the case with the composition of fatty acids –, volatile compounds, and oxidative stability, which are important for their application and consumption (Zhang et al., 2023).

The main (traditional) method for extracting oil from oilseeds is mechanical pressing (cold pressing) (FIGURE 1.9a) (Chañi-Paucar et al., 2022; Ferreira et al., 2022; Ohale et al., 2022; Santoso; Iryanto; Inggrid, 2014; Wang et al., 2023a). The extraction of vegetable oils in the Amazon region, particularly through mechanical pressing, represents a vital economic activity for local communities, especially those organized in cooperatives. This method, which relies on the application of pressure to extract oil from seeds and nuts, is widely adopted due to its simplicity, cost-effectiveness, and alignment with sustainable practices. Unlike solvent extraction, mechanical pressing does not require chemical additives, preserving the natural composition of the oils and ensuring their suitability for food, cosmetic, and pharmaceutical applications.

One of the primary advantages of mechanical pressing is its direct benefit to small-scale producers. Many Amazon communities depend on the commercialization of these oils as a crucial source of income, fostering economic development while promoting the sustainable use of forest resources. The establishment of cooperatives enhances collective bargaining power, improves access to markets, and facilitates the adoption of best practices in oil production. Additionally, the environmentally friendly nature of mechanical extraction aligns

with the conservation goals of the Amazon, minimizing chemical waste and reducing the ecological footprint of oil processing.

Despite these advantages, the mechanical pressing of vegetable oils presents a significant limitation: its relatively low extraction yield. Compared to solvent-based techniques, mechanical pressing is less efficient in recovering oil from raw materials, often leaving a considerable amount of residual oil in the pressed cake. This inefficiency can lead to economic losses for producers and necessitates the use of large quantities of raw materials to achieve commercially viable production levels. Consequently, optimizing mechanical extraction parameters, exploring hybrid extraction approaches, or enhancing valorization strategies for by-products become essential to improving the profitability and sustainability of this process.

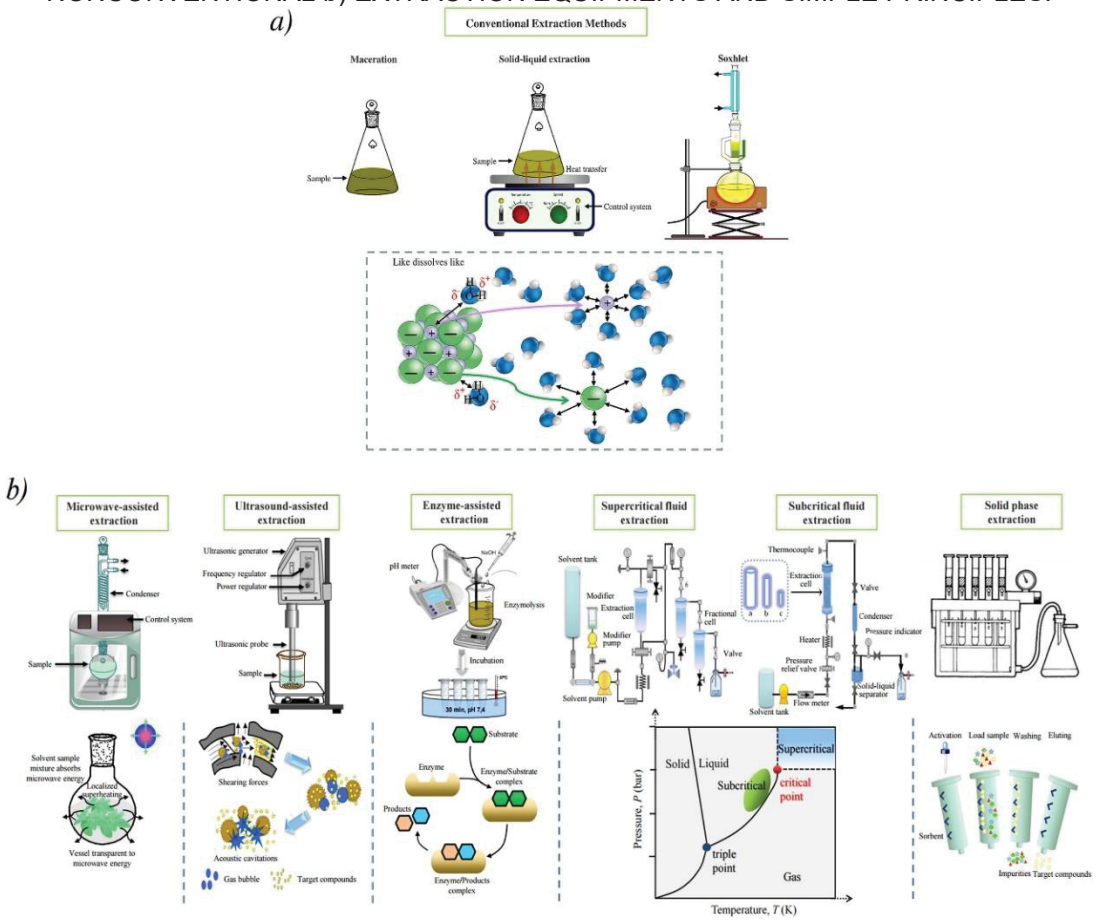
Solvent extraction method is widely used to obtain high lipid yield from oilseeds. This method provides high extraction of the desired material compared to pressing or maceration, as it promotes contact of the solvent with the compound to be extracted in a continuous contact, allowing complete separation. The method commonly uses organic solvents with polar affinity for oil extraction, such as hexane and petroleum ether (Fornasari et al., 2017).

The significance of the solvent extraction method extends beyond its foundational role in the vegetable oil industry; it is also integral to scientific research and food analysis (Soxhlet method), cosmetics, and pharmaceuticals. Its versatility in employing several solvents allows it to adapt to a wide range of extraction requirements, solidifying its status as an indispensable tool in extraction process worldwide. However, the technique is not without drawbacks. Prolonged extraction times to reach maximum yield, the use of high temperatures and toxic solvents (often derived from petroleum) are notable concerns. Additionally, potential loss of nutritional quality, alteration of flavors, and diminished oxidative stability, further complicates its application (Ferreira et al., 2022). These challenges are increasingly pronounced in the context of rising consumer awareness and demand for high-quality oils and environmentally sustainable extraction methods (Gong; Pegg, 2015).

### 1.4.1. New approaches for vegetable oil extraction

Due to problems mainly in extract quality, extraction yield, process cost (time and energy), environmental protection and human health, researchers have explored extraction alternatives as replacements for traditional methods (Zia et al., 2022). Supercritical (SupFE) and subcritical (SubFE) fluids, ultrasound-assisted and microwave-assisted extractions, as well as enzyme-assisted extraction are the latest unconventional techniques explored for vegetable oil extraction (FIGURE 1.9b) (Castejón; Luna; Señoráns, 2018; De Souza et al., 2020; Menezes et al., 2023; Nie et al., 2021; Zhuang et al., 2018).

FIGURE 1.9 – SCHEMATIC REPRESENTATIONS OF CONVENTIONAL a) AND NONCONVENTIONAL b) EXTRACTION EQUIPMENTS AND SIMPLE PRINCIPLES.




Source: Zhang et al. (2019).

These emerging technologies are not conventionally restrictive, they seek to align with sustainable strategies, which promote the improvement of efficiency, economic guidelines for reducing energy consumption and, also, the incentive for

more ecological, innovative and economical industries (Chemat et al., 2017). As discussed by Chemat et al. (2017), emerging extraction methods must meet some factors: (i) reduction of the consumption of organic solvents; (ii) lower energy consumption; (iii) reduction of time; (iv) reduction of unit operations; (v) generate products with high added value; (iv) generate natural products and not hire human beings.

#### 1.4.1.1. Supercritical fluid extraction (SupFE)

SupFE is a method that uses any substance under temperature and pressure conditions above its critical points. Under these conditions, the supercritical fluid has properties that are intermediate between those of a gas and a liquid, i.e., it has the density of a liquid (which allows it to dissolve substances) and the viscosity and diffusivity of a gas (facilitating penetration into solids). In general, the extraction yield will mainly depend on the gas flow, size of sample, temperature, pressure, extraction time and others.

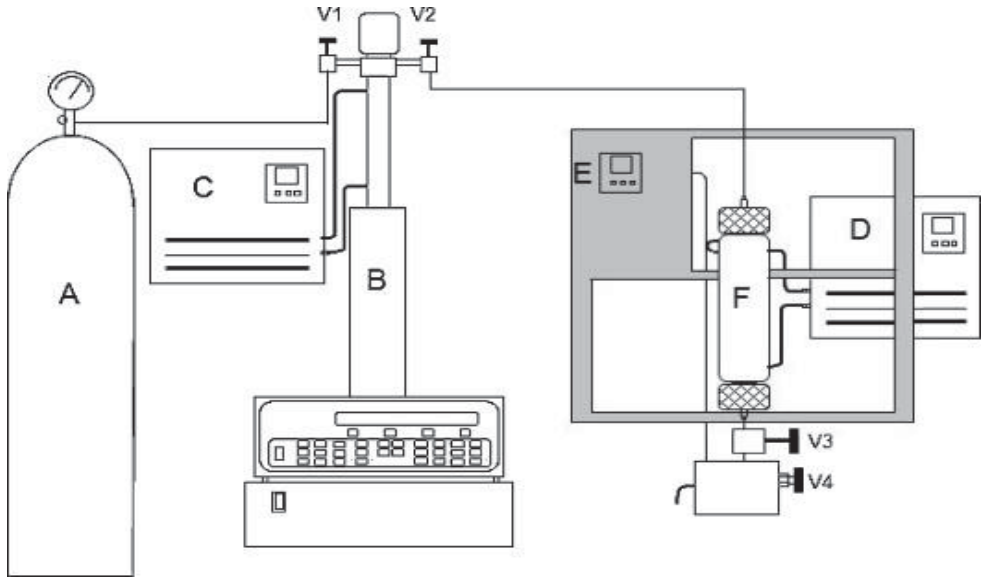
The most commonly used fluid under supercritical conditions is carbon dioxide ($CO_2$), due to its relatively low critical point (31 °C and 73 atm), being non-toxic, cheap, nonflammable, non-polluting properties, and easy to remove after the process (Yousefi et al., 2019). However, a limiting factor for its use is that high extraction pressures are required to obtain oil with supercritical $CO_2$, therefore involving high capital, operating, and maintenance costs (Azevedo et al., 2022). Moreover, it is associated with a low solubility of natural extracts compared with other apolar solvents (propane) (BATISTA et al., 2025; JESUS et al., 2013).

#### 1.4.1.2. Subcritical fluid extraction (SubFE)

In recent decades, the SubFE method has been widely used and improved for the purpose of extracting vegetable oils (Díaz-Reinoso et al., 2023; Zhang et al., 2019). The principle of this method is to extract lipid compounds from solid matrices using fluids with pressure and temperature below the critical point, which it can keep the fluid in a liquid state (Ferreira et al., 2022).

Although many works use water as a subcritical solvent (Díaz-Reinoso et al., 2023; Md Sarip et al., 2023; Zhu et al., 2024), propane (critical pressure and temperature of 369.67 K and 4.3 MPa, respectively) has attracted attention due to its complete solvation of TAGs through the reduction of viscosity and density while increasing diffusivity and solubility (ZHANG et al., 2024). Although it is extremely flammable and requires an explosion-proof apparatus for its application (in contrast to supercritical $CO_2$), propane is generally recognized as safe (GRAS) for food contact and allowed as an extraction solvent in many countries (Wenceslau et al., 2021). Furthermore, due to their low bubble point (example propane: -42.1 °C), subcritical fluid solvents can be easily separated from oils through evaporation and can even be reused using a gas recycling system. Regarding its apparatus, an extraction system using compressed propane basically consists of a gas cylinder, syringe pump, thermostatic baths, temperature controller on the micrometric valve, extractor, and flow control valves. FIGURE 1.10 shows a diagram that represents a laboratory-scale unit.

FIGURE 1.10 – EXPERIMENTAL MODULE OF EXTRACTION WITH SUBCRITICAL PROPANE: (A) GAS CYLINDER; (B) SYRINGE PUMP; (C AND D) THERMOSTATIC BATHS; (E) TEMPERATURE CONTROLER IN THE MICROMETRIC; (F) EXTRACTOR; (V1), (V2, AND (V3) NEEDLE VALVES; (V4) MICROMETRIC VALVE OF FLOW RATE CONTROLLER.




Source: Santos et al. (2015).

### 1.4.1.3. Ultrasound-assisted extraction

Ultrasound-assisted extraction has achieved high levels of application in the extraction of vegetable oils, either in combination with other techniques or used alone (Garofalo et al., 2022; Nie et al., 2021; Thilakarathna et al., 2023; Wang et al., 2023a; Wong; Tan; Geow, 2019). According to Chemat et al. (2017), ultrasound-assisted extraction can be completed in minutes with high reproducibility, reducing solvent consumption, simplifying handling and processing, providing greater final purity, eliminating wastewater post-treatment and consuming only a fraction of the fossil energy required for a conventional extraction method.

The mechanism behind ultrasound-assisted extraction is primarily driven by the phenomenon of cavitation, which is induced either directly by an ultrasound probe or indirectly via a bath sonicator. Cavitation generates high shear forces within the medium, resulting in the formation and subsequent implosion of cavitation bubbles at the product surface. These implosions cause surface flaking, macroturbulence, erosion, particle fragmentation, and micromixing. As a result, the cellular structure of the material collapses, thereby accelerating the mass transfer of the target compounds into the solvent (Thilakarathna et al., 2023).

On the other hand, the ultrasound-assisted extraction presents concerns regarding its process. Among the drawbacks, the cavitation phenomenon can generate an increase in the extraction temperature and, therefore, degrade thermally sensitive compounds. Another effect of sound waves is the possible erosion of equipment materials, which can contaminate the desired material. Furthermore, in some cases, ultrasound may not be efficient in extracting oils from certain plant matrices with low cavitation capacity or more rigid cellular structures, resulting in lower yield. Regarding the apparatus, the cost of the equipment, increased scale, and increased energy consumption are the disadvantages.

#### 1.4.1.4. High shear dispenser or Ultra turrax-assisted extraction (UTAE)

UTAE is a widely used method for the extraction of bioactive compounds from different matrices (Li et al., 2023a). This method is known as a high-speed

shearing disperser machine (3000 to 30,000 rpm) and can provide extremely strong shear and thrust forces on the material (Xu et al., 2016).

With a rotor-stator system, this equipment allows for dispersing, homogenizing, mixing, and stirring in one-step as so-called “one-vessel solution”. The rapid rotation of the rotor creates turbulence in the liquid or mixture, generating high shear energy, promoting the rupture of cells in biological matrices, releasing compounds such as proteins, polyphenols, lipids or other metabolites (Chang et al., 2023). In addition, there is the cavitation process, where the high speed creates small bubbles in the liquid medium that, when they collapse, release large amounts of energy. This cavitation facilitates the breakdown of cells or tissues, increasing the contact area between the solvent and the material, accelerating the extraction. Consequently, it is hypothesized that high speed stirring may also be suitable for the target compounds extraction due to the high mass transfer speed and forces with appropriate extraction solvent.

Among the advantages of the method, it can be mentioned the high extraction efficiency in a short time interval, as well as greater flexibility and control of parameters (rotation, extraction time and temperature can be adjusted to optimize extraction). On the other hand, the high rotation speed can cause an increase in temperature, which can degrade heat-sensitive compounds.

Earlier studies indicated that UTAE was widely applied in homogenizing the immiscible liquid/liquid systems (emulsions), bioactive compounds extraction, and the dispersion of raw powder crystals into a liquid phase (Cabrera-Trujillo et al., 2018; Xu et al., 2016). On the other hand, despite the high availability on the market, there are few reports exploring this method for extracting vegetable oils. Therefore, there is a gap to be explored.

## 1.5. State of the art

### 1.5.1. Ultra-turrax assisted extraction (UTAE)

As mentioned before, the UTAE is a high-shear homogenizer widely used for rapid mixing, dispersing, and emulsification of liquid and semi-solid samples. Common applications include sample preparation in pharmaceuticals, food

processing, cosmetics, and chemical industries, as well as in academic and industrial research for creating stable emulsions, suspensions, or dispersions.

Some studies explored the use of UTAE as a pre-process or in combination with other technologies to intensify the extraction of bioactive compounds. For instance, according the Xu et al. (2016), UTAE based ultrasound-assisted extraction (ultra turrax ultrasound-assisted extraction), was firstly designed to extract the main organic acids from honeysuckle. The results showed that Ultra turrax and ultrasound-assisted extraction could get fast extraction speed and uniform particle distribution and due to the high extraction efficiency could be applied in industrial production.

Vázquez-Vázquez et al. (2024) evaluated the extraction of antioxidant phenolics from fresh berries and their waste using UTAE and Ultrasonication. The researchers have founded that UTAE 14,000 r/min, 5 min combined with 1 h ultrasonication led to total phenolics and total flavonoids concentration of 42.29 $mgGAE.g^{-1}dw$, 21.85 $mgRE.g^{-1}$ for blueberry, 97.68 $mgGAE.g^{-1}dw$, 27.62 $mgRE.g^{-1}$ for raspberry and 44.28 $mgGAE.g^{-1}dw$, 16.5 $mgRE.g^{-1}$ for blackberry. In waste, 72.01 $mgGAE.g^{-1}dw$, 41.61 $mgRE.g^{-1}$ from blueberry, 101.18 $mgGAE.g^{-1}dw$, 19.76 $mgRE.g^{-1}$ from raspberry and 96.60 $mgGAE.g^{-1}dw$, 28.90 $mgRE.g^{-1}$ from blackberry were obtained. Syringic acid and epicatechin were the main recovered compounds with 1945 $mg.g^{-1}dw$ and 2028 mg $g^{-1}dw$ from fresh raspberry and 884 mg $g^{-1}dw$ and 966 mg $g^{-1}dw$ from blackberry waste.

The use of UTAE to boost the encapsulation of beneficial compounds is also found in the literature. For instance, the combining UTAE and ultrasonic homogenization to achieve higher vitamin E encapsulation efficiency in spray drying was developed by Siqueira et al. (2024). The results showed encapsulation efficiency was 73.73 % in the first stage (UTE/spray drying).

Sturm et al. (2018) studied UTAE for the extraction of pesticides from egg and milk samples. UTAE already successfully applied for the extraction of plant materials, has also proved to be suitable for the analysis of pesticides in eggs and milk. In comparison to the matrix solid-phase dispersion, the extraction is less time-consuming at excellent extraction efficiency. Further advantages are the flexibility of the extraction conditions with respect to the pH value and water

activity ($a_w$). So, even strongly acidic pesticides such as phenoxy carboxylic acids can be extracted.

Regarding the extraction of vegetable oils, it is noted in the literature that its use has a scarce application and exploration base for intensification of extraction using UTAE. Thus, due to the benefits of UTAE in other extraction areas, the possibility of also intensifying the extraction of vegetable oils is being considered, improving the extraction yield, processing time and maintaining the quality of the compounds present in the oil.

### 1.5.2. Oleogels (OGs) and emulsion gels (EGs)

OGs and EGs represent innovative structuring systems that offer significant potential in the development of healthier food products by enabling the replacement of TFAs and SFAs with healthier lipids, thus aligning with global health recommendations for reducing the risk of cardiovascular diseases. These systems provide versatility in texture, stability, and functionality, making them suitable for a wide range of food applications, including spreads, dressings, baked goods, and meat analogs. Despite their promise, the development of OGs and EGs faces significant challenges, including achieving consumer-acceptable textures, maintaining functional stability during processing and storage, and ensuring cost-effective scalability. Additionally, understanding the interaction between structural components, such as gelling agents, emulsifiers, and bioactive additives, presents complexities requiring advanced characterization techniques and multidisciplinary research approaches. Furthermore, regulatory approval and consumer perception of novel ingredients and formulations are critical barriers that must be addressed to facilitate their widespread adoption. Overcoming these challenges will be pivotal for leveraging the full potential of these systems in promoting healthier dietary patterns.

In this scenario, Sejwar et al. (2024) developed carnauba wax-based soybean OGs. According to the rheology results, the optimized sample's crossover point occurs at a larger strain percentage (59.2 %) than the control sample's (8.9 %), indicating that the product exhibits solid behavior over a wider strain percentage. The Linear viscoelastic region (LVR) appears between 0.01 to 0.01 %, presenting unstable structure increasing the strain.

Thakur et al. (2022) worked developing the optimization and characterization of soybean oil-carnauba wax OGs. Regarding the oil results, the OBC increased with the increasing concentration of carnauba wax with the lowest value was shown by the OG prepared at 5 % carnauba wax (68.76 %) and the highest value was observed at 10 % carnauba wax (99.73 %). According to the researchers, carnauba wax melts at a higher temperature (84 °C) and, consequently, the number of hydrogen bonding sites are increased, which creates a network with enhanced physical properties. For rheology, as evident, at low strain values G''/G' < 0.1, which indicates that the gel is quite stable. With increasing strain, the G′ value starts decreasing which signifies the structural breakdown. The cross-over point, which signifies the permanent structural breakdown, occurred at 361.4 ± 0.6 Pa and a strain of 12.01 ± 0.2 %. After the cross-over point, the G'' > G′ and the sample behave like fluid. In addition, differential scanning calorimetry (DSC) data exhibited a single broad peak around 75 °C, signifying its melting temperature, whereas soybean oil did not show any such melting peak. The range between the onset of melting temperature (70 °C) and the peak (81.4 °C) indicates that the structure of carnauba wax oleogel is complex.

Airoldi et al. (2022) explored the potential use of carnauba wax-OG to replace saturated fat in ice cream. All OGs formed stable and firm gels over 60 days, irrespective of the concentrations (6 %, 8 %, and 10 %) or storage temperatures (5 °C and 25 °C). The OBC of OGs containing 8 % and 10 % carnauba wax (~ 90 %) was significantly greater ($p < 0.05$) than that of OGs with 6 % carnauba wax (~ 80 %). Additionally, the DSC thermograms with 6 % carnauba wax presented a melting peak at 79.1 ± 0.6 °C and $\Delta_{Hm}$ of 7.0 ± 0.1 J/g. These endothermic peaks are associated with the saturated long-chain monofunctional waxes esters and fatty alcohols in carnauba wax that melts around 82 °C. For polarized light microscopy (PLM), the carnauba wax 6 % OG sample exhibited platelet-like crystals in tiny aggregation. The rise of the carnauba wax ratio (8 % and 10 %) forms an aggregation of the needle-like crystals from a nucleation center with several clusters that form large crystal structures, demonstrating a growth in the diameter mean and in the crystalized area ($p < 0.05$). This increase promotes gelation within the continuous network, achieved by cross-linking crystals and oil trapping.

Buitimea-Cantúa et al. (2021) studied the effect of quality of carnauba wax on microstructure, textural, and rheological properties of soybean oil-based organogels. The visual appearance of organogels revealed that the three samples at concentrations above 4.5 % w/w formed solid organogels. According to the authors, the gelling behavior is governed by the nature and chain length of their chemical components, mainly esters of C24 and C28 carboxylic acids and C32 and C34 straight-chained primary alcohols. For the color parameters, L*, a*, and b* ranged between 20.1 ± 0.2 to 25.4 ± 0.4, -1.7 ± 0.3 to 2.4 ± 0.3, and -2.4 ± 0.4 to 5.0 ± 0.5, respectably.

Aliasl Khiabani et al. (2020) worked with characterization of carnauba wax/adipic acid OG. Fourier transform infrared (FTIR) spectrum shows specified peaks related to the intramolecular or intermolecular hydrogen bonding are responsible for the formation of the semi-crystalline structure of OGs. Thus, for the researchers, there are possible interaction (hydrogen bonds) between the hydroxyl groups of carnauba wax and the carboxylate groups of Adipic acid in a soybean oil matrix (3400 to 3850 $cm^{-1}$). DSC thermogram presented peaks at 85 and 150 °C for carnauba wax and Adipic acid melting temperature, respectively. In the thermogram of carnauba wax, the large range between the onset of melting temperature (60 °C) and the peak point (85 °C) indicated the more complex structure of carnauba wax. Additionally, the graphic regarding the strain sweep showed the G′ were higher than the G″ of all OG samples, indicating the solid-like behavior of oleogel samples. By increasing the strain, G' and G“ decreased until reaching the crossover point at which large deformations occurred.

Wang et al. (2025) evaluated the fabrication, microstructure, rheological properties and interactions of soft solid OGs of hazelnut oil body (concentration from 20 to 50 %) using Xanthan gum and gelatin as gelling agents. The authors presented samples with a pure bright yellow appearance and – when the hazelnut oil body concentration exceeded 30 wt % - the (OBC of OGs began to drop from 100 % to 73.93 %. Notably, the OG with 50 wt % hazelnut oil body had the lowest oil binding capacity (~ 70 wt %), along with slight structural collapse and oil leakage. This result may be due to the fact that the interfacial network formed by xantham gum-gelatin (1.5 wt %) was not sufficient to bind all the oil droplets, leading to partial droplet aggregation and spillage. Regarding the rheology, the critical stress values for OGs with 40 wt % and 50 wt % hazelnut oil body were

around 10 Pa while those for OGs with 20 wt % and 30 wt % hazelnut oil body exceeded 100 Pa. On the other hand, only OGs with 40 and 50 wt % had a stable LVR, indicating better structure. For thermal properties, xantham gum-gelatin displayed an exothermic peak at approximately 108.2 °C. This peak disappeared in hazelnut oil body- xantham gum-gelatin due to interactions between hazelnut oil body and xantham gum-gelatin, which altered the xantham gum-gelatin structure.

Using capillary bridges interactions, Miao et al. (2024) fabricated starch-based OGs for application as edible inks in 3D food printing. At a secondary fluid content of 0.05, the capillary bridges formed between the starch particles were not enough to maintain the structure of the solid/liquid/liquid system. As a result, a flowable mixture is formed. This effect is probably because there was insufficient water present to form capillary bridges between all the starch particles The capillary suspension transformed from a fluid-like to gel-like state when secondary fluid content was increased to 0.2. For rheology results, at secondary fluid content = 0.1, G′ was greater than G″ at all frequencies, suggesting that a space-spanning network was formed by the starch particles, resulting in the formation of a semi-solid oleogel. As the secondary fluid content value increased from 0.1 to 0.4, the G′ and G″ values increased and then decreased, with a maximum value at secondary fluid content = 0.25. This result suggests that there is an optimum intermediate water content required to form strong capillary network structures. Regarding the OBC, the oil loss maximum was around 10 % with 38 % of starch. Samples with 19 % starch reached oil loss around 60 %.

The emulsion-templated OG based on gelatin and Tamarind seed polysaccharide complex was prepared by Xie et al. (2023). The oil loss of gelatin OG was 18.2 %, which was significantly higher than that of gelatin+0.1 % Tamarind seed polysaccharide OG (8.0 %), gelatin+0.3 % Tamarind seed polysaccharide OG (6.9 %), and gelatin+0.5 % Tamarind seed polysaccharide OG (3.2 %). This indicate that the addition of polysaccharide could improve the properties of OGs.

EGs from gelatin and sodium alginate as pork fat substitute was explored by Lee et al. (2025). Rheology results showed that pork fat and gelatin-sodium alginate EGs have a higher G′ modulus than G″ modulus, indicating solid-like properties. In addition, no changes in the G′ and G″ moduli with increasing shear

rate (0.1 to 10 1/s) were observed for either the EG or pork fat. In the DSC data, both pork fat and EG showed peak onset at $T_1$ and peak temperature at $T_2$. The melting temperature was observed at 35.07 °C for pork fat and 42.62 °C for EG, indicating a higher melting temperature for the EG. Since a 3D gelatin network may dominate EG structure, it seems to melt at approximately 42 °C which is the melting temperature of gelatin (data not shown).

## 1.6. Conclusion

Therefore, in light of the increasing global pressure to reduce the consumption of trans and saturated fats, promote healthier dietary patterns, and identify innovative raw material sources for the food industry, the development of new methods for structuring vegetable oils, such as OGs and EGs, represents a promising alternative. These structured systems not only mimic the functional and technological properties of conventional solid fats but also open avenues for creating products with improved nutritional profiles, addressing consumer demand for healthier options without compromising sensory quality.

Moreover, considering the unique chemical compositions of Amazon oilseeds, which are rich in unsaturated fatty acids, bioactive compounds, and essential nutrients, there is a significant opportunity for their integration into such structured lipid systems. Their application can simultaneously promote healthier eating habits and contribute to the socio-economic development of the Amazon region, aligning local resources with global trends in functional and sustainable food innovation.

However, the extraction of these oils remains a critical challenge. Conventional techniques, such as mechanical pressing or solvent-based methods, are often associated with low yields, long processing times, and degradation of heat-sensitive compounds. These drawbacks not only reduce efficiency and increase environmental impact but also generate economic losses for local communities that rely on oilseed processing as a source of income. In this context, the adoption of novel and more efficient extraction strategies becomes essential to ensure that the potential of Amazon oilseeds can be fully realized.

Altogether, these aspects highlight that advancing sustainable extraction technologies and applying Amazon oils in structured lipid systems address multiple global and regional needs. This approach contributes to reducing harmful fats in the food supply, supports healthier diets, and fosters innovation in food design. At the same time, it enhances the economic value of underutilized Amazon resources, promotes environmental preservation, and strengthens the bioeconomy of the region, thus providing benefits that extend from local communities to the international consumer market.

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# CHAPTER II

## Ultra turrax-assisted extraction of Amazon oils: Optimization and comparison with Soxhlet and compressed propane extraction

This chapter consists of the manuscript entitled "Ultra turrax-assisted extraction of Amazon oils: Optimization and comparison with Soxhlet and compressed propane extraction", **PUBLISHED** in the journal "*Chemical Engineering and Processing - Process Intensification*", which authorizes the main author to use the content to incorporate into his thesis. This article consists of an innovative study through the intensification of the extraction of Brazil nut and TAO using solvent extraction assisted by a high shear disperser (Ultra-Turrax). For this, the Plackett-Burmann methodology was used to evaluate whether the process parameters (extraction temperature, extraction time, solvent/solid ratio, type of solvent, sample, and Ultra-turrax rotation) were significant in the extraction. After this step, the significant process variables were used to optimize the extraction using the response surface methodology. Finally, under optimized process conditions, the oils obtained were compared with traditional (Soxhlet) and unconventional (compressed propane) extraction methods in relation to yield, fatty acid composition, thermal stability properties, bioactive compounds, and antioxidant activity.

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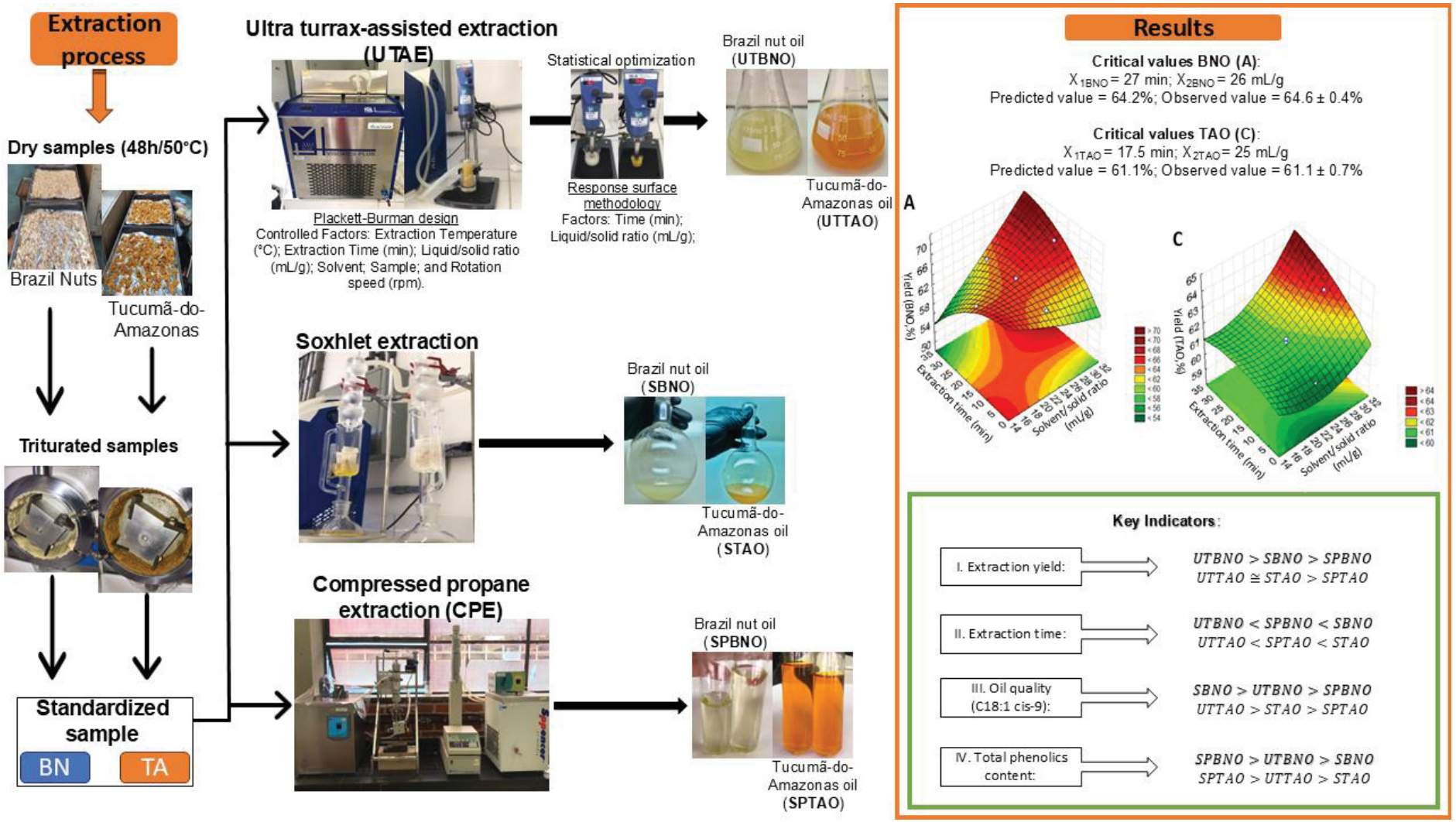
## Graphical Abstract



# Ultra turrax-assisted extraction of Amazon oils: Optimization and comparison with Soxhlet and compressed propane extraction

## 2.1. Abstract

The Amazon region, while rich in biodiversity, experiences low socio-economic development. One promising solution involves using Amazon oilseeds, which offer high nutritional value and industrial potential. However, traditional extraction methods have limitations, such as high energy use, solvent consumption, and low yields. This study optimized the extraction of BNO and TAO using UTAE, comparing it with conventional and non-conventional methods. Using the Plackett-Burman design (PBD), only extraction time and solvent/solid ratio were significant factors. Optimizing these variables through response surface methodology achieved high yields: 64.6 % for BNO and 61.1 % for TAO, outperforming Soxhlet and compressed propane extraction (CPE) by up to 43.4 %. UTAE produced comparable oleic acid levels in BNO (37.0 - 37.8 %) and TAO (66.1 - 68.2 %). Additionally, the total phenolic content was higher in both CPE (7.87 ± 0.77 and 26.71 ± 0.63 mg GAE.100 $g^{-1}$) and UTAE (4.57 ± 1.00 and 19.63 ± 1.68 mg GAE.100 $g^{-1}$) compared to Soxhlet (1.25 ± 0.37 and 10.97 ± 1.92 mg GAE.100 $g^{-1}$) for BNO and TAO, respectively. These results highlight UTAE's efficiency for oil extraction.

Keywords: *Bertholletia excelsa*; *Astrocaryum aculeatum*; Plackett-Burman design; Response surface methodology.

## 2.2. Introduction

Amazon rainforest occupies a total area of approximately 6.3 million $km^2$ distributed across eight countries: Brazil, Bolivia, Colombia, Ecuador, Guyana, Peru, Venezuela, Suriname and French Guiana, of which approximately 4.1 million $km^2$ (60 %) are in Brazilian territory. Despite occupying approximately 49 % of the Brazilian territory and having an extraordinarily rich biodiversity, the region has one of the lowest rates of social and economic development in the country (Dos Reis et al., 2023; IBGE, 2024). Furthermore, deforestation rates are

causing the disappearance of native species. One of the ways to overcome this situation is to promote the bioeconomy in the region, through the encouragement of sustainable agriculture based on the exploitation of Amazon oilseeds. Pesce (2009) cataloged approximately 84 species of these oilseeds, which have unique compositions and nutraceutical properties as they are composed of essential fatty acids and fat-soluble bioactive compounds that help maintain human health (Jaramillo-Vivanco et al., 2022).

Among the important matrices for strengthening the sustainable development of traditional communities in the Amazon, BN (*Bertholletia excelsa*) and TA (*Astrocaryum aculeatum*) stand out, which in the state of Amazonas alone, produced around 14 thousand tons and 367.8 tons, respectively. Oils constitute approximately 50–70 % of the dry mass of these almonds, making them a rich source of UFAs (primarily oleic and linoleic acids), as well as essential minerals and phenolic compounds. These components are associated with numerous health benefits, such as lowering blood TAG and cholesterol levels, and exhibiting antioxidant and anti-inflammatory properties (Cardoso et al., 2017; Machado et al., 2022) . Consequently, these almonds hold significant potential for industrial applications and could contribute to income generation for local communities.

In most industries, the extraction of these oils is carried out using conventional extraction methods, which present numerous challenges, including excessive solvent consumption, that are now subject to stringent regulations under European Directives and the Registration, Evaluation, Authorization, and Restriction of Chemicals framework (Ordóñez-Santos; Pinzón-Zarate; González-Salcedo, 2015). Besides, we can highlight other drawbacks, such as prolonged extraction times, lesser yield, higher energy consumption, and degradation of heat sensitive bio-active compounds (Arumugam; Baskar; Sriram, 2024). Therefore, industries have a great interest in using more environmentally friendly solvents and innovative extraction techniques. Recent attention has turned to extraction methods such as ultrasound-assisted extraction, enzyme-assisted extraction, microwave-assisted extraction, SupFE, and compressed solvent extraction (or SubFE) as a means of addressing these limitations (Cuco et al., 2019; Ferreira et al., 2022; Liu et al., 2023a; Santos; Rodrigues; Silva, 2022;

Sawant Sanket et al., 2024; Thilakarathna et al., 2023; Wei et al., 2023). In this scenario, UTAE emerges as a new approach in the extraction of vegetable oils. This new approach to the extraction of vegetable oils focuses on increasing the solid-liquid interface area, which provides acceleration of the mass transfer kinetics between the target compound (oil matrix) and the extractive phase (solvent). This method has the potential to significantly increase extraction yields while reducing processing time and the use of toxic solvents. Moreover, it can contribute to improving the nutritional profile of extracted oils by increasing the UFA profile and minimizing the degradation of bioactive compounds caused by external factors such as exposure to the effects of temperatures and oxidation.

This chapter aims to evaluate a novel approach for intensifying the extraction of vegetable oils using solvents employing UTAE to improve the yield and quality of Amazon oils (from BN and TA). To achieve this objective, the study evaluated and optimized process parameters and compared this method with conventional Soxhlet and CPE focusing on extraction yield, fatty acid composition, thermal stability properties, bioactive compounds, and antioxidant activity.

## 2.3. Methodology

### 2.3.1. Materials

#### 2.3.1.1. Sample collection and preparation

The fruits BN and TA were purchased from producers who sell the fruits at the Açaí fair located in the municipality of Cametá, State of Pará, Brazil (2°14'54.6 "S 49°29'54.3"W). Whole fruits were sanitized with a sodium hypochlorite solution (200 ppm/15 min). Their pulps were separated from the skins and seeds. These samples were dried in an oven with air circulation (Soc. Fabbe Ltda) for 48 hours at 50 °C. The final moisture content of the pulps was approximately 1 %, determined by drying in an oven at 105 °C until constant weight according to Association of Official Analytical Chemists International method 930.15 (AOAC, 2005). Thus, the dried samples were ground in a Willye-type macro mill (Tecnal). The crushed materials obtained were packaged in low-density polyethylene bags, vacuum sealed, and stored in a freezer at 268.15 K until use.

### 2.3.2. Methods

The present study was divided into three stages. In the first step, a PBD (Plackett; Burman, 1946) was applied to identify the main factors influencing the yield of vegetable oil extraction through UTAE. In the second step, only the factors that had a significant effect on the oil extraction yield of BN and TA in the first step were tested, validated, and optimized using a central composite rotatable design (CCRD). In the third stage, BNO and TAO obtained under optimized conditions in the UTAE process were compared to oils obtained by Soxhlet and using CPE in terms of extraction yield, thermal stability properties, fatty acid composition, bioactive compounds, and antioxidant activity.

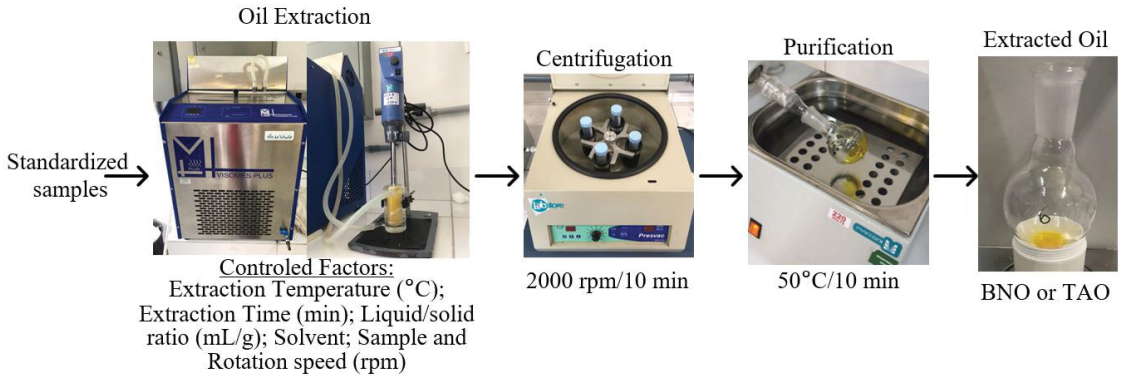
#### 2.3.2.1. Ultra turrax-assisted extraction (UTAE)

UTAE extraction was carried out using a jacketed glass cell coupled to a thermostatic bath (RW-1025G, Lab Companion) under constant agitation promoted by the Ultra-turrax disperser (IKA T25 D, Staufen, Germany) (FIGURE 2.1). The system was then centrifuged (Thermo Fisher Scientific Fresco 21 Micro Centrifuge) at 2000 rpm for 10 min. Finally, the supernatant generated by centrifugation was purified in a rotary evaporator (MA120/THV, Marconi) at 50 °C for 10 min under vacuum (400 mmHg) and then taken to the oven at 60 °C to remove remaining solvent residues. The oils were kept in an amber glass vessel and stored at −5 °C until further analysis and were coded as UTBNO and UTTAO for BNO and TAO, respectively. The percentage extraction yield was calculated on dry basis, according to Eq. (2.1).

$$Y_{(\%db)} = \left( \frac{m_o}{m_s(1 - \frac{U_s}{100})} \right) 100 \quad \text{(Eq. 2.1)}$$

Where: $Y_{(\%db)}$ is the yield in dry basis, $m_o$ is the obtained extract mass, $m_s$ is the sample mass used, and $U_s$ is the percentage moisture of the freeze-dried BN and TA pulp.

FIGURE 2.1 – ULTRA TURRAX-ASSISTED EXTRACTION (UTAE).




### 2.3.2.2. Soxhlet extraction

Soxhlet extraction yields were determined in triplicate, using n-hexane (Neon, 99.8 % purity) as solvent. Approximately 5 g of comminuted BN and TA samples and 250 mL of solvent were used, being refluxed to its boiling point in a Soxhlet extractor apparatus (UNIGLAS, Campinas, São Paulo, Brazil) for 6 h. After extraction, the solvent was removed from the oil using a rotary vacuum evaporator (MA120/THV, Marconi) at 50 °C for 10 min under vacuum (400 mmHg) to remove remaining solvent residues. The oil was kept in an amber glass vessel and stored at −5 °C until further analysis. The percentage extraction yield was calculated on dry basis, according to Eq. (2.1). Furthermore, the oils obtained from BN and TA were coded as SBNO and STAO, respectively.

### 2.3.2.3. Compressed propane extraction (CPE)

Extractions were carried out using a laboratory-scale supercritical extraction apparatus as described in detail in previous studies by our research groups using n-propane with 99.5 % purity (White Martins S.A., Araucária, PR, Brazil) (Freitas et al., 2024b). Thus, 30 g of BN and 26 g of TA of raw materials were used for each extraction under the following conditions used in previous oilseed extraction studies: solvent flow rate 2 $mL.min^{-1}$ at 100 bar; extraction temperature of 60 °C; static and dynamic periods of 30 min and 60 min, respectively. The oil obtained from BN was coded as CPBNO and TA as CPTAO

and the overall extraction yield of crude oil was calculated using Eq. (2.1), as described in Section 2.3.2.1.

#### 2.3.2.4. Plackett-Burman Design (PBD)

The UTAE process represents an innovative approach for the extraction of vegetable oils, wherein parameters capable of influencing the extraction yield were initially investigated using the PBD to screening variables and offer foundation for further optimization. The process conditions (extraction temperature, extraction time, solvent/solid ratio, type of solvent, sample, and Ultra-turrax rotation) were defined according to the statistical design defined in Table 2.1 and Table 2.2. Firstly, it was evaluated whether the yield could be affected using different types of samples. To this parameter, two vegetable matrices, BN and TA, characterized by distinct fatty acid compositions as described in the literature (Santos; Rodrigues; Silva, 2022; Schons et al., 2017), were selected. Subsequently, the type of solvent was the second factor investigated, with n-hexane (Neon, 99.8% purity) and isopropanol (Neon, 99.5% purity) being chosen due to their divergent polarities and boiling points.

Regarding the quantitative factors that could be evaluated and controlled during the process, the influence of temperature on the extraction yield was studied. A jacketed cell was used, with the lower (-1) and upper (+1) levels set at 10 °C and 50 °C, respectively. These temperatures were selected within a more moderate range to prevent the degradation of thermosensitive compounds and the loss of solvent by evaporation. Another crucial factor in the application of this new methodology was the duration of the process, with the levels primarily defined based on the upper limit of 30 minutes to ensure a viable range for process optimization, prevent equipment damage (such as the degradation of the tip due to the solvent), and minimize energy consumption. The rotation speed of the equipment was also examined, with the lower-level set at the minimum possible rotation of the equipment (3000 rpm) and the upper level at three times that intensity (9000 rpm). The last controllable parameter was the solid/solvent ratio, where the lower level (1 g/15 mL) was stipulated as the minimum amount possible to promote sample and solvent economy while ensuring adequate dispersion without damaging the glassware and equipment.

2.3.2.5. Statistical optimization using response surface methodology

For the purposes of experimental design, mathematical modeling, statistical analysis, and determination of surface response contour plots, Statistica software (version 8.0, USA) was used in the optimization project of the present research. Optimization design through response surface methodology is a collection of mathematical and statistical techniques that are efficient for modeling and analyzing problems in which responses are impacted by many variables, with the aim of evaluating and applying a mathematical model to obtain a relationship between a set of factors (Ye et al., 2024).

To evaluate and optimize operational parameters, a CCRD experimental design was implemented. This design involved two factors, each with five levels, to evaluate the response behavior of these factors in the extraction yield of vegetable oils. The levels were coded as -1.41, -1, 0, +1 and +1.41, and the two significant factors were previously defined according to topic 2.3.2.4. The vegetable oil extraction yield was considered the response value. To analyze the experimental data, an empirical response surface model was used.

A quadratic polynomial equation, represented by Eq. 2.2, was used to adjust the experimental data and elucidate the relationship between the factors and the response variable. Based on Eq. 2.2, its linear ($\beta_1$ and $\beta_2$), quadratic ($\beta_{11}$ and $\beta_{22}$), and interaction ($\beta_{12}$) effects were determined. $\beta_0$ is a constant term, and $X_1$ and $X_2$ are the levels of the independent variables. All experiments were randomized.

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \qquad \text{(Eq. 2.2)}$$

Furthermore, multiple regression analyses were performed to estimate the coefficients and establish mathematical equations that can effectively predict the response variables. Additionally, an analysis of variance (ANOVA) was performed to evaluate the significance of the model ($p < 0.05$). The coefficient of determination ($R^2$) was used to evaluate the model fit.

2.3.3. Characterization

2.3.3.1. Fatty acids analysis

The fatty acid profile of oils was determined after the preparation of samples according to the official method (Ce 266) to convert the oils into fatty acid methyl esters (Fetzer et al., 2021). Fatty acid methyl esters were analyzed using a Shimadzu chromatograph (GC 2010 Plus), a capillary column (SH-Rtx-Wax (Shimadzu): 30 m x 0.32 mm x 0.25 µm), flame ionization detector, and split injection mode (1:10). The injector and detector temperatures were 240 °C and 250 °C, respectively. The oven temperature was programmed to start at 100 °C and held for 5 min, followed by an increase up to 240 °C at a rate of 4 °C.min$^{-1}$ and maintained at this temperature for 5 min. The carrier gas was Helium at 32.5 cm$^3$.min$^{-1}$. Fatty acid methyl esters were identified by comparison with retention times of a standard mixture of fatty acid methyl esters (Supelco, MIX FAME 37, St. Louis, MO 63103, USA). The quantification of fatty acid was conducted by area normalization procedure. Results were expressed as a percentage of each fatty acid present in the sample.

2.3.3.2. Thermal stability

The thermal decomposition of BNO and TAO obtained was evaluated by thermogravimetry analysis (TGA). This was performed on a TGA 4000 Perkin Elmer (Waltham, USA) using a procedure based on the method proposed in Micić et al. (2015). The DSC was performed on a DSC 8500 Perkin Elmer (Waltham, USA) using $N_2$ as the purge gas. Around 5 mg of oil were weighed and sealed into a hermetic aluminum pan. The following time-temperature program was used: equilibration at 20 °C for 5 min to ensure complete temperature homogenization of the sample and then heated to 50 °C at 5 °C.min$^{-1}$, after that hold for 5 min followed by cooling to −80 °C at −5 °C/min to induce crystallization and holding at this temperature for 5 min, subsequently heated from −80 to 50 °C at 5 °C.min$^{-1}$ to obtain the melting profile. Each sample was measured in duplicate. Peak, onset, and endset temperatures were calculated using Originlab software.

2.3.3.3. Bioactive compounds

The total phenolic content (TPC) of the BNO and TAO were determined according to the Folin–Ciocalteu method (Singleton; Orthofer; Lamuela-

Raventós, 1999). Firstly, the samples were prepared by mixing 100 mg of oil with 1 mL of methanol: water (80:20), shaken and centrifuged for 10 min at 3000 rpm. To determine the TPC, 0.4 mL of the supernatant and 0.1 mL of methanol were mixed with 2.5 mL of Folin-Ciocalteu reagent (diluted 1:10 in distilled water). The mixture was kept in the darkness for 3 min. Afterward, 2 mL of 7.5 % (w/v) sodium carbonate aqueous solution was added to the mixture and incubated in the dark for 2 h. Then, the absorbance was measured at 760 nm in an UV-1800 Shimadzu Spectrophotometer (Kyoto, Japan). All assays were performed in triplicated. The quantitative results were calculated using a gallic acid calibration curve and were expressed as mg of gallic acid equivalents (GAE) per 100 g of sample (mg GAE.100 $g^{-1}$).

Total flavonoids content (TFC) of samples was determined based in the method proposed by Zhishen; Mengcheng; & Jianming (1999), with some modifications. Aliquots (0.2 mL) of samples, prepared as previously described, and 1.8 mL of distilled water, and 0.12 mL of $NaNO_2$ (5% w/v) were added to amber bottles and mixed. After 5 min, 0.12 mL of $AlCl_3$ (10% w/v) was added; and after 6 min, 0.8 mL of NaOH (1 mol.$L^{-1}$) and 0.96 mL of distilled water were added. Absorbance was measured at 510 nm in UV/Vis Spectrophotometer (UV-1800 Shimadzu). The Catechin was used as the standard for a calibration curve and the results were expressed as mg of catechin equivalent (CE) per 100 g of sample.

#### 2.3.3.4. Antioxidant activity

The DPPH• assay and ABTS method were performed based at method described by Brand-Williams; Cuvielier; & Berset (1995) and Re et al. (1999), respectively, with some modifications describe by Fetzer et al. (2021).

#### 2.3.3.5. Statistical analysis

The experimental design was performed using Statistica 7.0 software (Statsoft Inc., Tulsa, OK, USA), where data were analyzed (ANOVA). The graphs were created using Origin 8.6 (OriginLab, Northampton, MA, USA) software.

## 2.4. Results and discussion

### 2.4.1. Plackett-Burman design (PBD)

The PBD methodology is an extremely useful and efficient tool for identifying significant factors among many variables that influence the process using a few experimental runs. Thus, it allows you to simultaneously test several factors at different levels in a process, as well as understand the impact of each factor in comparison to the others and identify which ones are important to optimize the system (Spadi et al., 2021). In this work, six variables (extraction temperature, extraction time, solvent/solid ratio, solvent type, sample type, and rotation) were chosen at two levels (-1 for the lower level and +1 for the higher level) (TABLE 2.1). The influence of these parameters resulted in sixteen executions using the PBD, which had yields varying between 52.62 and 77.41 % (TABLE 2.2).

TABLE 2.1 – FACTORS TESTED USING THE PLACKETT–BURMAN DESIGN AT HIGHER (+1) AND LOWER (−1) LEVELS.

| **Parameters** | **Factor Code** | **Level** | |
|---|---|---|---|
| | | -1 | +1 |
| Extraction Temperature (°C) | F1 | 10 | 50 |
| Extraction Time (min) | F2 | 3 | 30 |
| Liquid/solid ratio ($mL.g^{-1}$) | F3 | 15 | 30 |
| Solvent | F4 | Hexane | Isopropanol |
| Sample | F5 | BN | TA |
| Rotation speed (rpm) | F6 | 3000 | 9000 |

TABLE 2.2 – PLACKETT-BURMAN DESIGN MATRIXES FOR SIX VARIABLES AND THE OBSERVED RESPONSE FOR EXTRACTION YIELDS FOR BRAZIL NUTS (BNO) AND TUCUMÃ-DO-AMAZONAS (TAO).

| | Factor | | | | | | |
|---|---|---|---|---|---|---|---|
| **Extraction** | **Extraction temperature (°C)** | **Extraction Time (min)** | **Solvent/ solid Ratio ($mL.g^{-1}$)** | **Solvent** | **Sample** | **Rotation speed (rpm)** | **Yield (%)** |
| 1 | 50 | 3 | 15 | Hexane | TA | 3000 | 57.25 |
| 2 | 50 | 30 | 15 | Hexane | BN | 9000 | 65.16 |
| 3 | 50 | 30 | 30 | Hexane | BN | 3000 | 69.11 |
| 4 | 50 | 30 | 30 | Isopropanol | BN | 3000 | 63.82 |
| 5 | 10 | 30 | 30 | Isopropanol | TA | 3000 | 64.29 |
| 6 | 50 | 3 | 30 | Isopropanol | TA | 9000 | 62.60 |
| 7 | 10 | 30 | 15 | Isopropanol | TA | 9000 | 55.33 |
| 8 | 50 | 3 | 30 | Hexane | TA | 9000 | 64.10 |
| 9 | 50 | 30 | 15 | Isopropanol | BN | 9000 | 63.64 |
| 10 | 10 | 30 | 30 | Hexane | TA | 3000 | 77.41 |
| 11 | 10 | 3 | 30 | Isopropanol | BN | 9000 | 63.00 |
| 12 | 50 | 3 | 15 | Isopropanol | TA | 3000 | 54.06 |
| 13 | 10 | 30 | 15 | Hexane | TA | 9000 | 57.02 |
| 14 | 10 | 3 | 30 | Hexane | BN | 9000 | 62.79 |
| 15 | 10 | 3 | 15 | Isopropanol | BN | 3000 | 52.63 |
| 16 | 10 | 3 | 15 | Hexane | BN | 3000 | 58.86 |

The *p*-value is the probability value that denotes the significance of the yield, and its p value less than 0.05 is considered the significant value. In general, factors with confidence intervals greater than 95 % or significance levels less than 0.05 are selected as significant factors. By analyzing TABLE 2.3, we can identify that the extraction yield was significantly affected by the solvent/solid ratio (0.0032) and extraction time (0.0316), being these two factors are the most critical factors. Furthermore, the Pareto chart (FIGURE 2.2) is an excellent way to visualize these PBD results and it indicates the significant factors for the extraction process. Therefore, the analysis of FIGURE 2.2 confirms the data presented in TABLE 2.3.

FIGURE 2.2 – PARETO CHART INDICATING THE SIGNIFICANT PARAMETERS.

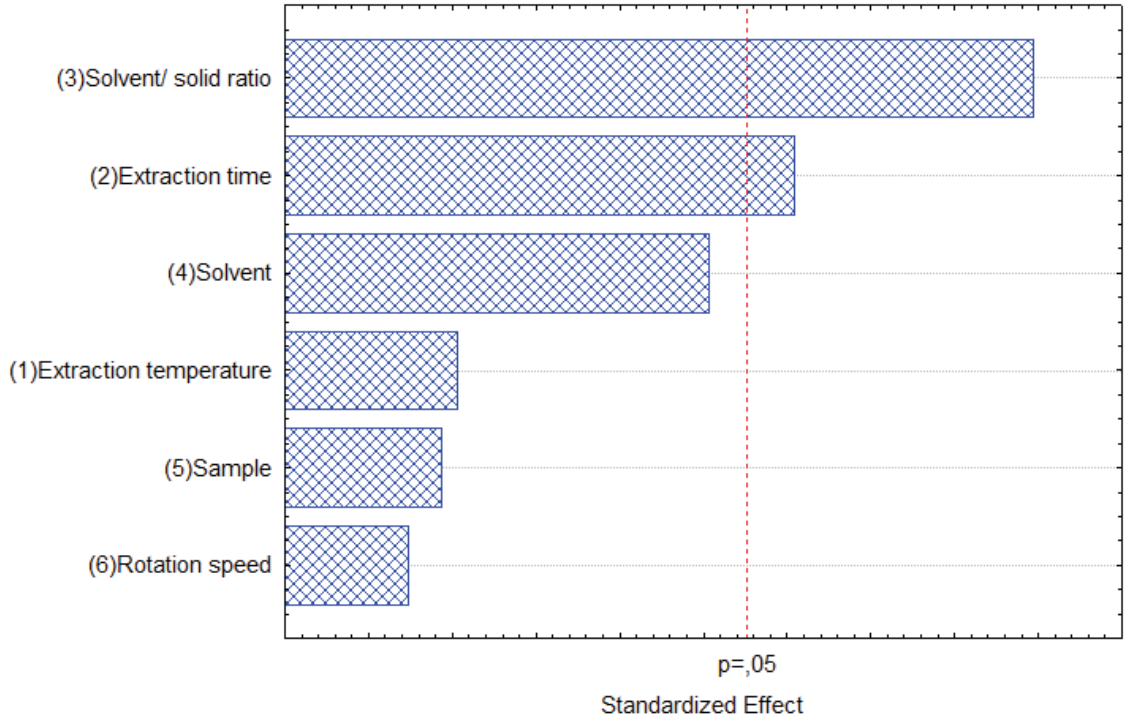



TABLE 2.3 – ANALYSIS OF VARIANCE (ANOVA) OF PBD PROCESS.

| Factor | SS* | Df* | MS* | F* | p* | $R^2$ |
|---|---|---|---|---|---|---|
| **(1) Extraction temperature** | 4.4310 | 1 | 4.4310 | 0.27947 | 0.609839 | 74.88 |
| **(2) Extraction time** | 102.4144 | 1 | 102.4144 | 6.45928 | 0.031632 | |
| **(3) Solvent/solid ratio** | 249.4820 | 1 | 249.4820 | 15.73485 | 0.003271 | |
| **(4) Solvent** | 65.2864 | 1 | 65.2864 | 4.11762 | 0.073030 | |
| **(5) Sample** | 3.0276 | 1 | 3.0276 | 0.19095 | 0.672423 | |
| **(6) Rotation speed** | 0.9025 | 1 | 0.9025 | 0.05692 | 0.816774 | |

*Sum of Square (SS); Degree of freedom (Df); Mean square (MS); F-Value (F); P-Value (p).

### 2.4.2. Response surface methodology to optimize oil extraction

According to the CCRD experimental design principle (Box; Wilson, 1992), a response surface analysis experiment can be designed using two factors and five levels to optimize the vegetable oil extraction process using the UTAE method. Based on the results of screening different parameters by PBD, F2 and F3 factors were considered significant for the vegetable oil extraction process.

Other factors were considered no significant ($p<0.05$) and were fixed for the method optimization process. Therefore, due to its low boiling point, n-hexane was chosen to preserve bioactive compounds during the oil purification process.

Regarding rotation speed and temperature, the lowest rotation speed and ambient temperature were chosen in order to reduce energy consumption. Regarding the samples, both were chosen for the optimization process, despite the process not showing significant differences ($p<0.05$) in the extraction yield. Therefore, the minimum (3 min and 15 $mL.g^{-1}$) and maximum (30 min and 30 $mL.g^{-1}$) values for extraction time and solvent/solid ratio were chosen as the optimization range (-1.41 and +1.41) (TABLE 2.4).

TABLE 2.4 – CODED AND REAL LEVELS OF THE INDEPENDENT VARIABLES STUDIED IN THE EXPERIMENTAL DESIGN (CCRD).

| **Independent variables** | **Coded variables** | **Variables levels** | | | | |
|---|---|---|---|---|---|---|
| | | **-1.41** | **-1** | **0** | **+1** | **+1.41** |
| Extraction time (min) | $X_1$ | 3 | 7 | 16.5 | 26 | 30 |
| Solvent/solid ratio ($mL.g^{-1}$) | $X_2$ | 15 | 17 | 22.5 | 28 | 30 |

Using the Statistica 8.0 software a CCRD $2^2$ experiment was designed with eleven experimental trials, with three repetitions at the central point (TABLE 2.5). It is noteworthy that polynomial multivariate regression models emerged as the most appropriate approach to modeling the effect of parameters on the response. The significance of each process parameter was determined by the magnitude of the F test and the *p*-value. The corresponding yield parameters would be more meaningful if the absolute F-value was larger, and the *p*-value was smaller ($p < 0.05$). The final code-specific equations are represented as Eq. (2.3) and Eq. (2.4), respectively, providing a comprehensive mathematical representation of the relationship between the factors and the response.

The adequacy of the models was rigorously evaluated using ANOVA, with the corresponding results presented in TABLE 2.6. Therefore, it was possible to observe that the linear coefficient ($X_2$) and the interaction of effects ($X_1$ and $X_2$) Eq. (2.3) presented significant effects ($p<0.05$) in the BNO UTAE process, therefore having a significant and positive impact on the extraction process. For TAO extraction, the linear and quadratic coefficients and the interaction of both effects (Eq. 2.4) did not show significant influences ($p < 0.05$). Moreover, the non-significant lack of fit values (0.072 and 0.617 which are greater than 0.05 for BNO

and TAO, respectively) showed that the second-order polynomial models were valid for the present study.

TABLE 2.5 – EXPERIMENTAL DESIGN FOR CENTRAL COMPOSITE ROTATIONAL DESIGN.

| | Coded values | | Real values | | Yield* (%) | |
|---|---|---|---|---|---|---|
| | Solvent/solid ratio | Extraction time | Solvent/solid ratio ($mL.g^{-1}$) | Extraction time (min) | BN** | TA** |
| **1** | -1 | -1 | 17 | 7 | 64.2±1.3 | 60.7±0.1 |
| **2** | -1 | 1 | 17 | 26 | 60.0±0.6 | 61.3±0.9 |
| **3** | 1 | -1 | 28 | 7 | 61.1±1.0 | 60.8±0.1 |
| **4** | 1 | 1 | 28 | 26 | 65.5±0.1 | 62.4±1.0 |
| **5** | -1.41 | 0 | 15 | 16.5 | 63.9±0.2 | 61.0±0.1 |
| **6** | 1.41 | 0 | 30 | 16.5 | 68.2±1.3 | 63.2±0.7 |
| **7** | 0 | -1.41 | 22.5 | 3 | 63.6±1.2 | 60.5±1.2 |
| **8** | 0 | 1.41 | 22.5 | 30 | 63.9±1.0 | 61.6±1.4 |
| **9** | 0 | 0 | 22.5 | 16.5 | 64.6±1.4 | 61.3±1.2 |
| **10** | 0 | 0 | 22.5 | 16.5 | 63.9±0.8 | 61.6±0.3 |
| **11** | 0 | 0 | 22.5 | 16.5 | 64.9±1.3 | 60.6±0.6 |

* mean ± confidence interval at a 95 % level of significance.
**BN: Brazil nut; and TA: Tucumã-do-Amazonas.

$$Yield\ (CBO, \%) = 64.46 + 0.09 * X_1 - 0.89 * {X_1}^2 + 1.05 * X_2 + 0.25 * {X_2}^2 + 2.15 * X_1 * X_2 \quad \text{(Eq.2.3)}$$

$$Yield\ (TAO, \%) = 61.17 + 0.47 * X_1 - 0.14 * {X_1}^2 + 0.54 * X_2 + 0.40 * {X_2}^2 + 0.24 * X_1 * X_2 \quad \text{(Eq.2.4)}$$

The effect of interactions between the variables considered (extraction time ($X_1$) and solvent/solid ratio ($X_2$)) was subsequently analyzed through the description of contour (3D) and two-dimensional (2D) graphs in relation to these independent parameters. Thus, the response surface graphs obtained are shown in FIGURE 2.3, with FIGURE 2.3A–B and FIGURE 2.3C-D representing the effects of variables $X_1$ and $X_2$ correlated to the BNO and TAO samples, respectively. Therefore, it can be seen in FIGURE 2.3A-B that the extraction efficiency was greater for a combination of higher $X_1$ and $X_2$, for example, 68.2 ± 1.3 % for an extraction time of 16.5 min and solvent/solid ratio of 30 $mL.g^{-1}$. This is in line with the significant effects presented in the previous topic.

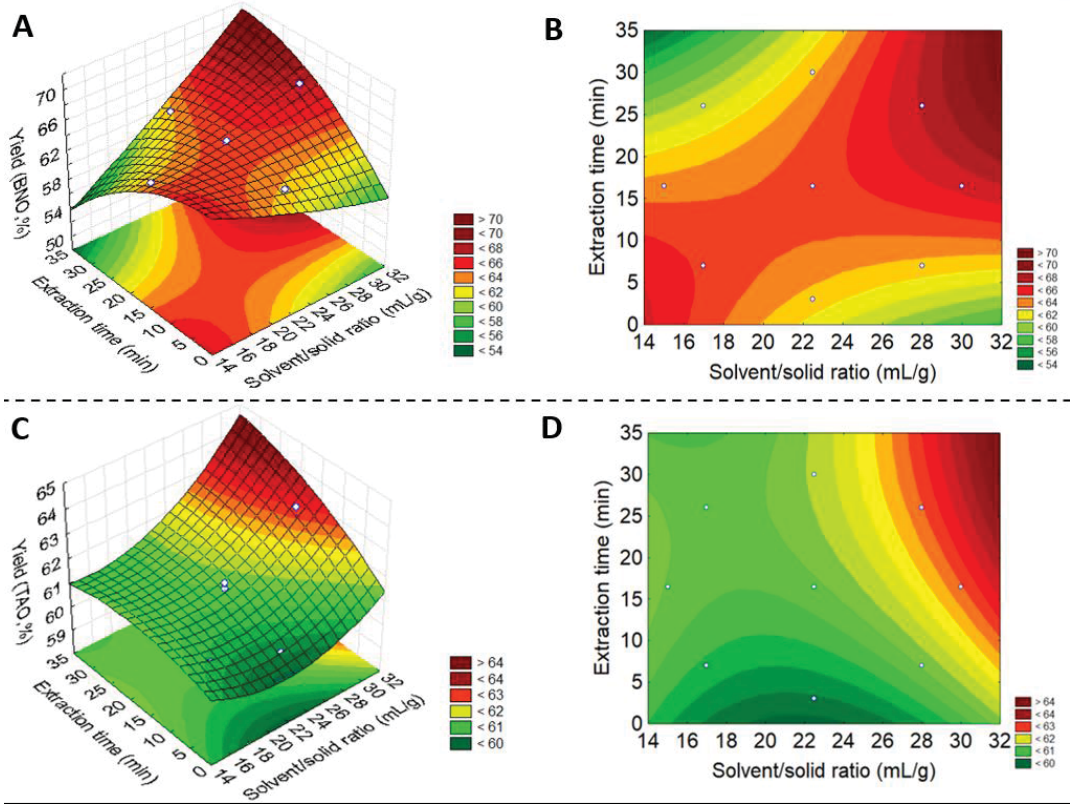
TABLE 2.6 – ANALYSIS OF VARIANCE APPLIED TO BNO AND TAO EXTRACTION STUDIES.

| Factor | SS | Df | MS | F | *p* | $R^2$ |
|---|---|---|---|---|---|---|
| | | | **BNO** | | | |
| (1) Solvent/solid ratio(L) | 8.88863 | 1 | 8.88863 | 31.11739 | 0.030666* | 74.20 |
| Solvent/solid ratio(Q) | 0.37151 | 1 | 0.37151 | 1.30058 | 0.372269 | |
| (2) Extraction time(L) | 0.07202 | 1 | 0.07202 | 0.25214 | 0.665402 | |
| Extraction time(Q) | 4.50384 | 1 | 4.50384 | 15.76710 | 0.057964 | |
| 1L by 2L | 18.59292 | 1 | 18.59292 | 65.09025 | 0.015018* | |
| Lack of Fit | 11.15182 | 3 | 3.71727 | 13.01347 | 0.072201 | |
| Pure Error | 0.57130 | 2 | 0.28565 | | | |
| Total SS | 45.44668 | 10 | | | | |
| | | | **TAO** | | | |
| (1)Solvent/solid ratio(L) | 2.345153 | 1 | 2.345153 | 8.387415 | 0.101413 | 83.04 |
| Solvent/solid ratio(Q) | 0.947467 | 1 | 0.947467 | 3.388607 | 0.207002 | |
| (2)Extraction time(L) | 1.835298 | 1 | 1.835298 | 6.563924 | 0.124522 | |
| Extraction time(Q) | 0.114338 | 1 | 0.114338 | 0.408928 | 0.587987 | |
| 1L by 2L | 0.239639 | 1 | 0.239639 | 0.857066 | 0.452295 | |
| Lack of Fit | 0.623769 | 3 | 0.207923 | 0.743634 | 0.617112 | |
| Error | 0.559207 | 2 | 0.279604 | | | |
| Total SS | 6.977365 | 10 | | | | |

Sum of Square (SS); Degree of freedom (Df); Mean square (MS); F-Value (F); P-Value (p); Brazil nut oil (BNO); Tucumã-do-Amazonas oil (TAO).
*The highlighted effects indicate that the variable had a significant effect ($p<0.05$).

For FIGURE 2.3C-D related to the optimization of the TAO extraction process, a higher yield (63.2 %) was also observed for a higher combination of variables $X_1$ (16.5 min) and $X_2$ (30 mL.g$^{-1}$). On the other hand, unlike BNO, both variables show no significant influences ($p < 0.05$). Regarding the critical values of the process ($X_{1BNO}$ = 26.91 min; $X_{2BNO}$ = 25.94 mL.g$^{-1}$; $X_{1TAO}$ = 24.71 min; $X_{2TAO}$ = 17.59 mL.g$^{-1}$), it is noted that there are no significant differences ($p < 0.05$) between observed value (64.6 ± 0.4 % and 61.1 ± 0.7 %) and predicted value (64.2 % and 61.1 %) for BNO and TAO, respectively. Therefore, for both BNO and TAO, the models are effective.

FIGURE 2.3 – THREE-DIMENSIONAL RESPONSE SURFACE-INTERACTION EFFECTS OF VARIABLES ON THE YIELD. (A-B: THE INTERACTION BETWEEN EXTRACTION TIME AND SOLVENT/SOLID RATIO TO BNO; C-D: THE INTERACTION BETWEEN EXTRACTION TIME AND SOLVENT/SOLID RATIO TO TAO). COLORS: GREEN (LOWER RESPONSE VALUES IN THE SYSTEM) TO RED (HIGHEST RESPONSE VALUES IN THE SYSTEM).




2.4.3. Extraction yield

The yield obtained by the UTAE method for BNO (64.6 %) in its optimized condition (26 mL of solvent.g$^{-1}$ of BN for 27 min) was higher than those found by Soxhlet (54.9 %) and CPE (45.0 %) (TABLE 2.7). Regarding the optimized TAO condition (17.5 mL of solvent.g$^{-1}$ of TA for 25 min), the yield of UTAE (61.1 %) was similar to Soxhlet (61.6 %) and higher than that of CPE (57.3 %) (TABLE 2.7).

Furthermore, when comparing the solvent consumption for the Soxhlet method and the optimized conditions (17.5 mL.g$^{-1}$ TAO and 26 mL.g$^{-1}$ BNO), UTAE presented a reduction of 65 % (UTTAO) and 48 % (UTBNO) in relation to STAO and BNO, respectively. These disparities can be attributed to the enhanced interactions facilitated by UTAE between solvents and samples, primarily due to its superior dispersion performance, resulting in more efficient mass transfer within a shorter contact period. Hence, our findings suggest that UTAE represents a more cost-effective and environmentally friendly extraction

method, particularly given its reduced solvent usage, and shorter solvent/sample interaction time regarding the Soxhlet extraction.

### 2.4.4. Fatty acid composition

Fatty acid compositions of BNO and TAO oils from Soxhlet, UTAE, and CPE were determined by gas chromatography, and the results are detailed in TABLE 2.7. Considering the BNO extract and its different extraction methods, the similar presence of SFA and UFA can be noted, with emphasis on the greater proportion of MUFAs. Oleic acid (C18:1 cis-9) was the UFA with the highest concentration, varying from 37.04 % (SBNO) to 37.79 % (CPBNO), while linoleic acid varied from 33.89 % (UTBNO) to 35.30 % (SBNO), both totaling approximately 72 % of the fatty acid composition in the BNO samples. Regarding SFA, the presence of palmitic acid (C16:0) and stearic acid (C18:0) were identified, which varied between 13.97 % (CPBNO) to 15.68 % (UTBNO) and 12.72 % (SBNO) to 13.68 % (CPBNO), respectively.

The fatty acid profiles of the TAO samples are presented in TABLE 2.7, and, similar to the BNO results, the same fatty acids were identified. Notably, TAO exhibits a high content of oleic acid (C18:1 cis-9) ranging from 67.64 % to 68.15 %, along with a lower concentration of palmitic acid (C16:0) (4.33 % to 7.53 %) compared to BNO. Moreover, a comparison of extraction methods revealed similar variations in the SFA and UFA composition of TAO, indicating that the UTAE method can extract vegetable oils with equivalent nutritional quality to both conventional and unconventional methods.

In comparison to the other works, it can highlight that the results revealed a good nutritional value for the oil samples, independent of the extraction technique. For instance, the characterization of fatty acids from BNO in different extraction processes (Soxhlet, pressed oil, and $CO_2$ supercritical) presented by Santos et al. (2012), the UTAE method presented higher values (TABLE 2.7) in relation to the concentration of palmitic acid (12.63 % to 14.94 %), stearic acid (10.23 % to 11.93 %), and oleic acid (29.76 % to 36.26 %). Besides, the characterization of TAO extracted using the supercritical fluid ($CO_2$) (15 MPa and 60 °C and 35 MPa and 40 °C) by Carvalho (2022), it was possible to extract a maximum of 5.85 % of oleic acid and 2.25 % of linoleic acid. On the other hand,

in the present work, no other fatty acids were identified, such as myristic acid and linolenic acid.

TABLE 2.7. EXTRACTION YIELDS AND FATTY ACID COMPOSITION OF BNO AND TAO EXTRACTED BY DIFFERENT METHODS.

| | BNO (%) | | | TAO (%) | | |
|---|---|---|---|---|---|---|
| | SBNO | UTBNO | CPBNO | STAO | UTTAO | CPTAO |
| Extraction Yields | 54.9 | 64.6 | 45.0 | 61.6 | 61.1 | 57.3 |
| Palmitic (C16:0) | 14.64 | 15.68 | 13.97 | 7.53 | 5.96 | 4.33 |
| Stearic (C18:0) | 12.72 | 12.85 | 13.68 | 16.41 | 15.34 | 17.81 |
| Oleic (C18:1 cis-9) | 37.79 | 37.56 | 37.04 | 67.64 | 68.15 | 66.13 |
| Linoleic (C18:2) | 34.83 | 33.89 | 35.30 | 8.41 | 10.53 | 11.71 |
| SFA[a] | 27.36 | 28.53 | 27.65 | 23.94 | 21.30 | 22.14 |
| UFA[b] | 72.62 | 71.45 | 72.34 | 76.05 | 78.68 | 77.84 |

Brazil nut oil (BNO); Tucumã-do-Amazonas oil (TAO); BNO extracted by Soxhlet (SBNO); TAO extracted by Soxhlet (STAO); BNO extracted by Ultra turrax-assisted extraction (UTBNO); TAO extracted by Ultra turrax-assisted extraction (UTTAO); BNO extracted by Compressed propane (CPBNO); and TAO extracted by Compressed propane (CPTAO).
[a]Satured fatty acids.
[b]Unsatured fatty acids.

Thus, the difference between the UTAE method and the supercritical fluids, UTAE, enzymatic, and microwave-assisted extraction methods is that it can obtain excellent UFA results in a reduced time without the need to change some process conditions, such as temperature, pH, and pressure. Hence, the findings consistently underscored a high nutritional value in the UTAE samples. This nutritional richness is attributed to the presence of oleic acid and linoleic acid, both of which possess potential benefits in mitigating inflammatory processes as well as reducing the risk of cardiovascular and cerebrovascular diseases (Liu et al., 2023b).

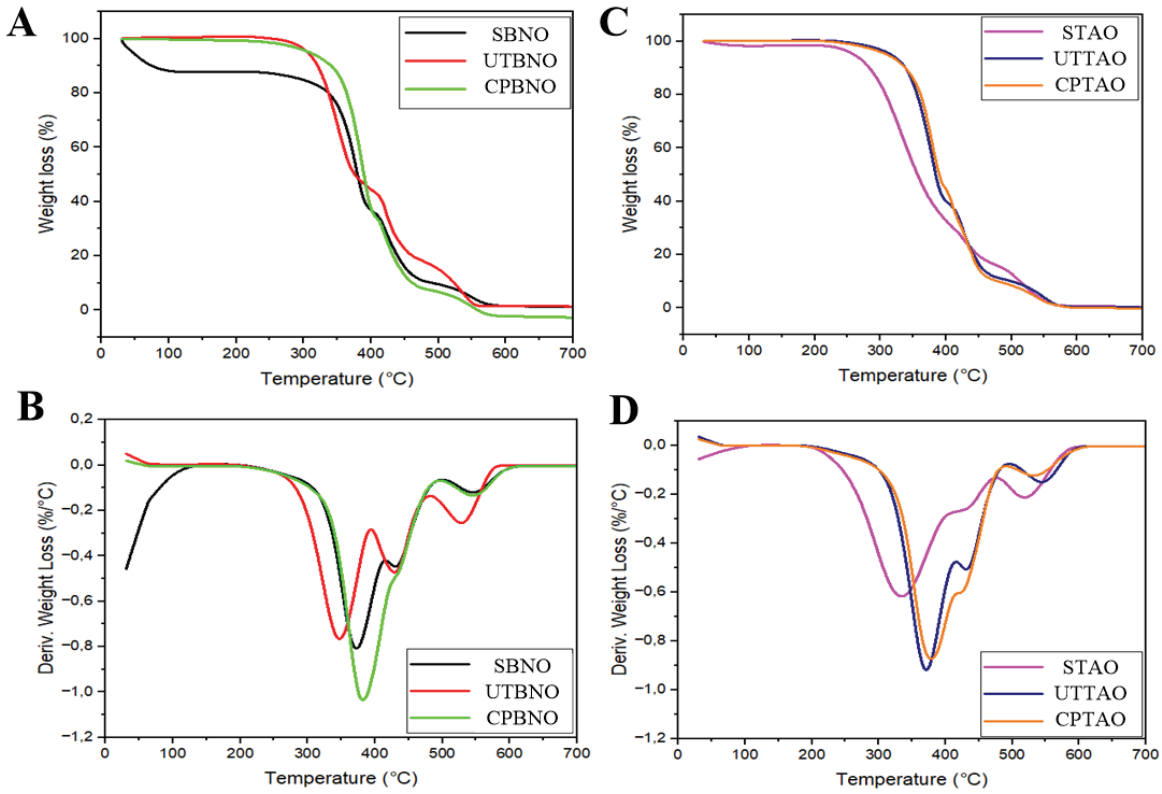
### 2.4.5. Thermal behavior

#### 2.4.5.1. Thermogravimetric analysis (TGA)

The thermogravimetric analysis (TGA/DTG) presented in FIGURE 2.4 describes the thermal decomposition patterns of extracts from the Soxhlet (SBNO and STAO), UTAE (UTBNO and UTTAO) and CPE (CPBNO and CPTAO) extraction processes. For BNO samples (FIGURE 2.4 A-B), weight loss was observed in the 100 °C range of the SBNO curve, which may be linked to the

presence of hexane, even after drying process. Moreover, thermal degradation occurs in three distinct phases (Santos et al., 2002): initially from 246.58 to 386.11 °C, followed by a second stage from 386.11 to 415.01 °C, and a final phase of 415.01 to 569.93 °C. Similarly, the TAO samples (FIGURE 2.4 CD) exhibit a comparable pattern of thermal degradation in three stages: the first ranging from 208.64 to 404.17 °C, the second from 404.17 to 480.23 °C, and the third from 480.23 to 595.14 °C.

FIGURE 2.4 – TGA/DTG CURVES OF HEATING: (A) TGA-BNO; (B) DTG-BNO; (C) TGA-TAO; AND (D) DTG-TAO, AT HEATING RATE OF 10 °C/MIN IN AIR FLOW 50 ML/MIN. OILS EXTRACTED BY SOXHLET (SBNO AND STAO), ULTRA TURRAX-ASSISTED (UTBNO AND UTTAO) AND COMPRESSED PROPANE (CPBNO AND CPTAO) PROCESS.




Santos et al. (2012) reported that the first two stages are associated with the decomposition of various UFAs, while the third stage pertains to the degradation of SFAs. Notably, at the end of the second stage for BNO samples (FIGURE 2.4A-B), the CPE method exhibited a more pronounced degradation peak, likely due to the higher linoleic acid content (35.30 %) compared to UTBNO (33.89 %) and SBNO (34.83 %) (TABLE 2.7). Similarly, for TAO samples (FIGURE 2.4C-D), the higher concentrations of linoleic acid in UTTAO (10.53 %) and CPTAO (11.71 %) relative to STAO (8.41 %) contributed to more intense

degradation peaks at the end of the second stage. In the third stage, the elevated palmitic acid levels in UTBNO (15.68 %) and STAO (7.53 %) also resulted in more pronounced degradation peaks (TABLE 2.7).

2.4.5.2. Differential scanning calorimetry (DSC)

Cooling and heating curves of BN and TA oil using Soxhlet, UTAE, and CPE were determined by differential scanning calorimetry method (FIGURE 2.5). The temperatures for cooling and heating and enthalpy are also shown in TABLE 2.8. As can be seen from FIGURE 2.5A, the nucleation phenomenon for these samples showed similar behavior with a slightly major intensity in the Soxhlet sample oil (SBNO and STAO). Moreover, the data presented in TABLE 2.8 shows that cooling starts last in the UTAE samples. Specifically, on the BNO curve (FIGURE 2.5A), two peaks between -3 to -70 °C can be observed, indicating a crystallization of these samples. This first peak can be attributed to an oil fraction that contains mainly SFAs such as palmitic and stearic acid (Fetzer et al., 2021; Teixeira et al., 2018).

Melting curves from Soxhlet, UTAE, and CPE (FIGURE 2.5 B) show the melting area that starts at around -31 °C and complete near 13 °C. This peak endothermic can be explained by the diverse TAGs and their different melting ranges present in the different oils (Thilakarathna et al., 2023). Among the BNO melting curves, the onset temperature ($TC_{onset}$) data (-30.52 °C) and the enthalpies of peaks 1 (120.47 $J.g^{-1}$) and peak 2 (215,25 $J.g^{-1}$) of Soxhlet extraction (SBNO) distinct behaviors in relation to UTBNO (-27.45 °C; peak 1, 251.07 $J.g^{-1}$; peak 2, 51.25 $J.g^{-1}$) and CPBNO (-27.23 °C; peak 1, 244.07 $J.g^{-1}$; peak 2, 43.27 $J.g^{-1}$), or may be caused by the presence of waxes extracted by the method (TABLE 2.8). These variation in enthalpy and onset temperatures across different extraction methods and samples can also be explained by differences in their UFA and SFA compositions, as suggested by Hu et al. (2020). This variation becomes more evident when observing the different intensities of the peaks in FIGURE 2.5B with the data in TABLE 2.7, where a higher concentration of oleic fatty acids increases the intensity of the peak in the -10 to 0 °C region and an increase in concentration of palmitic and linoleic acids generates more notable peaks in the region between -30 to -10 °C.

FIGURE 2.5 – DSC CURVES SHOWING (A) CRYSTALLIZATION (50 TO −80 °C) AND (B) MELTING (−80 TO 40 °C) BEHAVIORS OF BNO AND TAO OBTAINED USING SOXHLET (SBNO-STAO), UTAE (UTBNO-UTTAO), AND CPE (CPBNO-CPTAO).

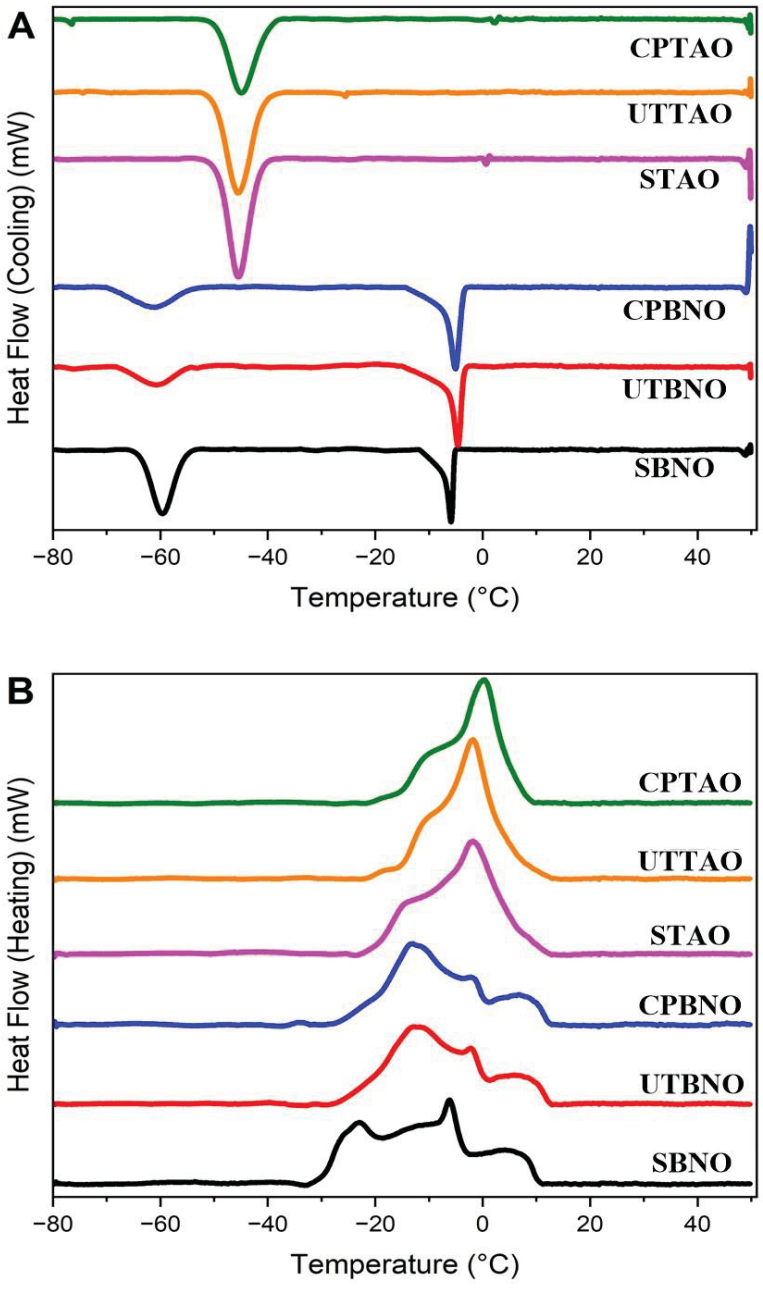


TABLE 2.8 – DSC PARAMETERS FOR COOLING (50 °C TO -80 °C) AND HEATING (-80 TO 40 °C) BEHAVIOR OF BNO AND TAO OILS OBTAINED BY SOXHLET, ULTRA TURRAX-ASSISTED EXTRACTION (UTAE), AND COMPRESSED PROPANE EXTRACTION (CPE).

| Sample | *Peak 1* | | | | *Peak 2* | | | | Peak 3 | | | | $H_{PM}$ | $H_{Total}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $TC_{onset}$ °C | $PC_M$ °C | $TC_{endset}$ °C | $P_{width}$ °C | $TC_{onset}$ °C | $PC_M$ °C | $TC_{endset}$ °C | $P_{width}$ °C | $TC_{onset}$ °C | $PC_M$ °C | $TC_{endset}$ °C | $P_{width}$ °C | $J.g^{-1}$ | $J.g^{-1}$ |
| **Cooling** | | | | | | | | | | | | | | |
| *SBNO* | -65.43 | -59.54 | -53.96 | 4.65 | -10.92 | -5.89 | -5.02 | 1.28 | -- | -- | -- | -- | -78.58 | -113.84 |
| *UTBNO* | -67.60 | -60.66 | -54.89 | 7.30 | -13.62 | -4.60 | -3.35 | 1.72 | -- | -- | -- | -- | -57.85 | -89.52 |
| *CPBNO* | -71.25 | -60.99 | -53.82 | 8.84 | -10.96 | -5.06 | -3.56 | 2.07 | -- | -- | -- | -- | -61.63 | -104.63 |
| *STAO* | -51.54 | -45.45 | -39.74 | 4.32 | -- | -- | -- | -- | -- | -- | -- | -- | -137.80 | -137.80 |
| *UTTAO* | -51.03 | -45.63 | -40.15 | 5.16 | -- | -- | -- | -- | -- | -- | -- | -- | -135.98 | -135.98 |
| *CPTAO* | -50.92 | -44.97 | -38.94 | 5.44 | -- | -- | -- | -- | -- | -- | -- | -- | -105.26 | -105.26 |
| **Heating** | | | | | | | | | | | | | | |
| *SBNO* | -30.52 | -22.98 | -19.71 | 9.164 | -17.98 | -6.16 | -3.14 | 14.54 | -0.63 | 4.13 | 10.52 | 10.92 | 215.25 | 416.04 |
| *UTBNO* | -27.45 | -12.975 | -4.89 | 14.72 | -3.43 | -2.22 | 0.36 | 4.05 | 1.73 | 5.80 | 12.59 | 9.65 | 251.07 | 363.70 |
| *CPBNO* | -27.23 | -12.99 | -4.9 | 14.14 | -3.16 | -2.23 | 0.62 | 4.29 | 1.54 | 6.73 | 12.46 | 9.74 | 244.06 | 348.08 |
| *STAO* | -21.47 | -1.79 | 11.95 | 14.93 | -- | -- | -- | -- | -- | -- | -- | -- | 400.09 | 400.09 |
| *UTTAO* | -19.54 | -1.85 | 8.82 | 9.90 | -- | -- | -- | -- | -- | -- | -- | -- | 378.12 | 378.12 |
| *CPTAO* | -20.03 | 0.31 | 8.51 | 8.80 | -- | -- | -- | -- | -- | -- | -- | -- | 325.69 | 325.69 |

Brazil nut oil (BNO); Tucumã-do-Amazonas oil (TAO); BNO extracted by Soxhlet (SBNO); TAO extracted by Soxhlet (STAO); BNO extracted by Ultra turrax-assisted extraction (UTBNO); TAO extracted by Ultra turrax-assisted extraction (UTTAO); BNO extracted by Compressed propane (CPBNO); and TAO extracted by Compressed propane (CPTAO).

$TC_{onset}$, onset temperature; $PC_M$, temperature for the main peak; $TC_{endset}$, endset temperature; $P_{width}$width of the peak; $H_{PM}$enthalpy of the main peak; $H_{Total}$total enthalpy.

2.4.6. Bioactive compounds

Phenolic compounds, common secondary metabolites in plants, exhibit a broad spectrum of biological activities. Additionally, TPC in plant oils can effectively inhibit lipid peroxidation and enhance crude oil stability. For the TPC results (TABLE 2.9), higher concentrations were observed in samples extracted by CPE (7.87 ± 0.77 and 26.71 ± 0.63 mg GAE.100 $g^{-1}$ for CPBNO and CPTAO, respectively). However, UTAE showed higher TPC concentrations (4.57 ± 1.00 and 19.63 ± 1.68 mg GAE.100 $g^{-1}$ for UTBNO and UTTAO, respectively) compared to the data from Soxhlet (1.25 ± 0.37 and 10.97 ± 1.92 mg GAE.100 $g^{-1}$ for SBNO and STAO, respectively).

To the TFC results, the values of UTBNO (209.85 ± 7.27 mg CE.100 $g^{-1}$) and UTTAO (698.17 ± 10.37 mg CE.100 $g^{-1}$) were lower than those obtained by Soxhlet (242.76 ± 6.40 and 859.61 ± 36.11 mg CE.100 $g^{-1}$ to SBNO and STAO, respectively) and CPE (224.28 ± 6.39 and 847.33 ± 27.73 mg CE.100 $g^{-1}$ to CPBNO and CPTAO, respectively), suggesting a greater exposure of these compounds to oxidative species during the UTAE process. On the other hand, regardless of the extraction method, both BNO and TAO presented TFC concentrations similar to or higher than those reported in the literature. For instance, Guex et al. (2022) identified a concentration of 2.76 ± 0.10 mg/g rutin equivalent (RE) in Tucumã pulp extracts obtained with ethanol and Cardoso et al. (2017) and Yang (2009) presented a total of 54.3 to 107.8 ± 6.0 mg/100 g of TFC in BNs.

TABLE 2.9 – COMPARISON OF TOTAL POWER CONSUMPTION, YIELD, BIOACTIVE COMPOUNDS, AND ANTIOXIDANT ACTIVITY CONTENT BETWEEN EXTRACTION PROCESSES.

| Approach | TPC (mg GAE.100 $g^{-1}$) | | TFC (mg CE.100 $g^{-1}$) | | DPPH (µmol TEAC.100 $g^{-1}$) | | ABTS (µmol TEAC.100 $g^{-1}$) | |
|---|---|---|---|---|---|---|---|---|
| | BNO | TAO | BNO | TAO | BNO | TAO | BNO | TAO |
| Soxhlet | 1.25±0.37 | 10.97±1.92 | 242.76±6.40 | 859.61±36.11 | 683.94±0.15 | 1920.03±0.16 | 504.68±1.01 | 1328.25±13.55 |
| UTAE | 4.57±1.00 | 19.63±1.68 | 209.85±7.27 | 698.17±10.37 | 671.88±0.15 | 1913.39±1.14 | 416.83±3.93 | 1268.25±6.47 |
| CPE | 7.87±0.77 | 26.71±0.63 | 224.28±6.39 | 847.33±27.73 | 682.86±0.03 | 1927.70±1.21 | 470.63±1.33 | 1325.75±7.14 |
| Approach | TPC (mg GAE.100 $g^{-1}$) | | TFC (mg CE.100 $g^{-1}$) | | DPPH (µmol TEAC.100 $g^{-1}$) | | ABTS (µmol TEAC.100 $g^{-1}$) | |
| | BNO | TAO | BNO | TAO | BNO | TAO | BNO | TAO |
| Soxhlet | 1.25±0.37 | 10.97±1.92 | 242.76±6.40 | 859.61±36.11 | 683.94±0.15 | 1920.03±0.16 | 504.68±1.01 | 1328.25±13.55 |
| UTAE | 4.57±1.00 | 19.63±1.68 | 209.85±7.27 | 698.17±10.37 | 671.88±0.15 | 1913.39±1.14 | 416.83±3.93 | 1268.25±6.47 |
| CPE | 7.87±0.77 | 26.71±0.63 | 224.28±6.39 | 847.33±27.73 | 682.86±0.03 | 1927.70±1.21 | 470.63±1.33 | 1325.75±7.14 |

*Based on average extraction time for BNO and TAO.
Mean ± confidence interval at a 95% level of significance of Total Phenolics Content (expressed as mg of Gallic Acid Equivalents per 100 g of sample), Total Flavonoids (expressed as mg of catechin equivalent (CE) per 100 g of sample), Antioxidant Activity by DPPH (expressed as µmol of Trolox Equivalents Antioxidant Capacity per 100 g of sample), and Antioxidant Activity by ABTS (expressed as µmol of Trolox Equivalents Antioxidant Capacity per 100 g of sample).

2.4.7. Antioxidant activity

For the DPPH results (TABLE 2.9), similar BNO and TAO results were observed for the antioxidant activities of Soxhlet (683.94 ± 0.15 and 1920.03 ± 0.16 μmol TEAC.100 $g^{-1}$), UTAE (671.88 ± 0.15 and 1913.39 ± 1.14 μmol TEAC.100 $g^{-1}$), and CPE (682.86 ± 0.03 and 1927.70 ± 1.21 μmol TEAC.100 $g^{-1}$), respectively. For the ABTS results (μmol TEAC.100 $g^{-1}$), a similar trend was observed, with higher antioxidant activity in SBNO (504.68 ± 1.01) and STAO (1328.25 ± 13.55) compared to UTBNO (416.83 ± 3.93), UTTAO (1268.25 ± 6.47), CPBNO (470.63 ± 1.33), and CPTAO (1325.75 ± 7.14). On the other hand, the ABTS results in this work still exhibit values that are higher or comparable to those reported by Gomes & Torres (2016) (2.97 ± 0.04 to 4.07 ± 0.28 μmol TEAC.$g^{-1}$ of BN cake) and Santos; Rodrigues; & Silva (2022) (27.36 ± 1.15 to 193.93 ± 2.52 μM Trolox.$g^{-1}$ of Tucumã oil).

Considering the concentrations of bioactive compounds and their correlation with antioxidant activity, it can be inferred that the UTAE method generated a slight oxidation of bioactive compounds, mainly flavonoids. This may justify a slight reduction in the antioxidant activity of UTBNO and UTTAO oils in relation to SBNO and STAO. For example, DPPH obtained for UTBNO and UTTAO were 1.76 % and 0.35 % lower than those obtained for Soxhlet, respectively.

Therefore, although UTAE has proven to be efficient in improving oil yield (increased mass transfer) and its UFA profile in a short extraction period, the use of UTAE resulted in a slight increase in the oxidation of bioactive compounds, probably due to the greater air solubilization caused by the high rotation of the process. This effect may also have been intensified by the optimized extraction yield chosen for the BN and TA oils, suggesting that longer UTAE times increase oxidation. In addition, the viscosity of hexane is a relevant factor, since less viscous solvents allow greater incorporation of air into the solution. Thus, the choice of a nonpolar solvent with higher viscosity could maintain similar extraction yields, avoiding the degradation of the bioactive compounds present in the oils.

## 2.5. Conclusion

The current work has explored UTAE method as an innovative approach to enhance oil extraction from Amazon sources, particularly BN and TA, through

process optimization and comparison with conventional Soxhlet and CPE methods. Firstly, ANOVA applied to test the availability of PBD has revealed that the solvent/solid ratio and extraction time were the significant process parameters of yield extraction. Under optimized conditions (BNO 26 mL.g-1 for 27 min; and TAO 17.5 mL.g-1 for 25 min), resulted in higher UTAE extraction yields (64.89 % and 61.13 %) compared to Soxhlet (54.85 % and 61.56 %) and CPE (45.04 % and 57.30 %) methods for BNO and TAO, respectively, indicating its effectiveness in extracting oils from these raw materials.

The findings also revealed that oils extracted using UTAE methods preserved their fatty acid profiles and thermal stability properties comparable to those obtained by CPE. Additionally, applying UTAE to other oilseed matrices, incorporating environmentally friendly solvents, conducting an economic analysis (energy costs and scalability), and assessing tribological impacts will expand the scope of this study. Thus, further research and implementation of this approach could contribute to the promotion of the bioeconomy and support the livelihoods of traditional while preserving its rich biodiversity and mitigating the environmental impacts of conventional extraction methods.

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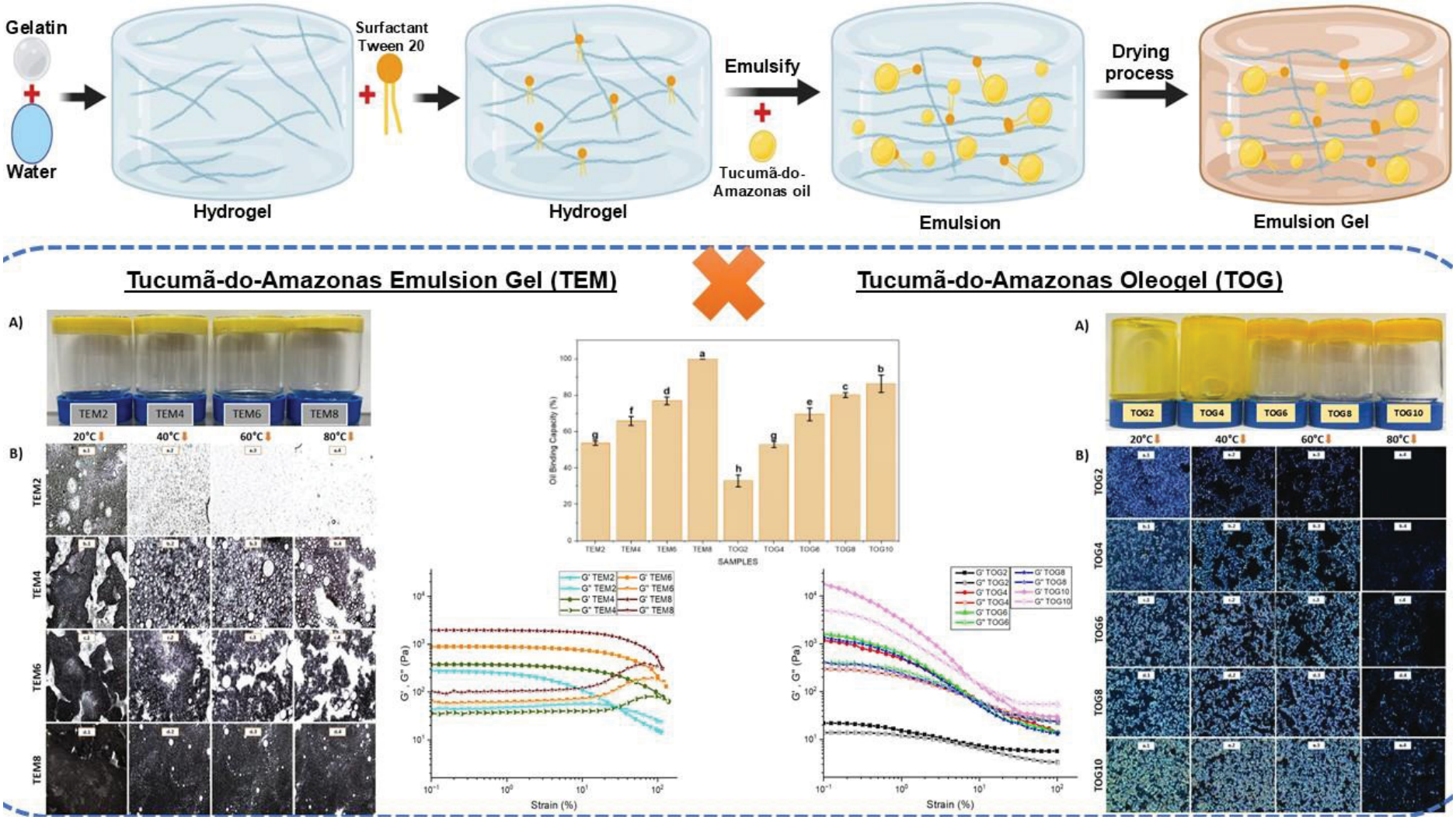
# CHAPTER III

## Development and characterization of Amazon Tucumã oil-based oleogels and emulgels with carnauba wax and gelatin

This chapter consists of the manuscript entitled "Development and characterization of Amazon Tucumã oil-based oleogels and emulgels with carnauba wax and gelatin: structure, color, thermal stability and rheological properties", which it has **UNSUBMITTED status**. This article consists of the use of TAO obtained under optimized conditions (identified in Chapter II) for the production of OG and EG. For this purpose, carnauba wax and gelatin were the structuring agents used in various concentrations to produce the OGs and EGs, respectively. In addition, these obtained materials were characterized using morphology, color, OBC, thermal stability, and rheological methods.

## Graphical Abstract



## Development and characterization of Amazon Tucumã oil-based oleogels and emulgels with carnauba wax and gelatin

### 3.1. Abstract

This study aimed to develop and characterize oleogels and emulgels using TAO, rich in UFA, carotenoids, and tocopherols. carnauba wax and gelatin were used as gelling agent in the OGs and EGs, respectively. The effects of different gelling agent concentrations and structuring methods on OBC, color, morphology, thermal behavior, and rheological properties were evaluated. The results showed that higher concentrations of carnauba wax increased OBC and promoted the formation of visibly denser crystalline networks in the OGs. The EGs presented greater oil retention capacity (~100 % TEM8), even at lower concentrations, and superior viscoelastic properties, indicating a more cohesive and stable protein network. Both the method and the concentration of the gelling agents influenced the color and microstructure: systems with wax presented an opaquer appearance, while those with gelatin resulted in more translucent systems due to the protein network. The results indicated that these systems have promising potential for the inclusion of Amazon functional oils in food products, as they can help increase stability, nutritional value, and the sustainable use of local resources.

Keywords: Oleogelation; Amazon oil; *Astrocaryum aculeatum*; Fat replacers; and Structured lipids.

### 3.2. Introduction

In recent years, the consumption of TFA and SFA is strongly associated with several disorders (increased risk cardiovascular diseases, systemic inflammation and elevated low-density lipoprotein cholesterol levels in blood) (Frías et al., 2023; Hirata et al., 2017, 2023; Hwang, 2020; Liu et al., 2024). In response, international organizations such as WHO have been launched global campaigns and guidance to reduce the intake of these compounds and promote healthy fat diets (such as UFAs and PUFAs), with the potential to save an estimated 183,000 lives annually (WHO, 2023, 2024). Similarly, regulatory agencies such as the U.S. FDA and the European Food Safety Authority have classified industrial TFAs as not GRAS and established limits for their intake,

respectively (EFSA, 2018; FDA, 2018). In addition to governmental pressure, there is growing consumer awareness regarding healthier dietary habits, prompting the food industry to seek alternatives to raw materials with high concentrations of SFAs and TFAs (Miró-Colmenárez et al., 2024; Zhang et al., 2025). This scenario presents additional challenges, as conventional vegetable fats exhibit unique sensory and textural properties (spreadability, creaminess, stability, and desirable mouthfeel) that are difficult to replicate in food formulations (Thakur et al., 2022). Moreover, market uncertainties – including price volatility, diplomatic conflicts, and environmental concerns (FAO, 2025; Sarpong, 2024) – have further intensified the instability surrounding the supply of essential raw materials, such as cocoa butter and palm oil, compelling also the food manufacturers to pursue innovative and sustainable fat-replacement strategies.

New strategies have led to the exploration of healthier oils and advanced fat mimetics that meet industrial and health demands without compromising attributes of food products (Aliasl khiabani et al., 2020; Ferdaus et al., 2024; Ghorghi et al., 2023; Jing et al., 2022). Among these alternatives, OGs have emerged as promising solutions (Mahmud; Ferdaus; Silva, 2024; Silva et al., 2023). OGs are structured lipid-based systems which entrap a high proportion of liquid oil (generally greater than 90 %) within a network formed by low concentrations of gelling agent. Furthermore, the OG process does not change the fatty acid composition (rich in UFAs and PUFAs) and keep the low TFA and SFA concentrations (Huang et al., 2023). Although the OGs have been applied at laboratory scale to different food products showing promise in replacing solid and semi-solid fats (Almeida et al., 2024; Tarté et al., 2020; Wolfer et al., 2018), there are still challenges such as physical properties, the specific affinity with lipophilic compounds, and healthy concerns – elevated caloric content (9 kcal/g), food-grade gelling agents that comply with regulatory standards, and high lipid digestion (Sabet et al., 2024). For that reason, researchers have developed advanced healthy structures to avoid these drawbacks using amphiphilic systems (Lin; Kelly; Miao, 2020; Xie et al., 2023; Zampouni et al., 2024).

A new innovative approach is called EGs, which represent a promise versatile class of gels formed by incorporating gelling agents into emulsions (such as O/W, W/O, etc.), leading to three-dimensional networks with potential improved physical stability and ability to incorporate both hydrophobic and

hydrophilic compounds. In this sense, proteins and polysaccharides have been applied as emulsions to produce food products considering also their sustainability, degradability, biocompatibility and low cost (Wang et al., 2025; Yan et al., 2024). Zare et al. (2024) reported the alginate/whey protein isolate-based emulgel as efficient alternative replacement for margarine in processed cheese, presenting higher physical properties and greater thermal stability. Therefore, it's speculated that EGs may have better structural and physical properties than OGs.

Besides, the exploration of underutilized vegetable oils, particularly those from biodiversity-rich regions such as the Amazon, holds untapped potential for innovation in fat-structuring systems. TA is an oil-rich Amazon fruit with a high proportion of UFAs, bioactive compounds, and favorable oxidative stability (Carvalho et al., 2024; Machado et al., 2022). However, its industrial application is limited due to its propensity for oxidation and thermal degradation (Ferreira et al., 2021; Santos et al., 2023). In this scenario, transforming oil into structured systems, OGs and EGs emerge as a promising alternative to preserve its bioactive compounds, increase stability and enable its application in functional and higher value-added food formulations.

The objective of this study was to develop OGs and EGs using TAO as a liquid base, and carnauba wax and gelatin as gelling agents, respectively. The impacts of various concentrations of the gelling agents and the method used on the structure, color, oil retention capacity, heat resistance, and rheological behavior of the developed systems were specifically analyzed. These findings offer industrial relevance, promote healthier formulations, and contribute to regional bioeconomy initiatives.

## 3.3. Methodology

### 3.3.1. Materials

Carnauba wax (melting point of 81–86 °C) was purchased from Fisher Scientific (Hampton, NH, USA), unflavored and colorless food grade gelatin from MP Biomedicals (Santa Ana, CA, USA), Tween® 20 from Sigma-Aldrich (St. Louis, MO, USA). Additionally, deionized water was used in all experiments.

### 3.3.2. Methods

#### 3.3.2.1. Extraction of Tucumã-do-Amazonas oils (TAO)

The TAO was extracted as described by our research group using n-hexane (Neon, 99.8 % purity) as solvent (Santos et al., 2025). Thus, UTAE was conducted using a T25 digital Ultra Turrax (IKA-Werke, Staufen, Germany) under the following process conditions: a time of 17.5 min and a solvent-to-solid ratio of 25 mL/g. The oils were stored in amber glass and kept at −5 °C until the development of TAO OGs (TOG) and TAO EGs (TEM). The TAO fatty acid composition was analyzed in our previous research, finding it comprises 21.30 % of SFA, primarily palmitic acid (C16:0) at 5.96 %, stearic acid (C18:0) at 15.34 % and 78.68 % of UFA, including oleic acid (C18:1) at 68.15 % and linoleic acid (C18:2) at 10.53 % (Santos et al., 2025).

#### 3.3.2.2. Preparation of Tucumã-do-Amazonas oil oleogel (TOG)

The TOGs were prepared according to the direct method based on the methodologies of Airoldi et al. (2022) and Moghtadaei et al. (2018), with adaptations. Thus, 15 g of the oil was heated to 85 °C under magnetic stirring (350 rpm). Once the oil temperature was reached, carnauba wax, as the gelling agent, was slowly added (2, 4, 6, 8, and 10 % w/w) and mixed until dissolution (5 min). After the complete incorporation of carnauba wax, the samples (in liquid form) were transferred to the test tubes. Then, the samples were kept in a static condition at room temperature (20 ± 4 °C) for 1 h to structure and stabilize. Finally, the samples were stored at 4 °C until characterization. The samples were coded as TOG2, TOG4, TOG6, TOG8, and TOG10, regarding the carnauba wax concentration.

#### 3.3.2.3. Preparation of Tucumã-do-Amazonas oil emulsion gel (TEM)

The EGs were prepared according to Wang et al. (2025), with modifications. First, TEMs were prepared by dissolving 1 % Tween 20 (a *sorbitan monolaurate)* in ultrapure water (v/v) at 25 °C. In the second step, gelatin was added in the following proportions: 2 %, 4 %, 6 %, and 8 % (m/v), and the mixture was left to wait for 30 minutes to complete the hydration. After hydration, TAO was added to the gelatin solution at a 1:1 (v/v) ratio and homogenized using a high-speed disperser (digital Ultra-Turrax, T25, IKA, Germany) at 10,000 rpm for

2 min. Then, the samples were dehydrated at 50 °C for 24 hours or until they reached approximately 35 % moisture content. After drying, the samples were homogenized again using the Ultra-Turrax under the same conditions described previously (10,000 rpm for 2 min). After complete dispersion, they were transferred to test tubes and maintained in a static condition at room temperature (20 ± 4 °C) for 1 h to facilitate structuring and stabilization. Finally, the samples were stored at 4 °C and then characterized. The samples were coded as TEM2, TEM4, TEM6, and TEM8, regarding the gelatin proportion.

### 3.3.3. Characterization

#### 3.3.3.1. Macroscopic appearance analysis

The macroscopic appearance analysis was developed according to Wang et al. (2024), with modifications. Thus, 5 g of the sample was poured into a test tube, cooled to 25 °C, and then stored in a refrigerator at 4 °C for 24 hours. After 24 h, the samples were then placed at 25 °C for 2 h, and the test tubes were inverted to observe the fluidity of the samples and determine whether they had gelified or not.

#### 3.3.3.2. Polarized light microscopy (PLM) observation

TOGs and TEMs microstructures were analyzed as mentioned by Silva et al. (2016), with adaptations. The samples were analyzed using a 10 × magnification. Images were recorded isothermally at 20 ºC and during heating at 40 °C, 60 ºC, and 80 °C at 5 °C/min using a Linkam thermal system (Linkam Scientific Instruments Ltd., Redhill, UK) attached to the microscope stage (Valoppi et al., 2023). Images of each sample were observed using Image Pro-Plus software version 7.0 for Windows.

#### 3.3.3.3. Color measurement

Color measurement was conducted according to Oyom et al. (2024) and the whiteness index (WI) values were calculated as mentioned by Li et al. (2023). These parameters were measured at three random surface areas of each sample.

#### 3.3.3.4. Oil binding capacity (OBC)

The OBC of the samples was determined by accelerated stability tests using the method mentioned by Ferdaus et al., (2022).

3.3.3.5. Thermal behavior

The thermal behavior of TOGs and TEMs was analyzed using a differential scanning calorimeter (DSC; TA Instruments, New Castle, DE, USA). Approximately 8–12 mg of each sample was accurately weighed and hermetically sealed in an aluminum pan, with a sealed empty pan as the reference. Before analysis, the samples were equilibrated at 20 °C for 1 minute to ensure stabilization within the DSC chamber. The samples were then cooled to −10 °C to facilitate complete crystallization. After holding at −10 °C for 10 minutes, the samples were subjected to a heating cycle, melting from −10 °C to 90 °C (TOGs) and from −10 °C to 200 °C (TEMs) at a controlled rate of 5 °C/min under a continuous nitrogen gas flow (50 mL/min). Key thermal parameters, including onset temperature (°C), peak height (W/g), peak temperature (°C), and melting enthalpy (ΔH, J/g), were determined from the melting curves. All measurements were performed in triplicate.

3.3.3.6. Rheological measurements

To evaluate the viscoelastic properties of the samples (24 h after production), dynamic oscillatory tests (strain sweep) were determined using a rheometer (Discovery HR-10, TA Instruments, New Castle, DE, USA). The upper fixture was a parallel plate (25 mm), and about 4 g of sample was put into the rheometer and trimmed to remove the excess dough outside of the fixture when the gap was 2050 μm. The strain sweep test was performed in the strain range 0.1–100 % (at a constant frequency of 1 Hz and a temperature of 25 °C) to determine the following parameters: 1. LVR; 2. Viscoelastic behavior in the LVR range; 3. Crosspoint of G' and G" (flow point, G ' = G").

3.3.3.7. Statistical analysis and visualization

ANOVA and Tukey's test were applied at a significance level of $p < 0.05$ using Statistica 7.0 software (Statsoft Inc., Tulsa, OK, USA). Graphical analysis was performed using OriginPro® 2025 (OriginLab Corp., Northampton, MA, USA).All determinations were expressed as mean ± confidence interval at a 95

% significance level of at least two measurements from three experimental replicates (n ≥ 2 × 2) if not otherwise specified.

## 3.4. Results and discussion

### 3.4.1. Macroscopic appearance analysis

FIGURE 3.1A illustrates the visual analysis of OGs (TOG) formulated with different concentrations of carnauba wax incorporated into TAO. After inversion of the system, a fluid-to-gel/semi-solid transition is observed starting at 6 % (w/w) carnauba wax, indicating the formation of a rigid crystalline network capable of preventing oil exudation, characterizing OG formation. This phase transition reflects the reduction in fluidity due to the more developed crystalline structure. With increasing carnauba wax concentration, the OGs become opaquer and firmer, suggesting a denser and more structured three-dimensional network (Qu et al., 2024). Furthermore, the oil composition influences the final structure, since TAO has a high content of UFAs (~70 %), as reported by (Santos et al., 2025). Previous studies demonstrate that gelling agent concentrations of 4–6 % are effective in structuring oils rich in UFAs, especially those with medium- and long-chain TAGs, such as oleic (C18:1) and linoleic (C18:2) acids (Buitimea-Cantúa et al., 2021; Wang et al., 2024).

FIGURE 3.2A shows that all gelatin concentrations enabled the formation of stable gels with a semi-solid consistency, even in the samples with the lowest content (TEM2), demonstrating the effectiveness of gelatin in structuring the oil phase. This behavior is due to the amphiphilic nature of gelatin, which allows its adsorption at the oil-water interface, stabilizing the dispersed droplets (Lee et al., 2025). As described by Lin; Roos; & Miao (2025), gelatin-based EGs are classified as “emulsion-filled gels,” where the continuous phase – composed of gelatin triple helices – establishes a structured network via physical self-assembly or covalent cross-linking. The incorporated oil droplets function as “active fillers,” increasing the mechanical strength and stability of the gel. As the gelatin concentration increases (e.g., in TEM6 and TEM8), the emulgels become visually denser and less translucent, reflecting homogeneous microstructures with smaller droplets and a more cohesive protein network, a typical result of protein-

stabilized emulsion systems (Dickinson, 2012; Vélez-Erazo et al., 2022; Xu et al., 2024).

FIGURE 3.1 – MACROSCOPIC APPEARANCE A) AND POLARIZED LIGHT MICROSCOPIC IMAGES (B) OF TUCUMÃ-DO-AMAZONAS OLEOGELS.

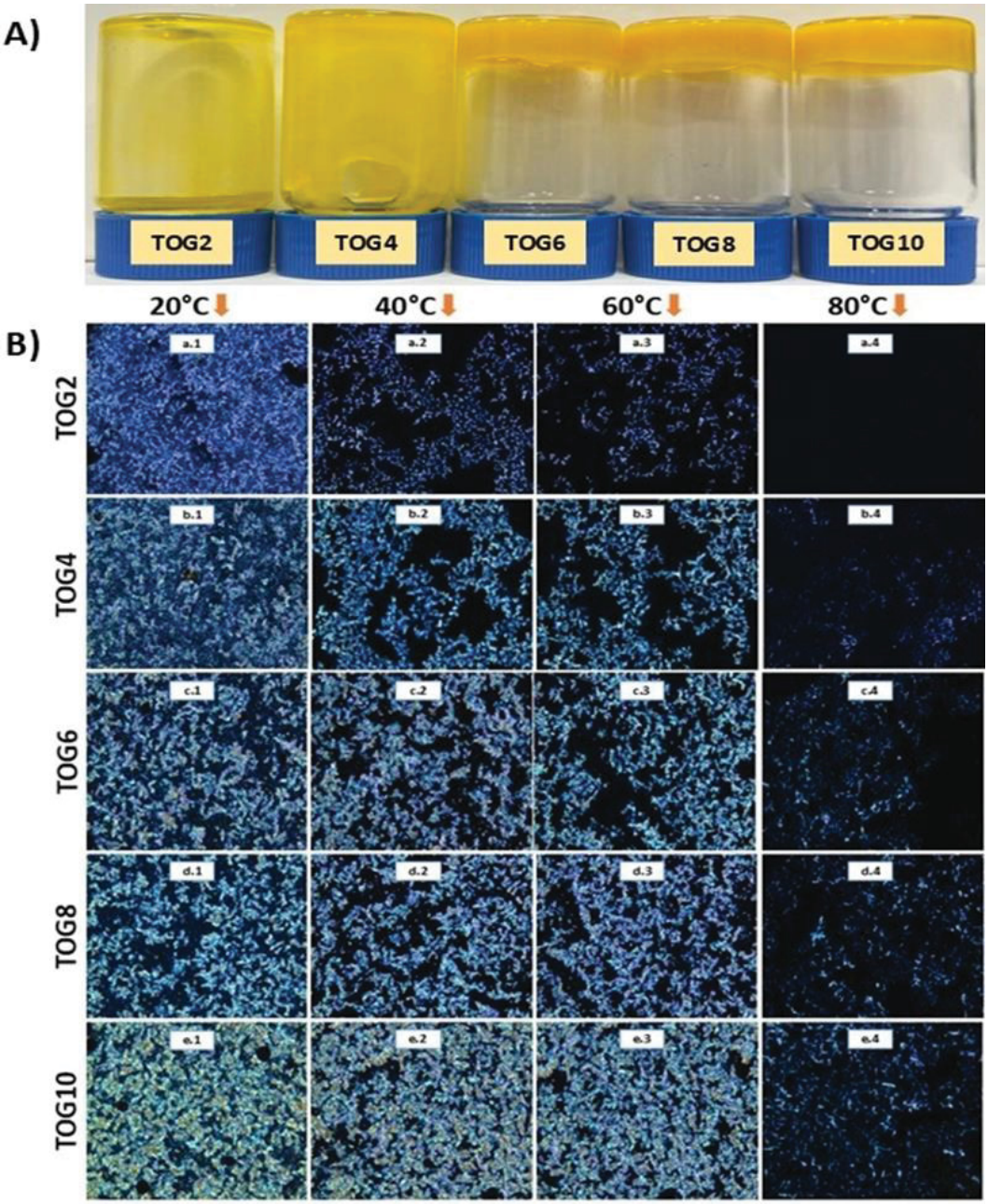
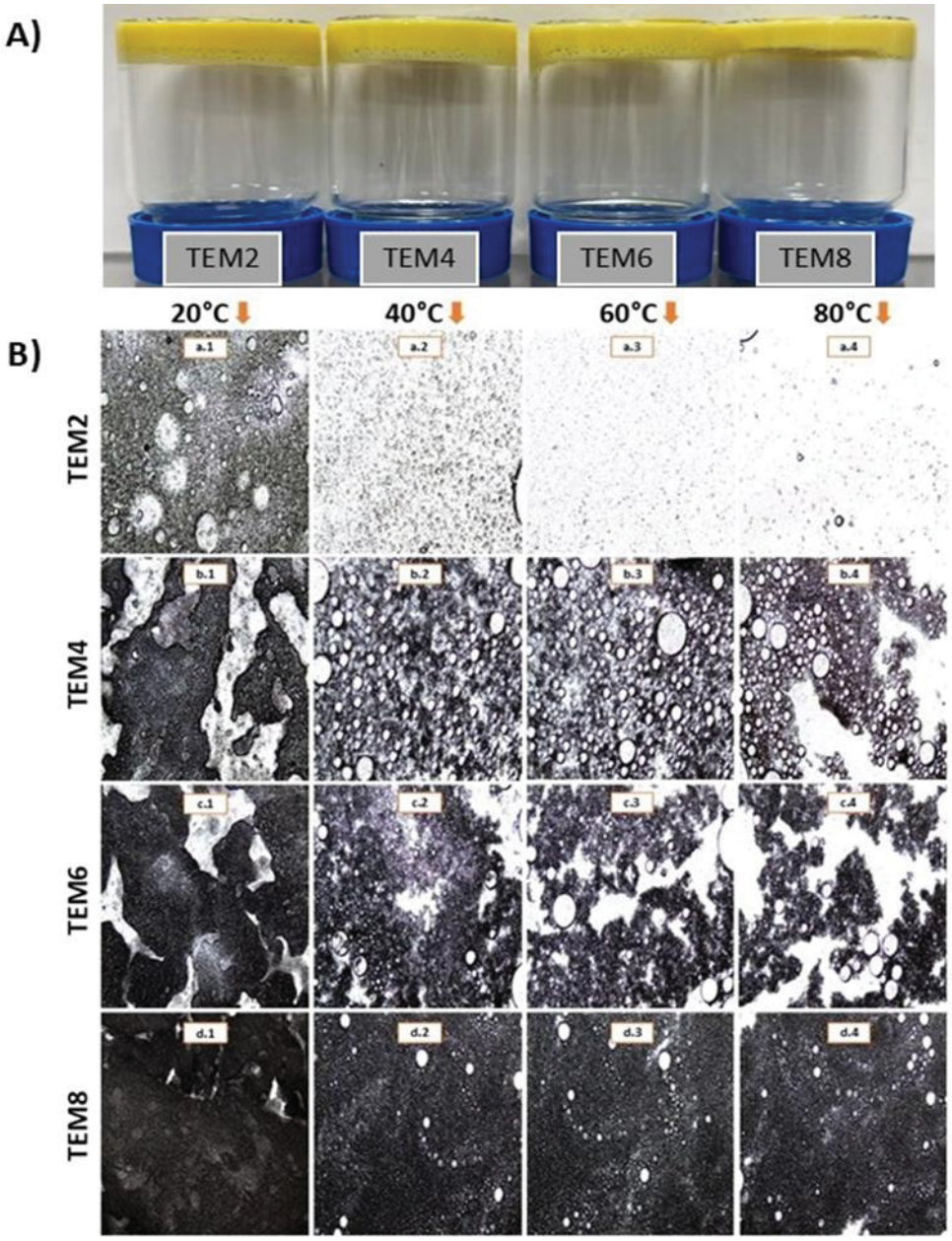


FIGURE 3.2 – MACROSCOPIC APPEARANCE A) AND POLARIZED LIGHT MICROSCOPIC IMAGES (B) OF TUCUMÃ-DO-AMAZONAS EMULGELS.




3.4.2. Polarized light microscopy observation (PLM)

Polarized micrographs of the OG samples, shown in FIGURE 3.1B, indicate that crystals are present in a more dispersed and fine form (TOG2-TOG4) and become dense aggregates with long needle-like or dendritic crystals as the carnauba wax concentration increases (TOG6-TOG10). This indicates the formation of a more compact three-dimensional network driven by physical attractive forces, such as van der Waals interactions (Shahamati et al.,

2024). These intermolecular interactions likely arise from aliphatic chains formed between the carnauba wax and TAO fatty acids. Notably, the crystals become more pronounced and compact at carnauba wax concentrations of 6 % or higher, immobilizing the liquid oil within the matrix, increasing firmness, and maintaining stability up to 60 °C (FIGURE 3.1B.c3 and e3) (Airoldi et al., 2022; Meng et al., 2025). This finding is consistent with the visual observations discussed in Section 3.4.1. As the temperature increased to the 60–80 °C range, a visible collapse of the crystalline structure in the OGs was observed, resulting in a loss of opacity, increased fluidity, and reduced firmness. This behavior reveals the low thermal stability of crystalline networks formed by waxes such as carnauba wax. This observation is consistent with the data of Öğütcü & Yilmaz (2014), who recorded primary melting temperatures between 58–60 °C for OGs structured with waxes, corroborating the decrease in structural integrity in this temperature range.

FIGURE 3.2B illustrates the microstructure of TEM formulations at different temperatures. It can be seen that the TEM2 micrographs (FIGURE 3.2B. a1) display spherical oil droplets dispersed within the continuous hydrogel phase at 20 °C. As the gelatin concentration increases, the droplets decrease in size, revealing denser, thicker, and more compact EGs, as can be seen in FIGURE 3.2B. c.2–d.2. This behavior is typical of protein-stabilized EGs, which form homogeneous and cohesive microstructures characterized by smaller droplets and a denser matrix, as reported by Zhang & Yu (2023) in gelatin-stabilized emulsions. This behavior is in line with that of Zeng et al. (2023), who found that the droplet sizes of gelatin-containing emulsions decreased as the emulsifier concentration increased from 1 % to 4 % (w/w), due to more effective surface coverage of the newly formed droplets. All samples exhibited EG formation and effective oil entrapment within the three-dimensional network, consistent with the findings of visual analysis. This phenomenon occurs due to the active surface of gelatin, containing charged and amphiphilic groups (e.g., $NH_2$- and OH-), which facilitate the formation of hydrogen bonds at the oil-water interface, increasing emulsion stability (Ahmad et al., 2024). At 40 °C, TEM2 shows a slight loss of network integrity (FIGURE 3.2B.a2), while TEM6 and TEM8 maintain their stable structures, suggesting that higher gelatin concentrations provide superior thermal resistance.

### 3.4.3. Color measurement

According to TABLE 3.1, the OGs (TOG) samples exhibited lower lightness (L*) and WI values compared to the EGs (TEM) samples, indicating a darker, more yellowish appearance. However, increasing gelling agent concentrations resulted in a significant increase in these parameters ($p < 0.05$), a behavior similar to that described by Qu et al. (2024) in wax-structured OG systems, in which higher gelling agent concentrations resulted in lighter, brighter OGs.

In contrast, the TEM samples exhibited higher L*, WI, and b* values, corresponding to a lighter, brighter yellow coloration. These differences are closely related to the structure of the matrix formed by each system. While carnauba wax promotes the formation of a dense, crystalline, and opaque lipid network, gelatin, in turn, forms a more translucent three-dimensional protein network, capable of scattering light more evenly and thus increasing the perception of gloss (Dimakopoulou-Papazoglou; Giannakaki; Katsanidis, 2023; Xu et al., 2020). A strong correlation ($r > 0.90$; $p < 0.05$) was observed between b* values and OBC, particularly in the TEM samples, suggesting that the increased yellowing may be related to more efficient emulsification and the presence of smaller droplets in a dense protein matrix. Furthermore, the tendency toward yellow (b*) in both systems can be attributed to the presence of carotenoids naturally present in TAO, which contribute to the orange-yellow color of the final product (Amorim et al., 2022; Santos et al., 2023).

These findings reinforce the importance of matrix structure and phase interactions in defining the appearance of OGs and EGs, highlighting the technological potential of using gelatin and natural waxes to modulate the color and stability of lipid systems.

TABLE 3.1 – COLOR PARAMETERS AND OIL BINDING CAPACITY (OBC) RESULTS FOR TUCUMÃ-DO-AMAZONAS OLEOGELS (TOG) AND EMULSION GELS (TEM).

| Samples | Color | | | |
|---|---|---|---|---|
| | $L^*$ | $a^*$ | $b^*$ | Whiteness index (WI) |
| TOG2 | 29.24 ± 2.33$^{g}$ | -0.65 ± 0.67$^{bc}$ | 11.90 ± 1.22$^{h}$ | 28.24 ± 2.15$^{e}$ |
| TOG4 | 32.38 ± 4.12$^{g}$ | -0.89 ± 0.11$^{c}$ | 13.81 ± 1.75$^{g}$ | 30.97 ± 3.70$^{e}$ |
| TOG6 | 37.78 ± 1.95$^{f}$ | -0.35 ± 0.46$^{b}$ | 16.07 ± 0.86$^{f}$ | 35.74 ± 1.92$^{d}$ |
| TOG8 | 39.97 ± 4.32$^{ef}$ | -0.60 ± 0.44$^{bc}$ | 19.94 ± 0.79$^{e}$ | 36.74 ± 4.33$^{d}$ |
| TOG10 | 41.80 ± 3.85$^{e}$ | 0.32 ± 0.25$^{a}$ | 22.82 ± 2.75$^{d}$ | 37.48 ± 4.01$^{d}$ |
| TEM2 | 45.71 ± 3.78$^{d}$ | -1.88 ± 0.16$^{d}$ | 21.04 ± 1.16$^{e}$ | 41.74 ± 3.70$^{c}$ |
| TEM4 | 52.60 ± 2.93$^{c}$ | -2.86 ± 0.09$^{e}$ | 25.50 ± 1.66$^{c}$ | 46.09 ± 1.78$^{b}$ |
| TEM6 | 58.92 ± 2.90$^{b}$ | -3.64 ± 0.35$^{f}$ | 28.65 ± 0.96$^{b}$ | 49.77 ± 1.79$^{a}$ |
| TEM8 | 65.13 ± 2.62$^{a}$ | -4.57 ± 0.26$^{g}$ | 31.05 ±1.09$^{a}$ | 53.08 ± 1.32$^{a}$ |

* Values are mean ± confidence interval at a 95 % level of significance;

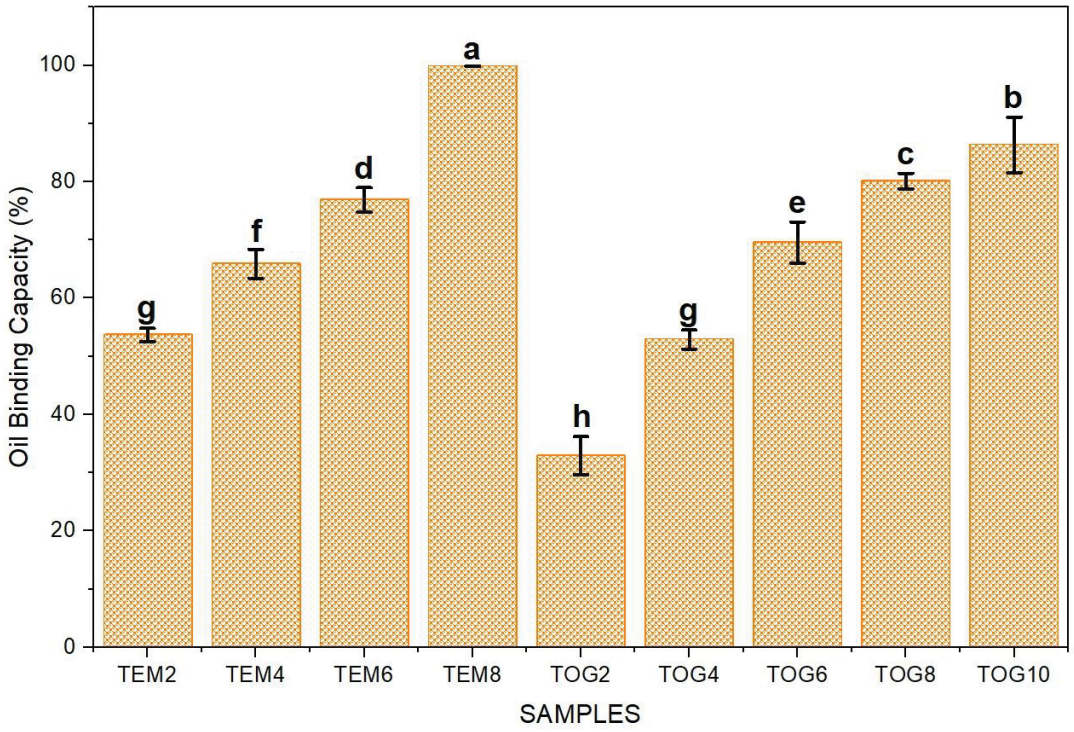
**Different letters superscripted within the same column represent significant differences ($p < 0.05$).

### 3.4.4. Oil binding capacity (OBC)

OBC increased significantly with gelling agent concentration in both systems. In OGs (TOG) formulated with carnauba wax, OBC ranged from 32.92 ± 3.26 % to 86.34 ± 4.75 %, indicating that higher wax concentrations reinforce the crystal lattice and reduce oil exudation (FIGURE 3.3). Previous studies, such as Airoldi et al. (2022), also report significant increases in OBC ($p < 0.05$) with 2 % increases in carnauba wax concentration. In EGs with gelatin (TEM), OBC ranged from 53.66 ± 1.15 % to 99.85 ± 0.02 % (FIGURE 3.3). At each concentration level, EGs presented higher OBC than OGs: for example, with approximately 4 % of gelling agent, the TOG presented an OBC of 52.87 %, while the TEM reached 65.87 %. These values suggest that the network formed by gelatin confers greater oil retention. Gelatin acts as an emulsifier in the oil-in-water (O/W) system, adsorbing at the interface, reducing interfacial tension, and forming a continuous hydrated network in the aqueous medium. At concentrations ≥4 %, this network retains water and oil efficiently, promoting

greater stability and reduced phase separation, as demonstrated by Zhang & Yu (2023). Furthermore, the statistical difference between the systems decreases at higher concentrations ($p < 0.05$), suggesting that both systems achieve high OBC with sufficient gelling agent.

FIGURE 3.3 – OBC OF OLEOGELS (TOG) AND EMULSION GELS (TEM). DIFFERENT SUPERSCRIPTED LETTERS REPRESENT DIFFERENT RESULTS ($p < 0.05$) ACCORDING TO TUKEY'S MULTIPLE COMPARISON TEST FOR THE ANALYZED SAMPLES.




### 3.4.5. Thermal behavior (DSC)

TABLE 3.2 shows the values related to DSC analysis. The data obtained for TAO showed an enthalpy of 10.76 $J.g^{-1}$ with an endothermic transition between -8.48 °C and 0.8 °C. This value is close to previous studies reporting the fusion of oleic acid-rich TAGs, characterized by the presence of a single peak, suggesting stability at low temperatures (Ferreira et al., 2021; O'Brien, 2008). Thermal analysis for TOG revealed two main transitions: one close to 0 °C, still related to TAGs but with small variations due to the presence of wax, and another between 62.52 °C and 67.02 °C (Onset temperature). This second peak is consistent with studies on OGs structured with waxes, being related to the melting

of the lipid crystal network of carnauba wax (Meng et al., 2025). It can be noted that both the temperature and the enthalpy of the second transition showed a progressive increase with the concentration of the gelling agent (Doan et al., 2017; Zhao et al., 2020). This progressive increase suggests that higher concentrations of the gelling agent enable greater formation of crystal networks, in which the supersaturation of the structuring agent can promote more efficient nucleation, generating a denser network with greater thermal stability in samples with a higher concentration of carnauba wax (Doan et al., 2017).

Like TAO, the TEM samples exhibited only one endothermic transition at temperatures ranging from 93.79 to 102.31 °C and presented higher enthalpy values, ranging from 416.29 to 655.90 $J.g^{-1}$. These results indicate that the EGs formed with TAO and gelatin enabled the construction of a thermally stable and highly cohesive structural network. According to Huang et al. (2018), the thermoreversible behavior of gelatin is associated with the difference in energy required for the association and dissociation of the junction zones between its filaments. This process directly influences the gelation and melting points, with higher gelation points tending to result in greater thermal stability. This mechanism supports the formation of cohesive structural networks in the EGs formulated with TAO and gelatin. During cooling, a structural rearrangement of the gelatin chains occurs, promoting the formation of α-helices stabilized by hydrophobic interactions and hydrogen bonds (Zeng et al., 2023). Mosleh et al. (2021) demonstrated that the transition enthalpy ($\Delta H_m$) correlates linearly with the triple-helix content in gelatin films, indicating that systems with higher $\Delta H_m$ values have more structured and thermally robust protein networks.

Furthermore, for the TEM samples, it was observed that the enthalpy decreased with increasing gelatin concentration, which may have occurred due to the reorganization of the polymer network. Studies conducted with wet gelatin films cross-linked with glutaraldehyde have shown that enthalpy can decrease with increasing covalent cross-linking due to the decrease in hydrogen bonds and the increase in covalent cross-links, which break endothermally and exothermally, respectively. Thus, the composition and predominant type of interaction in the formed network directly influence the thermal behavior of the system (Bigi et al., 2001). The DSC results confirm the data obtained in the

micrographs, reinforcing the difference in thermal stability between crystalline and protein networks.

TABLE 3.2 – THERMAL BEHAVIOR RESULTS FOR TUCUMÃ-DO-AMAZONAS OLEOGELS (TOG) AND EMULSION GELS (TEM).

| Samples | DSC | | | | |
|---|---|---|---|---|---|
| | Peak | | | | |
| | Onset (°C) | Endset (ºC) | Peak height (W/g) | Peak temperature (°C) | Enthalpy (J/g) |
| TAO | -8.48 ± 1.44 | -7.44 ± 1.71 | -0.09 ± 0.01 | 0.08 ± 0.02 | 10.76 ± 1.53 |
| TOG2 | 62.52 ± 1.18 | 77.54 ± 4.34 | -0.02 ± 0.00 | 71.32 ± 2.09 | 2.64 ± 0.64 |
| TOG4 | 64.83 ± 1.31 | 76.42 ± 1.38 | -0.04 ± 0.01 | 72.53 ± 1.06 | 5.75 ± 1.67 |
| TOG6 | 65.57 ± 1.22 | 78.91 ± 0.44 | -0.06 ± 0.09 | 74.67 ± 1.32 | 7.57 ± 3.62 |
| TOG8 | 66.82 ± 1.30 | 79.63 ± 1.30 | -0.09 ± 0.05 | 75.38 ± 1.02 | 14.10 ± 2.17 |
| TOG10 | 67.02 ± 1.44 | 80.26 ± 1.52 | -0.10 ± 0.08 | 76.36 ± 2.06 | 17.66 ± 3.96 |
| TEM2 | 101.07 ± 5.72 | 118.08 ± 19.89 | -2.686 ± 3.41 | 107.92 ± 18.92 | 655.90 ± 57.29 |
| TEM4 | 99.25 ± 0.61 | 116.87 ± 0.33 | -4.760 ± 1.24 | 103.72 ± 5.37 | 570.36 ± 55.57 |
| TEM6 | 102.31 ± 6.06 | 150.81 ± 0.97 | -6.835 ± 8,41 | 137.27 ± 5.35 | 416.29 ± 1.22 |
| TEM8 | 93.79 ± 2.67 | 145.75 ± 4.51 | -5.207 ± 3.17 | 104.41 ± 7.85 | 433.40 ± 52.34 |

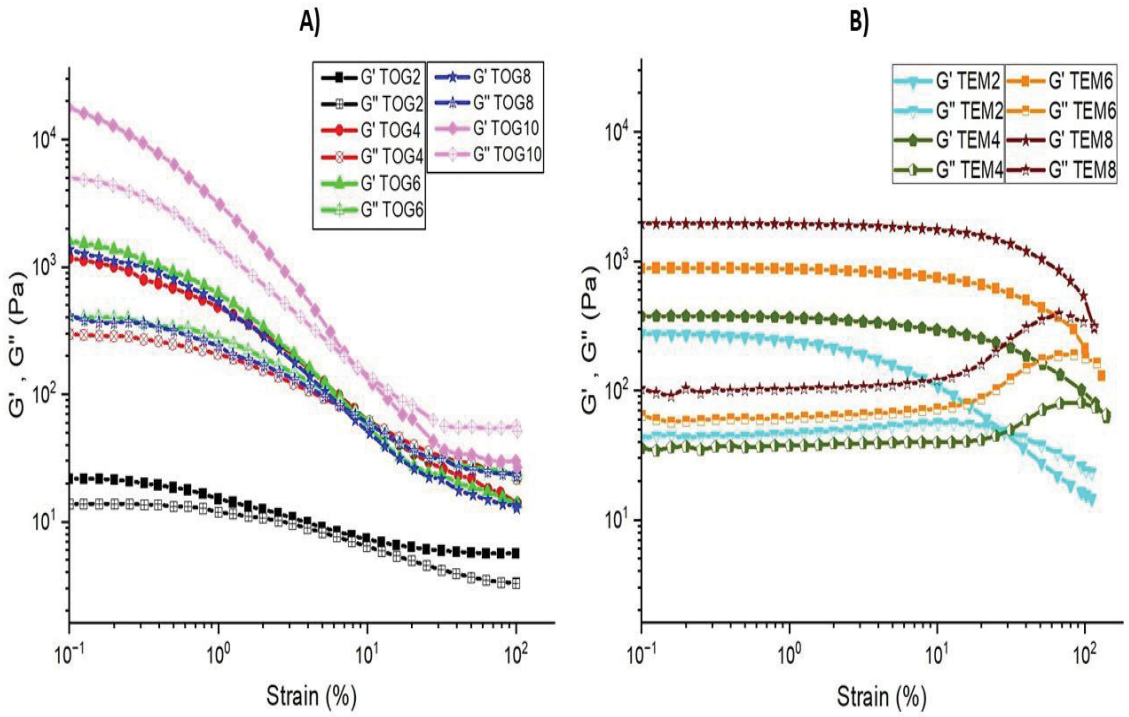
* Values are mean ± confidence interval at a 95 % level of significance;

**Different letters superscripted within the same column represent significant differences ($p < 0.05$).

### 3.4.6. Rheological measurements

Rheological analysis is essential in gel development as it provides comprehensive insights into the material's mechanical strength, structural stability, and functional performance. Assessing key rheological parameters enables precise characterization of texture and consistency, evaluation of structural integrity, optimization of formulations, and accurate prediction of performance under various processing and storage conditions. FIGURE 3.4 illustrates the relationship between the G′ and the G″ under varying strain for OGs (TOG) and EGs (TEM). At strain of 0.1 %, all TOG and TEM samples present the solid/semi-solid gel structure elastic component (G' > G'') (FIGURE 3.4). As strain increased for TOG, G′ progressively decreased, reflecting structural breakdown of fat-like crystalline network (FIGURE 3.4A) (Patel et al., 2013). Furthermore, marks the transition from solid-like to liquid-like behavior (crossover point, G' = G'), revealing the gel's stability limits at around 10 Pa. In addition, the FIGURE 3.4A shows a short range of LVR (0.1 to 0.2 Pa), where the gel maintains consistent mechanical properties before structural damage occurs. On the other hand, at FIGURE 3.4B, it is possible to notice different behavior regarding the LVR and crossover points of TEM. Thus, the LVR of TEM4, TEM6, and TEM8 occur in a strain range between 0.1 to 10 % and crossover points around 100 %. Moreover, even the TEM2 demonstrated a structure more stable (biggest LVR range and crossover) than TOG samples.

FIGURE 3.4 – RHEOLOGICAL BEHAVIORS OF OLEOGELS (OLG) AND EMULSION GELS (EG). PLOTS SHOW THE LOG-LOG PLOT OF THE STORAGE MODULUS (G′) AND LOSS MODULUS (G″) AS A FUNCTION OF APPLIED STRAIN FOR OG (A) AND EG (B), RESPECTIVELY.




Overall, the rheological results support the structural differences previously observed between the TOG and TEM systems. Carnauba wax-based OGs exhibited efficient crystalline networks but were mechanically more fragile, whereas gelatin-stabilized EGs showed markedly higher viscoelastic behavior and deformation resistance. These findings underscore the crucial role of the gelling agent nature and the type of continuous phase (lipid or aqueous) in determining the mechanical performance of the systems. Therefore, the rheological data complement the other analyses, providing a comprehensive assessment of the impact of physical structuring strategies and gelling agent concentrations on the performance of the formulated OGs and EGs.

## 3.5. Conclusion

The results of this study demonstrate that TAO has strong potential for the formulation of structured lipid systems using carnauba wax and gelatin as gelling

agents. carnauba wax promoted the formation of OGs with high OBC at higher gelling agent concentrations. However, these systems exhibited lower thermal and mechanical strength compared to gelatin-based EGs, based on analyses. In contrast, the EGs exhibited superior elasticity, OBC (~99.85 %), and thermal stability, attributed to their three-dimensional protein networks. Furthermore, color and visual appearance were influenced by both the concentration and the nature of the gelling agent used.

These results demonstrate the potential of TAO as a functional component in structured formulations, presenting advantageous lipid retention characteristics and rheological properties. This work also establishes a theoretical foundation for creating healthier alternatives to SFA and TFA in food. Furthermore, the production methods used provide advantages for industrial applications, such as high efficiency, rapid processing, and chemical adaptability.

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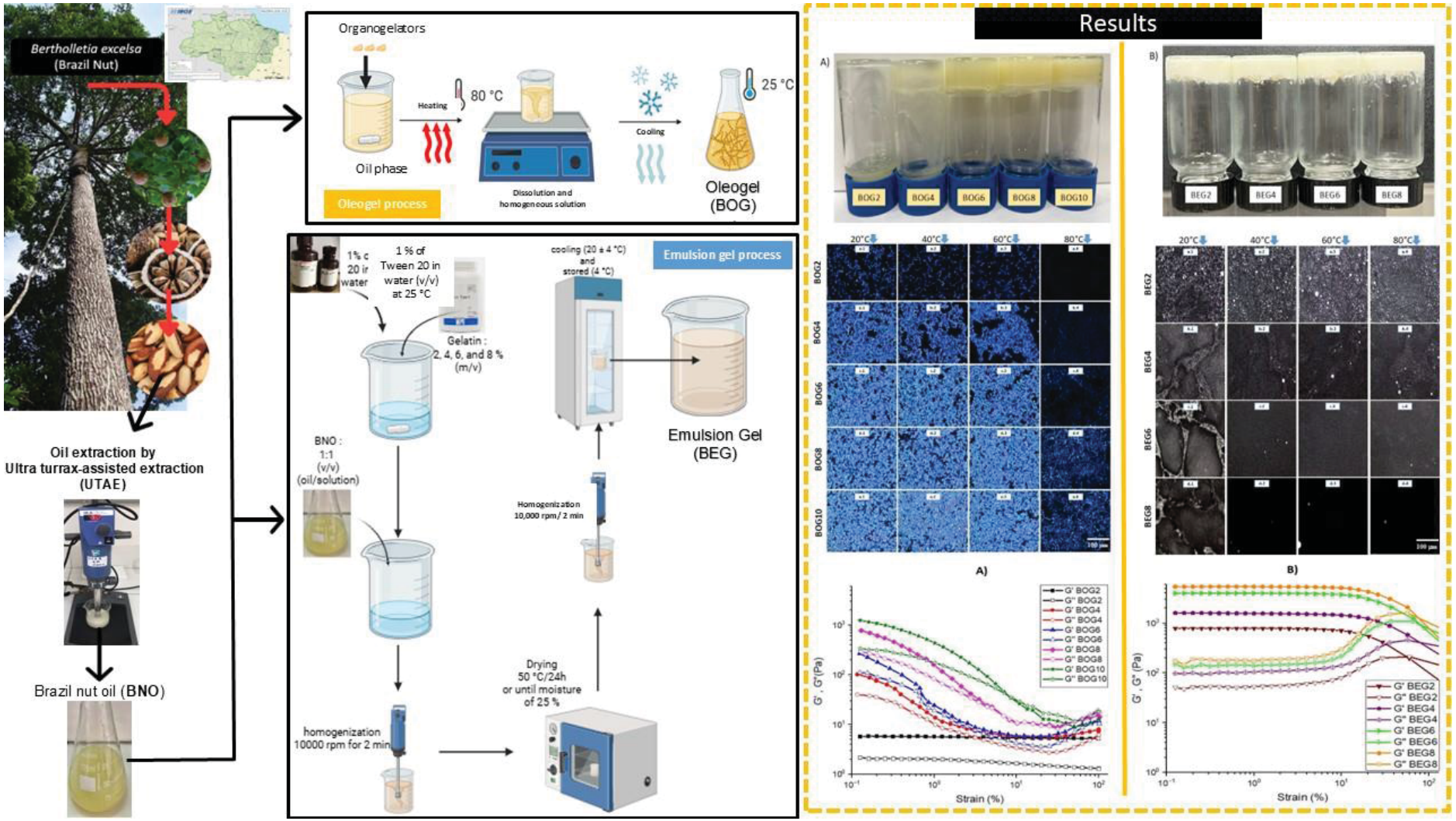
# CHAPTER IV

## Development and physical properties of Brazil Nut oil-based oleogel and emulsion gel

This chapter consists of the article entitled "Development and physical properties of BNO-based oleogel and emulsion gel", which it **has been submitted (Food hydrocolloids)**. This article consists of the use of BNO obtained under optimized conditions (identified in Chapter II) for the production of OG and EG. For this purpose, carnauba wax and gelatin were the gelling agents used in various concentrations to produce the OGs and EGs, respectively. In addition, these obtained materials were characterized using morphology, color, OBC, thermal stability, and rheological methods. Furthermore, based on the results presented in this chapter, this study will serve as a basis for Chapter V, where the Brazil nut emulsion gel (BEG) with 2 % of gelatin (w/w) will be applied as a substitute in the formulation of chocolate spread.

## Graphical abstract



# Development and physical properties of Brazil Nut oil-based oleogel and emulsion gel

## 4.1. Abstract

In the context of excessive dietary intake of SFA and TFA, OG and EG have emerged as promising alternatives for replacing harmful solid fats with UFA, thereby improving the nutritional profile of food products. In this study, BNO was structured with carnauba wax and gelatin to produce health-promoting OG and EG, intended as substitutes for solid fats in food applications. Visual analysis demonstrated that BNO can be effectively structured with 6 % carnauba wax, while 2 % gelatin was sufficient to induce the transition from liquid oil to a semi-solid material. PLM revealed the formation of crystalline structures in OG and emulsion-filled networks in EG, which were responsible for their structural stability. Regarding OBC, it increased significantly with higher carnauba wax concentrations, rising from 30.63 ± 1.34 % (BOG2) to 83.65 ± 1.90 % (BOG10) ($p < 0.05$). For OBC of EG, all samples obtained similar results, approximately 100 % ($p < 0.05$). Furthermore, rheology results showed that most samples presented gel behavior (G' > G", except 2 and 4 % carnauba wax in BNO), highlighting the presence of LVR and higher crossover point for EG samples in relation to OG. Overall, the incorporation of carnauba wax and gelatin into BNO proved to be a feasible approach for producing high-quality OG and EG, with promising potential for application in food products.

Keywords: *Bertholletia excelsa*; Amazon oil; Emulgel; fat replacers; and Structured lipids.

## 4.2. Introduction

Although solid fats contribute significantly to food structure and sensory properties (Zampouni et al., 2024), their consumption has been strongly linked to various health risks, particularly cardiovascular diseases (Frías et al., 2023; Guo; Cui; Meng, 2023; Langley et al., 2020; López-Pedrouso et al., 2021). Consequently, there are intense efforts to replace TFA and SFA with healthy oils (Blount; Ferdaus; Silva, 2025; Ferdaus et al., 2024; Mahmud; Ferdaus; Silva, 2024; Oyom et al., 2024; Silva et al., 2023). For instance, the WHO has issued

guidelines on SFA and TFA intake, which states that SFA intake should be less than 10 % of total daily energy consumption and replaced with PUFAs and MUFAs to minimize the incidence of cardiovascular diseases (WHO, 2023).

However, the simple substitution of SFA with unsaturated oils poses several challenges for the food industry, as solid fats contribute to key physical properties of food products (including texture, oxidative stability, taste, color, mouthfeel, crunchiness, and other) (Gao et al., 2024; Wang et al., 2024). In response, fat mimetics have emerged as promising alternatives to conventional fats (Ferdaus; Blount; Silva, 2022; Silva et al., 2023). These substitutes are created from proteins, healthy oils, or polysaccharides that aim to replicate the functional properties of solid fats while minimizing health risks (Cen; Li; Meng, 2024; Huang et al., 2023; Vélez-Erazo et al., 2022; Xie et al., 2023).

In this context, an approach known as organogelation has been developed using conventional oils to produce OG as a healthy substitute for solid fats (Guo; Cui; Meng, 2023; Manzoor et al., 2022). This concept is based on a high amount of liquid oil entrapped within a three-dimensional network using one or more organogelators, also called gelling agents, in a 10 % maximum amount (Huang et al., 2023). Carnauba wax has been used as a gelling agent due to its high melting point (81–86 °C), low solubility, and relative inertness and stability, as stated by the European Food Safety Authority (Susmita Devi et al., 2022).

EGs also have emerged as an alternative strategy for replacing solid fats with healthier vegetable oils, offering improved stability, safety, solubilized hydrophobic and hydrophilic components, and nutritional benefits compared to the OGs (Farjami & Madadlou, 2019; Lin et al., 2020; Wang et al., 2025). EGs are structured systems defined by gel-like networks and solid-like textural properties (Keramat; Golmakani, 2024), formed through the incorporation of gelling agents into various types of emulsions (O/W, W/O, W/O/W, or O/W/O). These agents promote the formation of a three-dimensional matrix that enhances the stability of the dispersed phase (Czapalay; Marangoni, 2024). For this purpose, gelatin, a non-globular, natural amphiphilic protein derived from collagen, exhibits excellent gelling, emulsifying, and film-forming capabilities (Yan et al., 2023). Due to its characteristics of gelation, biocompatibility, wide source, low cost, and nontoxicity, gelatin is considered a promising raw material for EGs application (Mao et al., 2022).

These innovations present an opportunity to utilize healthy vegetable oils that remain underexplored in industrial applications, particularly Amazon oils. Among the 84 oilseed species identified in the region by Pesce (2009) BN is recognized for its numerous health benefits, including reducing cardiovascular risk factors by lowering TAG and cholesterol levels, antioxidant and anti-inflammatory activity, and high composition of essential minerals (160 % of the recommended daily selenium intake) (Cardoso et al., 2016; Cominetti et al., 2012; Vasquez et al., 2021; Yang, 2009).

The objective of this study was to develop novel OGs and EGs using BNO as the continuous phase and carnauba wax and gelatin as gelling agents. Specifically, the study evaluated the effects of varying concentrations of gelling agent and processing methods on the morphology, color, OBC, thermal stability, and rheological properties of OGs and EGs. The findings of this research may support the use of Amazon oils as innovative structured oils, offering high OBC and desirable rheological characteristics, which provide theoretical support for reducing TFA and SFA in food products. Furthermore, the preparation methods employed for producing these gel materials offer significant potential for application in the food industry, given their efficiency, rapid processing times, and lack of chemical modifications. Additionally, this work supports the sustainable development of the Amazon region by promoting the responsible use of its natural resources, both environmentally and economically.

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## 4.3. Methodology

### 4.3.1. Materials

Carnauba wax (melting point of 81–86 °C) was purchased from Fisher Scientific (Hampton, NH, USA), unflavored and colorless food grade gelatin from MP Biomedicals (Santa Ana, CA, USA), Tween® 20 from Sigma-Aldrich (St. Louis, MO, USA). Additionally, deionized water was used in all experiments.

### 4.3.2. Methods

#### 4.3.2.1. Extraction of Brazil Nut oils (BNO)

The BNO was extracted as described by our research group using n-hexane (Neon, 99.8 % purity) as solvent (Santos et al., 2025). Thus, UTAE was conducted using a T25 digital Ultra Turrax (IKA-Werke, Staufen, Germany) under

the following process conditions: a time of 27 min and a solvent-to-solid ratio of 26 mL/g. The oils were stored in amber glass and kept at −5 °C until the development of Brazil Nuts oil oleogels (BOG) and Brazil Nuts oil emulgels (BEG). The BNO fatty acid composition was analyzed in our previous research, finding it comprises of 28.53 % of SFA, primarily palmitic acid (C16:0) at 15.68 %, stearic acid (C18:0) at 12.85 % and 71.45 % of UFA, including oleic acid (C18:1) at 37.56 % and linoleic acid (C18:2) at 33.89 % (Santos et al., 2025).

#### 4.3.2.2. Preparation of Brazil Nuts oil oleogels (BOG)

The BOGs were prepared according to the direct method based on the methodologies of Airoldi et al. (2022) and Moghtadaei et al. (2018), with adaptations. Thus, 15 g of the oil was heated to 100 °C under magnetic stirring (350 rpm). Once the oil temperature was reached, carnauba wax, as the oleogelator, was slowly added (2, 4, 6, 8, and 10 % w/w) and mixed until dissolution (5 min). After the complete incorporation of carnauba wax, the samples (in liquid form) were transferred to the test tubes. Then, the samples were kept in a static condition at room temperature (20 ± 4 °C) for 1 h to structure and stabilize. Finally, the samples were stored at 4 °C until characterization. The samples were coded as BOG2, BOG4, BOG6, BOG8, and BOG10, regarding the carnauba wax concentration.

#### 4.3.2.3. Preparation of Brazil Nut oil emulgels (BEG)

The EGs were prepared according to Wang et al. (2025), with modifications. First, BEGs were prepared by dissolving 1 % Tween 20 (a sorbitan monolaurate) in ultrapure water (v/v) at 25 °C. In the second step, gelatin was added in the following proportions: 2 %, 4 %, 6 %, and 8 % (m/v), and the mixture was left to wait for 30 minutes to complete the hydration. After hydration, BNO was added to the gelatin solution at a 1:1 (v/v) ratio and homogenized using a high-speed disperser (digital Ultra-Turrax, T25, IKA, Germany) at 10,000 rpm for 2 min. Then, the samples were dehydrated at 50 °C for 24 hours or until they reached approximately 25 % moisture content. After drying, the samples were homogenized again using the Ultra-Turrax under the same conditions described previously (10,000 rpm for 2 min). After complete dispersion, they were transferred to test tubes and maintained in a static condition at room temperature

(20 ± 4 °C) for 1 h to facilitate structuring and stabilization. Finally, the samples were stored at 4 °C and then characterized. The samples were coded as BEG2, BEG4, BEG6, and BEG8, regarding the gelatin proportion.

### 4.3.3. Characterization

#### 4.3.3.1. Macroscopic appearance analysis

The macroscopic appearance analysis was developed according to Wang et al. (2024), with modifications. Thus, 5 g of the sample was poured into a test tube, cooled to 25 °C, and then stored in a refrigerator at 4 °C for 24 hours. After 24 h, the samples were then placed at 25 °C for 2 h, and the test tubes were inverted to observe the fluidity of the samples and determine whether they had gelified or not.

#### 4.3.3.2. Polarized light microscopy observation (PLM)

BOGs and BEGs microstructure were analyzed as mentioned by Silva et al. (2016), with adaptations. The samples were analyzed using a 10 × magnification. Images were recorded isothermally at 20 ºC and during heating at 40 °C, 60 ºC, and 80 °C at 5 °C/min using a Linkam thermal system (Linkam Scientific Instruments Ltd., Redhill, UK) attached to the microscope stage (Valoppi et al., 2023). Images of each sample were observed using Image Pro-Plus software version 7.0 for Windows.

#### 4.3.3.3. Color measurement

Color measurement (L*a*b*) was conducted according to Oyom et al. (2024) and the WI values were calculated as mentioned by Li et al. (2021). These parameters were measured at three random surface areas of each sample.

#### 4.3.3.4. Oil binding capacity (OBC)

The OBC of the samples was determined by accelerated stability tests using the method mentioned by Ferdaus et al., (2022).

#### 4.3.3.5. Thermal behavior

The thermal behavior of BOGs and BEGs was analyzed using a differential scanning calorimeter (DSC; TA Instruments, New Castle, DE, USA).

Approximately 8–12 mg of each sample was accurately weighed and hermetically sealed in an aluminum pan, with a sealed empty pan as the reference. Before analysis, the samples were equilibrated at 20 °C for 1 minute to ensure stabilization within the DSC chamber. The samples were then cooled to −10 °C to facilitate complete crystallization. After holding at −10 °C for 10 minutes, the samples were subjected to a heating cycle, melting from −10 °C to 90 °C (BOGs) and from −10 °C to 200 °C (BEGs) at a controlled rate of 5 °C/min under a continuous nitrogen gas flow (50 mL/min). Key thermal parameters, including onset temperature (°C), peak height (W/g), peak temperature (°C), and melting enthalpy (ΔH, J/g), were determined from the melting curves. All measurements were performed in triplicate.

#### 4.3.3.6. Rheological measurements

To evaluate the viscoelastic properties of the samples (24 h after production), dynamic oscillatory tests (strain sweep) were determined using a rheometer (Discovery HR-10, TA Instruments, New Castle, DE, USA). The upper fixture was a parallel plate (25 mm), and about 4 g of sample was put into the rheometer. Trimmed to remove the excess dough outside of the fixture when the gap was 2050 μm. Initially, the strain sweep test was performed in the strain range 0.1–100 % (at a constant frequency of 1 Hz and a temperature of 25 °C) to determine the following parameters: 1. LVR; 2. Viscoelastic behavior in the LVR range; 3. Crosspoint of G' and G" (flow point, G ' = G").

#### 4.3.3.7. Statistical analysis and visualization

ANOVA and Tukey's test were applied at a significance level of $p < 0.05$ using Statistica 7.0 software (Statsoft Inc., Tulsa, OK, USA). Graphical analysis was performed using OriginPro® 2025 (OriginLab Corp., Northampton, MA, USA).All determinations were expressed as mean ± confidence interval at a 95 % significance level of at least two measurements from three experimental replicates ($n \geq 2 \times 2$) if not otherwise specified.

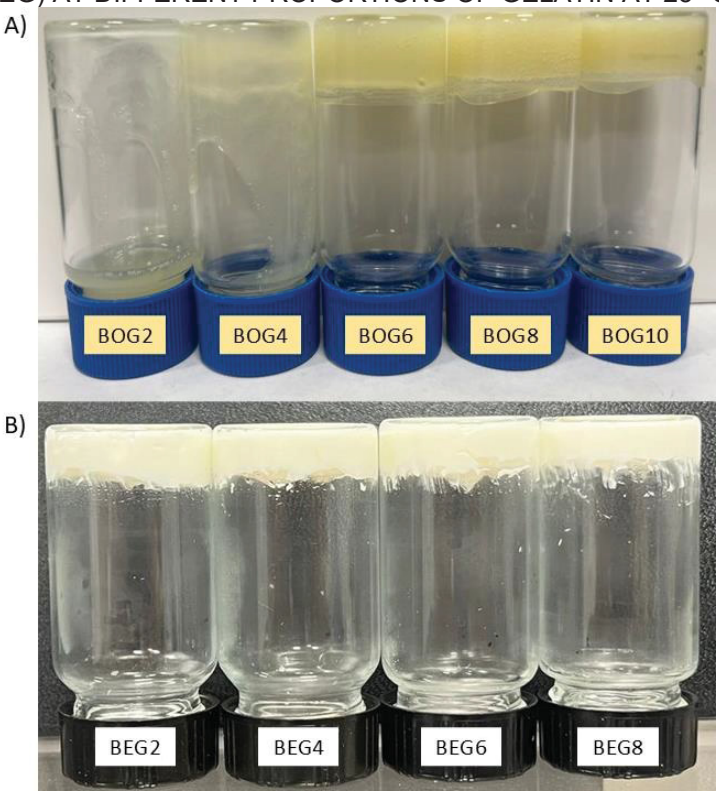
## 4.4. Results and discussion

### 4.4.1. Macroscopic appearance analysis

FIGURE 4.1A presents the results of the visual analysis of the BOGs prepared with the carnauba wax incorporated into BNO. After the system inversion, a transition from a liquid to a semi-solid state was observed, starting at a 6 % carnauba wax (w/w) concentration, indicating the formation of the OG. These results demonstrate the loss of fluidity of the system, which is related to the crystalline phase formed by the carnauba wax crystals. After dilution of the gelling agent and reduction of the solution temperature, this phase was formed through the crystallization of carnauba wax (nucleation) and self-assembly (mainly driven by branched methyl groups), creating a crystalline network capable of trapping BNO (Doan et al., 2015; Shahamati et al., 2024). Furthermore, considering the order of importance of chemical compounds in the oil gelation process (1. hydrocarbons; 2. fatty acids; 3. wax esters; and 4. fatty alcohols), the high concentrations of wax ester (58–62 %) and free fatty alcohol (30–34 %) in carnauba wax can justify a lower gelation temperature (25–45 °C) and a higher minimum concentration of gelling agent required to gelling vegetable oils compared to other waxes (Doan et al., 2015, 2017). Additionally, studies indicate that concentrations of 4–6 % carnauba wax are necessary to gel oils with elevated UFA levels, attributed to the presence of medium- and long-chain TAGs, such as oleic and linoleic acids (Buitimea-Cantúa et al., 2021; Wang et al., 2024b). The high UFA content in BNO (~ 70 %), reported previously by our research group (Santos et al., 2025), aligns with findings from previous studies.

On the other hand, BEGs were formed at all concentrations tested, indicating that gelatin was efficient in structuring the oil into semi-solid systems (FIGURE 4.1B). This result was achieved because gelatin is an amphipathic protein able to adsorb to the oil-water interface of oil droplets (Lee et al., 2025). According to Lin et al. (2025), gelatin-based EGs belong to the class of emulsion-filled gels, in which the continuous phase (triple helices) forms an ordered network structure through gelatin's physical self-assembly or covalent crosslinking. Meanwhile, the dispersed oil droplets serve as "active fillers", contributing to improved mechanical properties.

FIGURE 4.1 – MACROSCOPIC APPEARANCE: A) BRAZIL NUT OLEOGELS (BOG) WITH CARNAUBA WAX AT DIFFERENT CONCENTRATIONS; AND B) BRAZIL NUT EMULGEL (BEG) AT DIFFERENT PROPORTIONS OF GELATIN AT 25 °C.




4.4.2. Polarized light microscopic observation (PLM)

PLM analysis of the BOG samples (FIGURE 4.2) reveals homogeneous crystals and an increasingly aggregated structure with higher carnauba wax concentrations, suggesting the development of a more compact three-dimensional network driven by physical attractive forces, such as van der Waals interactions (Shahamati et al., 2024). These intermolecular interactions likely arise from aliphatic chains between carnauba wax and BNO fatty acids. Notably, the crystals become more pronounced and compact at carnauba wax concentrations of 6 % or higher, immobilizing the liquid oil within the matrix and maintaining stability up to 60 °C (FIGURE 4.2c–2e). This finding aligns with the visual observations discussed in Section 4.1.

FIGURE 4.2 – POLARIZED LIGHT MICROSCOPIC IMAGES (a), (b), (c), (d), AND (e) OF BRAZIL NUT OLEOGELS (BOG) AT DIFFERENT PROPORTIONS OF CARNAUBA WAX WITH DIFFERENT TEMPERATURE (THE NUMBERS FROM LEFT TO RIGHT INDICATE THE INCREASE IN TEMPERATURE: 1. 20 °C; 2. 40 °C; 3. 60 °C; AND 4. 80 °C). IMAGES SHOW 100 µm IN SCALE.

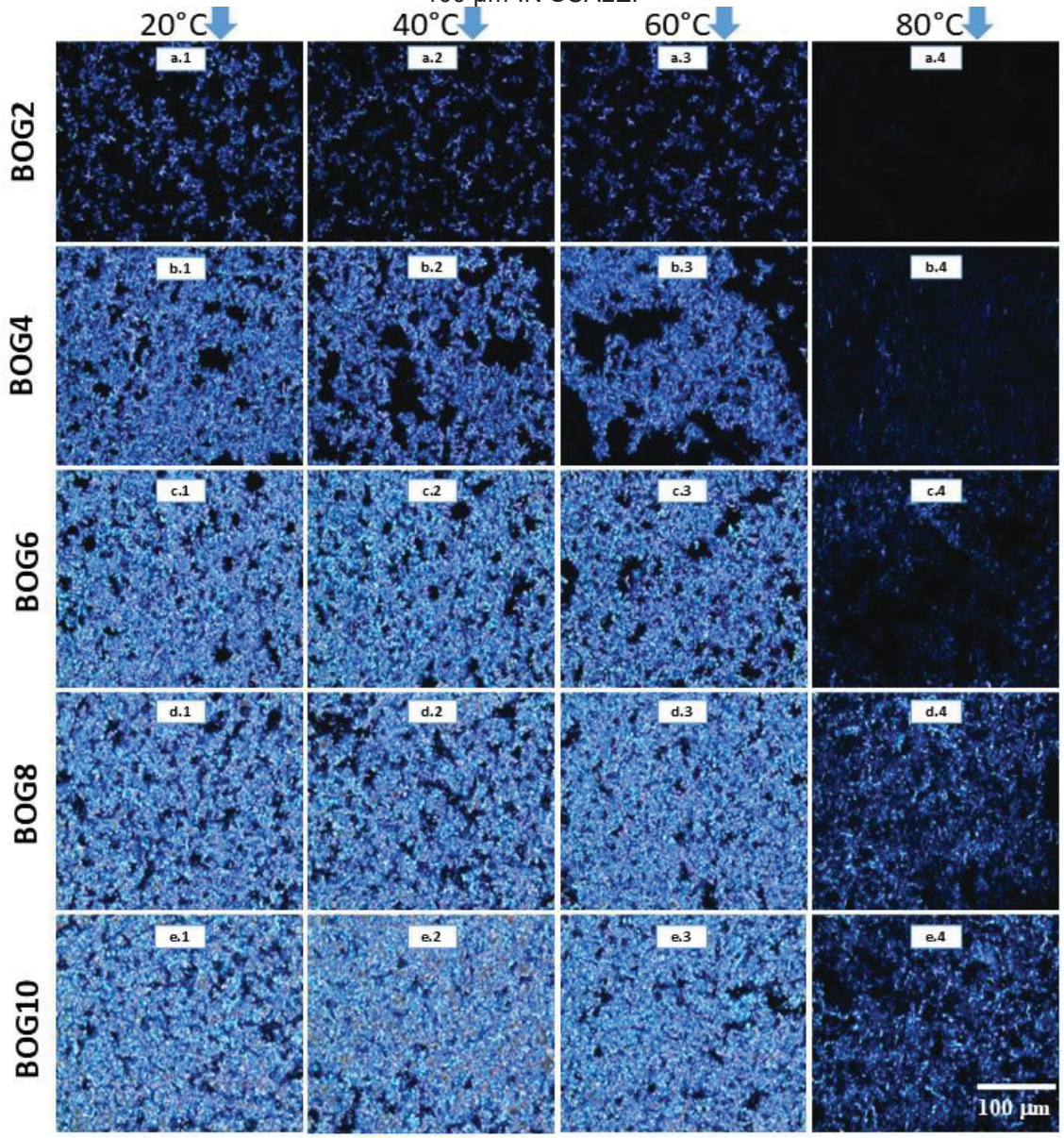
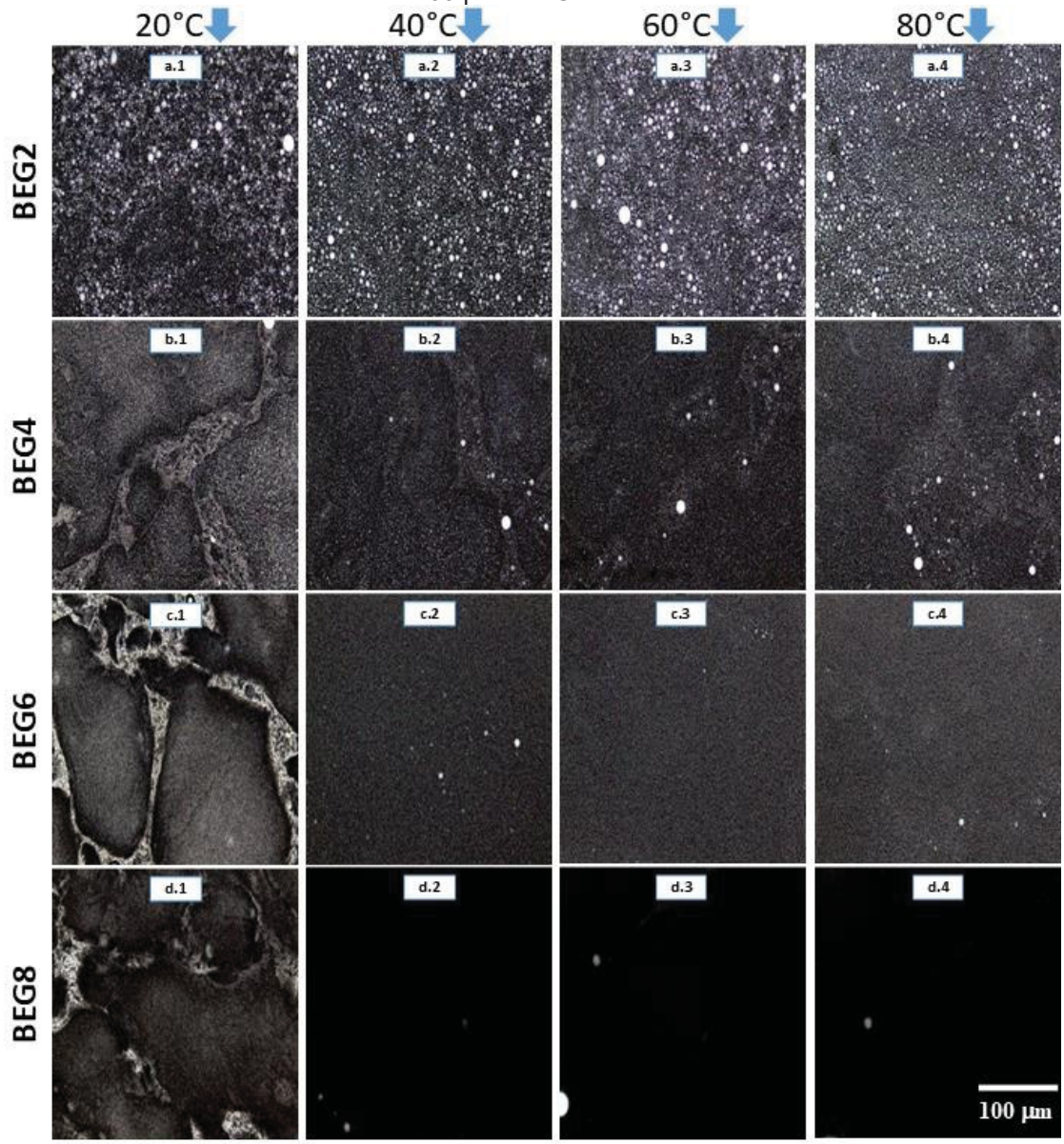



FIGURE 4.3 illustrates the microstructure of various BEG formulations at different temperatures. The micrographs of the BEG containing 2 % gelatin (FIGURE 4.3a) show spherical oil droplets dispersed within the hydrogel continuous phase at 20 °C. In contrast, micrographs of formulations with gelatin concentrations exceeding 4 % (FIGURE 4.3c–3d) reveal denser, thicker, and more compact EGs. All samples exhibited a structure similar to the emulsion-filled gels, with effective oil entrapment within the three-dimensional network,

consistent with the visual analysis findings. In this case, this phenomenon occurs due to the gelation mechanism of gelatin (cold-set after heat treatment). Thus, a self-assembly mechanism of gelatin happens, and helices are established (below 30 °C), which leads to aggregation and gelation (Farjami; Madadlou, 2019; Lin; Kelly; Miao, 2020; Nicolai, 2019).

Cracks observed in the images (FIGURE 4.3.b-d) likely result from the sample preparation process, where a coverslip was gently pressed onto the glass slide. Moreover, preliminary tests were conducted using stabilizers such as Tween 20, Tween 40, soy lecithin, glycerol, and Yucca schidigera extract in EGs formulated with conventional oils. Notably, Tween® 20 demonstrated effective gelatin stabilization by binding to gelatin's peptide bonds and protonated amino groups through hydrogen bonding (Tan et al., 2023). Additionally, all samples lost their structure after reaching 40 °C due to the protein denaturation (heat treatment above 40 °C, the polypeptide chains lose their rigid tertiary structure and become more mobile, increasing the solubility of gelatin) (Nicolai, 2019).

FIGURE 4.3 – POLARIZED LIGHT MICROSCOPIC IMAGES (a), (b), (c), (d), AND (e) OF BRAZIL NUT EMULGEL (BEG) AT DIFFERENT PROPORTIONS OF GELATIN AT DIFFERENT TEMPERATURE THE NUMBERS FROM LEFT TO RIGHT INDICATE THE INCREASE IN TEMPERATURE: 1. 20 °C; 2. 40 °C; 3. 60 °C; AND 4. 80 °C). IMAGES SHOW 100 µm IN SCALE.




4.4.3. Color measurement

TABLE 4.1 presents the measured color parameters of BOG and BEG, which are important parameters for quality control, indicators of stability, and also indicators of consumer acceptance. The luminosity values (L*) ranged from 34.87 ± 1.67 to 50.54 ± 2.00 and from 59.19 ± 4.16 to 69.04 ± 2.53 for BOG and BEG, respectively. Regarding the WI, it ranged from 34.87 ± 1.67 (BOG2) to 68.98 ± 2.54 (BEG10). These parameters presented a significant difference with an increase in the concentration of carnauba wax and gelatin ($p < 0.05$). Thus, with

the increase in the concentration of gelling agents, there is a greater formation of three-dimensional networks that intensify the physical effects. These results are aligned with visual and PLM results.

TABLE 4.1 – COLOR PARAMETERS RESULT FOR BRAZIL NUT OLEOGELS (BOG) AND EMULSION GELS (BEG).

| Samples | Color | | | |
|---|---|---|---|---|
| | $L^*$ | $a^*$ | $b^*$ | Whiteness index (WI) |
| BOG2 | 34.87 ± 1.67$^{g}$ | -0.87 ± 0.12$^{a}$ | 0.04 ± 0.21$^{fg}$ | 34.87 ± 1.67$^{g}$ |
| BOG4 | 39.35 ± 2.55$^{f}$ | -1.57 ± 0.22$^{b}$ | 0.57 ± 0.35$^{de}$ | 39.33 ± 2.54$^{f}$ |
| BOG6 | 45.10 ± 1.68$^{e}$ | -2.28 ± 0.10$^{c}$ | 2.15 ± 0.58$^{c}$ | 45.01 ± 1.67$^{e}$ |
| BOG8 | 46.14 ± 5.18$^{e}$ | -2.81 ± 0.54$^{d}$ | 3.89 ± 0.53$^{b}$ | 45.92 ± 5.11$^{e}$ |
| BOG10 | 50.54 ± 2.00$^{d}$ | -3.14 ± 0.40$^{e}$ | 4.65 ± 0.47$^{a}$ | 50.23 ± 1.92$^{d}$ |
| BEG2 | 59.19 ± 4.16$^{c}$ | -1.03 ± 0.04$^{a}$ | -0.36 ± 0.29$^{g}$ | 59.18 ± 4.16$^{c}$ |
| BEG4 | 61.62 ± 4.78$^{bc}$ | -1.40 ± 0.22$^{b}$ | 0.11 ± 0.29$^{f}$ | 61.59 ± 4.78$^{bc}$ |
| BEG6 | 64.56 ± 3.97$^{b}$ | -1.39 ± 0.10$^{b}$ | 0.27 ± 0.06$^{ef}$ | 64.53 ± 3.96$^{b}$ |
| BEG8 | 69.04 ± 2.53$^{a}$ | -1.56 ± 0.17$^{b}$ | 0.98 ± 0.27$^{d}$ | 68.98 ± 2.54$^{a}$ |

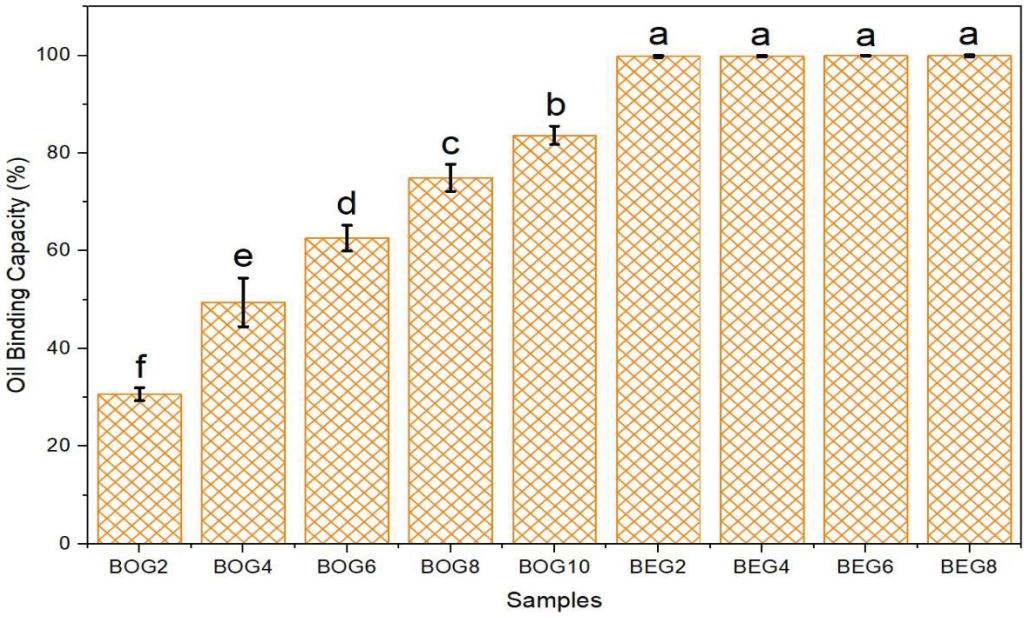
* Values are mean ± confidence interval at a 95 % level of significance;
**Different letters superscripted within the same column represent significant differences ($p < 0.05$).
***L* = color intensity, L* = 100 for lightness, and L* = 0 for darkness; +a* = increasing red, -a* = increasing green; +b* = increasing yellow, -b* = increasing blue.

For a* that represents the redness, the increase in the concentration of carnauba wax and gelatin impacted its reduction, ranging from -3.14 ± 0.40 to -0.87 ± 0.12 and -1.56 ± 0.17 to -1.03 ± 0.04, respectively. The yellowing (b*) of BOG10 was higher among the OG samples compared to the BEG samples. The results indicate that the natural yellowish color of carnauba wax (pigments) impacted the OG color. Carnauba wax contains compounds (mainly chlorophyll and carotenoids), which cause green and yellow-orange colorations (Buitimea-Cantúa et al., 2021; Roufegarinejad et al., 2024).

4.4.4. Oil binding capacity (OBC)

OBC is crucial in the development of OGs and EGs, as it allows the assessment of structural stability, texture, and functionality (Pakseresht et al., 2023; Shahamati et al., 2024). High OBC ensures effective oil retention, minimizing oil leakage and increasing gel strength and consistency (Chen et al., 2023). The OBC values for BOG increased significantly ($p < 0.05$) with increasing carnauba wax concentrations. For example, the OBC of BOG2 and BOG10 was found to be 30.63 ± 1.34 % and 83.65 ± 1.90 %, respectively (FIGURE 4.4). This result is consistent with the visual observations and PLM, which demonstrate that higher carnauba wax concentrations enhance the OG network's ability due to the high content of wax esters and hydrocarbons (Qu et al., 2024). Besides, according Doan et al. (2017), with more carnauba wax mass, the hydrogen bonding among long-chain free fatty alcohols in carnauba wax is more pronounced (such as $C_{30}$, $C_{32}$, and $C_{34}$). These findings also align with results reported by Thakur et al. (2022) and Shi et al. (2022), who reported that a high wax content enhances the OGs ability to retain liquid oil, resulting in a higher OBC.

FIGURE 4.4 – OBC OF OLEOGELS (BOG) AND EMULSION GELS (BEG). DIFFERENT SUPERSCRIPTED LETTERS REPRESENT DIFFERENT RESULTS ($p < 0.05$) ACCORDING TO TUKEY'S MULTIPLE COMPARISON TEST FOR THE ANALYZED SAMPLES.



In contrast, the results for BEG were significantly superior ($p < 0.05$) to those for BOGs. All BEG samples exhibited an OBC of approximately 100 % with no significant differences observed between them ($p > 0.05$). This behavior can be associated with the presence of high fatty alcohol content and uneven distribution of dendritic and aggregate-like crystals in carnauba wax (BOG), which form unstable networks and limit their ability to retain large amounts of oil in comparison to the gelatin (BEG) (Qu et al., 2024; Wang et al., 2023, 2024). Moreover, the amphiphilic protein structure and electrostatic interactions of gelatin, combined with the presence of Tween® 20 in BEG, enable the proteins and surfactant to migrate toward the lipid interface, reducing surface tension and preventing droplet coalescence (Taktak et al., 2021; Wu et al., 2024). In addition, the gelatin's active surface contains charged and amphiphilic groups (e.g., $NH_2$- and OH-), which facilitate hydrogen bond formation at the oil-water interface, enhancing emulsion stability (Ahmad et al., 2024), resulting in a more stable structure compared to BOGs.

### 4.4.5. Thermal behavior (DSC)

Given the widespread use of fat or shortening in food applications, it is critical to evaluate BOG and BEG melting profiles to ensure they closely replicate the thermal behavior of conventional fats, thereby preserving the desired properties of the final product (Sejwar et al., 2024). The BOG thermal profiles (TABLE 4.2) presented an endothermic melting peak ($T_o$ from 58.64 ± 1.83 to 65.48 ± 0.60 °C) is regarding the composition of carnauba wax and presented a high melting behavior compared with other waxes (Ferdaus; Blount; Silva, 2022) due to the high concentrations of wax ester and free fatty alcohol, as aforementioned (Doan et al., 2015, 2017).

For the BEG melting profile, one peak was observed with $T_o$ from 89.68 ± 1.05 to 103.74 ± 1.33 °C. As mentioned by Corredor-Chaparro et al. (2022), endothermic behavior between 85 °C and 120 °C suggests the evaporation of free water. Moreover, considering ΔH as enthalpy required for melting (Atik et al., 2022), BEG showed the highest data compared with BOG, demonstrating improved ability to maintain its thermal stability with increasing temperature (chemical structure and physical properties).

TABLE 4.2. THERMAL BEHAVIOR RESULTS FOR BRAZIL NUTS OLEOGELS (BOG) AND EMULSION GELS (BEG).

| Samples | Peak | | | | |
|---|---|---|---|---|---|
| | $TC_{onset}$ | $TC_{endset}$ | $H_E$ | $T_p$ | $\Delta H$ |
| | (°C) | (°C) | (W/g) | (°C) | (J/g) |
| BNO | -8.50 ± 1.89 | 2.57 ± 1.22 | -0.15 ± 0.01 | -0.77 ± 0.86 | 14.38 ± 0.86 |
| BOG2 | 58.64 ± 1.83 | 78.43 ± 1.14 | -0.01 ± 0.01 | 70.43 ± 2.98 | 1.51 ± 0.20 |
| BOG4 | 62.38 ± 1.34 | 80.63 ± 5.55 | -0.02 ± 0.02 | 71.61 ± 0.35 | 2.70 ± 0.50 |
| BOG6 | 63.67 ± 1.70 | 77.25 ± 9.99 | -0.04 ± 0.03 | 73.36 ± 0.40 | 5.86 ± 0.74 |
| BOG8 | 65.55 ± 0.60 | 79.27 ± 0.88 | -0.08 ± 0.01 | 73.98 ± 1.91 | 13.13 ± 2.01 |
| BOG10 | 65.48 ± 4.12 | 80.08 ± 0.53 | -0.11 ± 0.03 | 75.56 ± 0.96 | 17.74 ± 5.86 |
| BEG2 | 89.68 ± 1.05 | 123.75 ± 2.42 | -11.446 ± 0.60 | 96.95 ± 1.63 | 726.77 ± 3.59 |
| BEG4 | 97.53 ± 6.06 | 124.41 ± 0.18 | -5.070 ± 0.83 | 103.81 ± 4.65 | 706.41 ± 1.02 |
| BEG6 | 98.37 ± 0.53 | 128.89 ± 1.23 | -7.359 ± 8.42 | 106.79 ± 8.29 | 533.30 ± 4.24 |
| BEG8 | 103.74 ± 1.33 | 142.71 ± 9.37 | -6.418 ± 12.87 | 133.72 ± 12.97 | 491.48 ± 6.75 |

* Values are mean ± confidence interval at a 95 % level of significance.
$TC_{onset}$ – Onset temperature; $TC_{endset}$ – Endset temperature; $H_E$ – Peak height; $T_p$ – Peak temperature; and ΔH – Enthalpy.

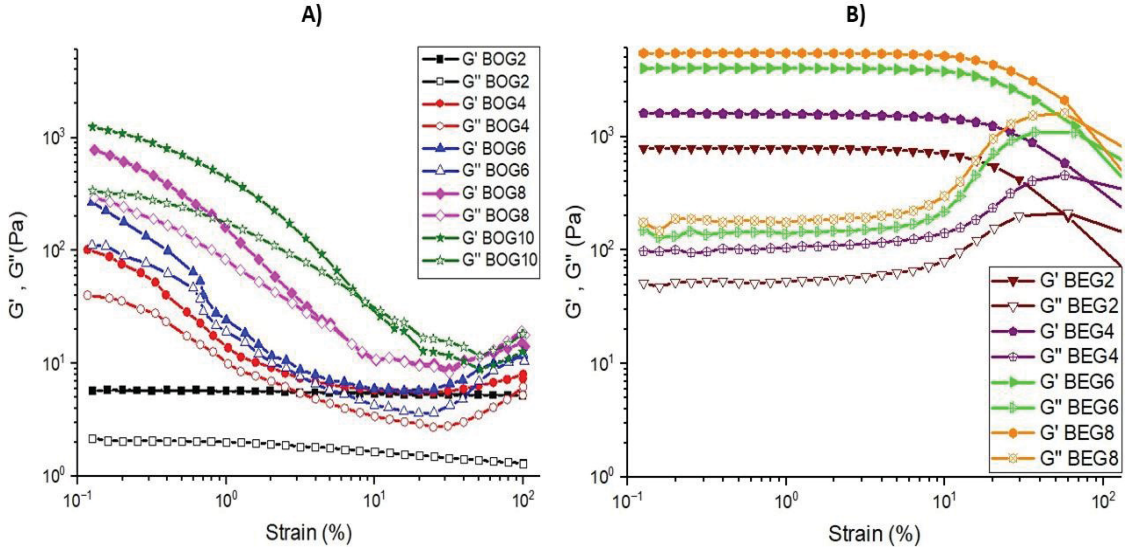
4.4.6. Rheology measurements

In the development of gels, rheological characterization is critical and provides comprehensive insights into their structural integrity and functional performance. By assessing key parameters, rheological analysis elucidates the textural properties, spreadability, and mechanical stability of these systems. This information is crucial for optimizing formulations to replicate the sensory and functional properties of conventional fats while ensuring product stability throughout processing, storage, and consumption. FIGURE 4.5 presents the relation of elastic component (G') and viscous component (G") on the oscillation strain applied to the BOGs and BEGs. It is possible to notice that for all BOG samples present, the G' prevails over the G" at small applied shear, reflecting that the BOGs had a predominantly elastic rather than viscous nature, characteristic of solid/semi-solid gels (FIGURE 4.5A). On the other hand, it does not correlate with a plateau ($G'_{LVR}$) or the LVR, except for BOG2, which exhibits stable elastic and viscous properties. With increasing strain, the G' value starts to decrease, indicating structural deformation or breakdown, which is consistent with the formation of a fat-like crystal network, as mentioned in the PLM topic (Patel et al., 2013). The BOG6 sample demonstrated the highest crossover point (G' = G'') at 11.7 Pa and 99 %; and the BOG8 and BOG10 showed the lowest point (around 10.8 ± 1.6 Pa and 7.0 ± 1.5 %). After the crossover point, the G" > G' and the sample behaves like a fluid (Thakur et al., 2022).

On the other hand, BEGs exhibited G' > G" (FIGURE 4.5B), with both moduli remaining independent of strain amplitude until the critical strain (>12 %). As the strain amplitude exceeded the critical LVR, G' sharply decreased while G" initially increased and subsequently dropped dramatically (FIGURE 4.5B), demonstrating a type III behavior (weak strain overshoot) (Zhao et al., 2022). Additionally, BEGs displayed a higher crossover point at approximately 100 %, signifying the transition from semi-solid-like to liquid-like behavior. This transition likely results from the disruption of intermolecular polymer bonds within the network structure under large deformations, leading to network yielding (Zhao et al., 2022). These results indicate that the BEGs developed in this study could effectively withstand higher stress and better maintain their original gel structure compared to BOGs. In comparison to the development of EG based on gelatin

and European eel oil, it was not possible to observe a stable structure (without LVR), but it presented a crossover point of approximately 650 % for the sample at 20 °C (Taktak et al., 2021).

FIGURE 4.5 – RHEOLOGICAL BEHAVIORS OF EMULSION GELS (BEG) AND OLEOGELS (BOG). PLOTS SHOW THE LOG-LOG PLOT OF THE STORAGE MODULUS (G′) AND VISCOUS MODULUS (G″) AS A FUNCTION OF APPLIED STRAIN FOR BOG (A) AND BEG (B).




## 4.5. Conclusion

OG and EG were developed by incorporating carnauba wax and gelatin as gelling agents into BNO, respectively. Visual analysis indicated that concentrations above 6 % carnauba wax were required to effectively structure BNO, whereas only 2 % gelatin was sufficient to induce a transition from the liquid to the semi-solid state. PLM revealed that crystal formation from carnauba wax was responsible for BNO structuring in OG. At the same time, the presence of emulsion-filled gels in EG samples indicated the formation of a more stable three-dimensional network compared to OG. This was further confirmed by OBC analysis, where OG exhibited a significant variation in OBC, ranging from 30.63 ± 1.34 % (BOG2) to 83.65 ± 1.90 % (BOG10) ($p < 0.05$, TABLE 4.1), whereas all EG samples maintained similar OBC values close to 100 %. Rheological analysis supported these findings, as only BOG6, BOG8, and BOG10 exhibited G’ > G”, while all EG samples demonstrated a semi-solid behavior (G’ > G”) with the presence of an LVR, indicating greater stability than OG. Future studies, such as

thermodynamic investigations, are needed to understand the thermal and structural stability of EG within the specific moisture range of approximately 25 %. Thus, these results suggest the use of an Amazon food matrix to develop OG and EG, which have potential as fat substitutes in food applications (depending on specific formulation requirements) for instance, BEG2 to develop chocolate spreads, highlighting its relevance for the socioeconomic development of the region.

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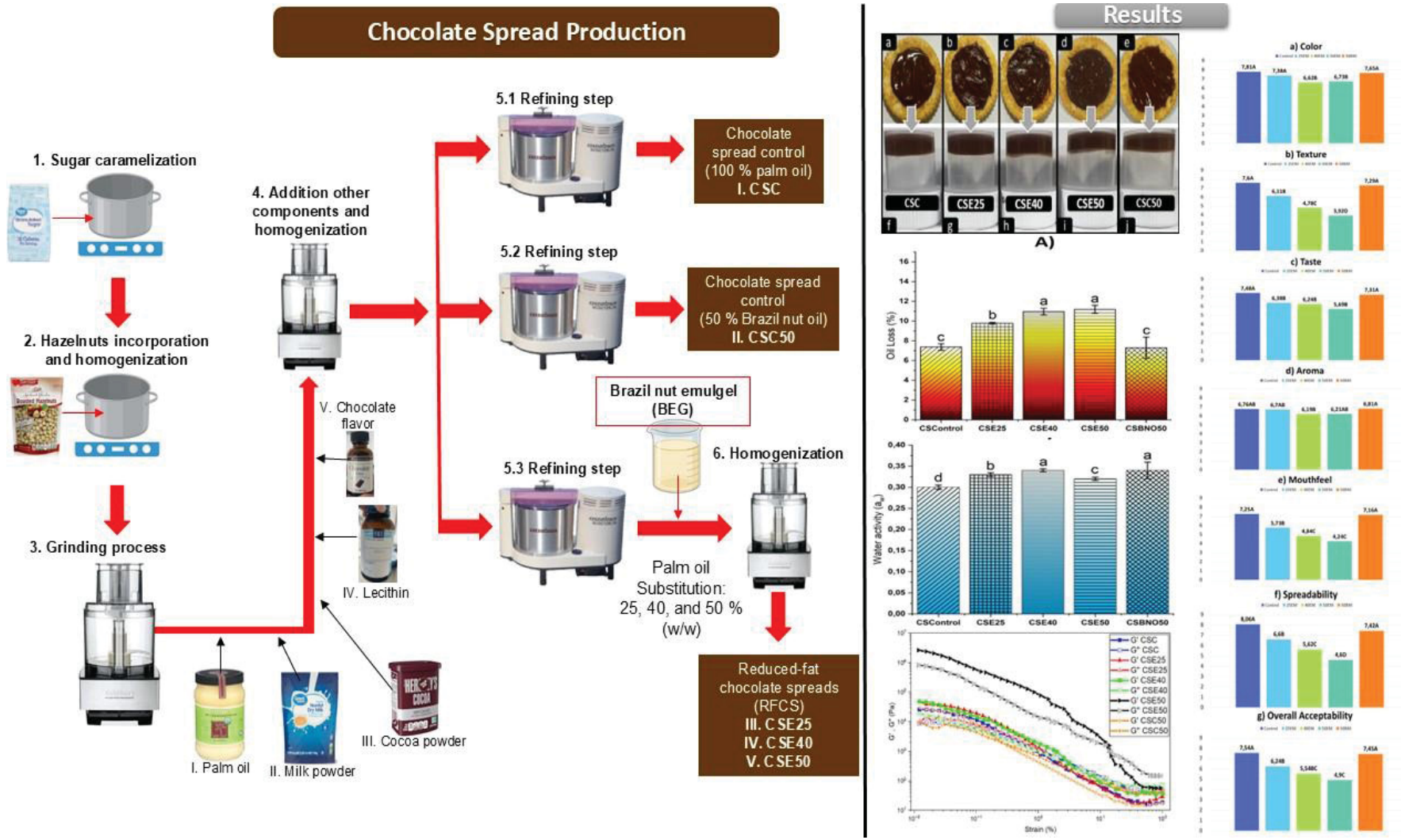
# CHAPTER V

## Brazil nut emulsion gel as an innovative strategy to develop reduced-saturated fat chocolate spreads: physicochemical and sensory evaluation

This chapter consists of the article entitled "Brazil nut emulsion gel as an innovative strategy to develop reduced-saturated fat chocolate spread: physicochemical and sensory evaluation," which **has not been submitted** yet. For this purpose, BEG2 obtained (Chapter III) was applied for the chocolate spread production with different palm oil substitution (proportions: 25/75, 40/60, and 50/50 of BEG2/palm oil). Moreover, chocolate spread with 100 % of palm oil and 50 % of BNO were produced as control samples. Besides, chocolate spread obtained were characterized using morphology, color, oil loss, thermal stability, rheological, texture, and sensory methods. The results obtained in this final paper will serve as a basis for further development/research on the potential of emulsion gels as viable substitutes for SFA and TFA in foods, specifically chocolate spread.

## Graphical abstract



**Brazil nut emulsion gel as an innovative strategy to develop reduced-saturated fat chocolate spreads: physicochemical and sensory evaluation**

## 5.1. Abstract

This study reports the successful development of reduced-fat chocolate spreads (RFCS) using BEG. BNO was incorporated into a gelatin solution at a 1:1 weight ratio (oil:solution) to produce BEG. RFCS were formulated by substituting palm oil with 25 % (CSE25), 40 % (CSE40), and 50 % (CSE50) BEG (w/w) and analyzed in comparison to control samples for physical, thermal, rheological, and sensory properties. All samples exhibited consistent RFCS with a visually uniform texture without graininess or separation. $a_w$ ranged from 0.30 ± 0.01 % (control, CSC) to 0.34 ± 0.02 % (CSE50). Rheological analysis indicated a gel-like structure (G' > G'') in all samples, with CSE25 and CSE40 showing similar behavior to CSC, while higher BEG incorporation increased crossover strain values. Texture measurements revealed firmness variations from 25.64 ± 4.96 N (CSC50) to 139.39 ± 18.83 N (CSE40) and spreadability from 6.29 ± 0.20 N.sec (CSC50) to 30.22 ± 4.12 N.sec (CSE40), demonstrating that BEG enhanced RFCS consistency. Sensory evaluation showed no significant differences ($p < 0.05$) in aroma and color between controls and CSE25, whereas significant differences ($p < 0.05$) were observed in texture, spreadability, mouthfeel, and taste. These findings highlight BEG's potential as a healthier alternative for replacing palm oil partially in chocolate spread formulations.

Keywords: *Bertholletia excelsa*; Amazon oil; Gelatin; structured lipid; and Fat replacer.

## 5.2. Introduction

Chocolate spread are semi-solid emulsified formulations primarily characterized by a smooth and homogeneous texture, allowing easy application on various foods such as pastries, breads, waffles, and pancakes. Palm oil, vegetable oil, or cocoa butter is used as a continuous phase in chocolate spread. This fat phase plays a crucial role in the chocolate spread's texture, consistency, and overall sensory attributes. However, beyond the rising global demand and cost of cocoa butter worldwide, the fat phase is primarily composed of SFA and TFA, which are linked to health problems in recent decades (Toshtay et al.,

2025). For instance, SFA tend to increase in low-density lipoprotein and decrease levels of high-density lipoprotein, known as one of the causes of atherosclerosis and cardiovascular disease (Almeida et al., 2024). Considering this perspective, there are global efforts to reduce SFA and TFA intake, and 53 countries have implemented policies aimed at eliminating industrially produced TFAs (WHO, 2024). These actions have improved the food environment for 3.7 billion people, or 46 % of the world's population, as compared to 6 % just 5 years ago, expecting to save approximately 183,000 lives a year (WHO, 2024).

Despite vegetable oils offering a viable alternative to animal fats by reducing SFA content and improving lipid profiles, direct substitution has resulted in quality degradation, textural challenges, and altered sensory properties (Lee et al., 2025). Various strategies, including OG, hydrogels, hybrid gels (bigels), and emulsions have been investigated to transform liquid vegetable oils into solid fats for use as a continuous-phase substitute in chocolate spread (Almeida et al., 2024; Bascuas et al., 2021; Ghorghi et al., 2023; Prakansamut et al., 2024; Tirgarian et al., 2023; Zampouni et al., 2023). A novel promising approach EG has emerged as a key research area in the food industry (Shahamati et al., 2024). This structuring method consists of a gel matrix incorporating an emulsion dispersion, where at least one phase, either continuous or dispersed, forms a three-dimensional network that enhances physical stability by encapsulating liquid oil and could also serve as carriers for functional substances (Almeida et al., 2024; Lee et al., 2025; Shi et al., 2025). Gelatin, an amphiphilic compound, has been applied as a gelling agent to create a three-dimensional network which has the ability to immobilize substantial amounts of water-oil (Zampouni et al., 2023). Upon heating, its triple-helix protein structure dissociates into random coils, which, during cooling, reassemble into well-ordered triple helices similar to collagen structure. These helices act as "junction zones", facilitating cross-linking and forming a structure responsible for the gel's texture and properties (Samui et al., 2021).

Regarding the vegetable oils with potential use in EG development, there are approximately 84 oilseed species in the Amazon region, identified by Pesce (2009), that present health benefits and, consequently, can be explored to enhance the lipid profile in chocolate spread (Barboza et al., 2022; Berto et al., 2015; Carvalho et al., 2024; Dos Santos et al., 2017; Pereira et al., 2019). In this

sense, BN offers a high concentration of proteins, fibers, bioactive compounds, and a healthy fatty acid composition (Cardoso et al., 2017). The presence of a high concentration of UFA not only can lower TAG and cholesterol levels to reduce cardiovascular risk factors but also exhibit antioxidant and anti-inflammatory properties and provide essential minerals (160 % of the recommended daily selenium intake) (Macan et al., 2024; Maranhão et al., 2011).

Thus, considering the health benefits of BNO and the lack of studies in the literature regarding the use of EGs on chocolate spread, it is necessary to investigate the potential of EGs as a SFA substitute. Consequently, this research aimed to develop a RFCS incorporating BEG. Specifically, the study investigated the effects of varying levels of palm oil substitution on chocolate spread through analyses of morphology, color, oil loss, $a_w$, thermal stability, rheology, texture, and sensory attributes. The findings support the use of structured EGs derived from Amazon oils as an innovative strategy to reduce SFA fat content in chocolate spread and other food products while maintaining desirable rheological properties. Furthermore, EGs offer a viable alternative for the food industry due to their efficiency, rapid processing, and the absence of chemical modifications. Additionally, this research fosters sustainable development in the Amazon by promoting responsible and economically advantageous utilization of its natural resources.

## 5.3. Methodology

### 5.3.1. Materials

Unflavored and colorless gelatin Type A was purchased from MP Biomedicals (Santa Ana, CA, USA), Tween® 20 from Sigma-Aldrich (St. Louis, MO, USA), Sugar and Nonfat Dry Milk from local market (NC, USA), Cocoa powder from Hershey's (PA, USA), Palm oil from Okonatur (Miami, FL, USA), Lecithin from Soybean from Tokyo Chemical Industry (TCI) (Tokyo, Japan), and Roasted Hazelnuts from Nature's Garden (NJ, USA). Deionized water was used and all other chemicals were of analytical grade.

### 5.3.2. Methods

#### 5.3.2.1. Extraction of Brazil Nuts oils (BNO)

The BNO was extracted as described in detail in the preview study by our research group using UTAE (Santos et al., 2025). The process was carried out under the following conditions: time and solvent/solid ratio of 27 min and 26 mL/g, respectively. The oils were kept in an amber glass and stored (−5 °C) until be applied to develop the BEG. BNO contained 28.53 % of SFA (15.68 % of palmitic acid and 12.85 % of stearic acid) and 71.45 % of UFA (37.56 % of oleic acid and 33.89 % of linoleic acid) (Santos et al., 2025).

5.3.2.2. Preparation of Brazil Nuts oil emulgels (BEG)

BEG preparation was started by dissolving 1 % of Tween® 20 in water (v/v) at 25 °C. In the second step, gelatin was added to the proportion of 2 % (m/v) and waited for 30 min to complete the hydration. After hydration, BNO was added to the gelatin solution at a ratio of 1:1 (v/v) and homogenized using a high-speed disperser (digital Ultra-Turrax, T25, IKA, Germany) at 10000 rpm for 2 min. Then, the samples were dehydrated at 50 °C for 24 h or until they reached 25 % moisture, approximately. After drying, the samples were homogenized again using the Ultra-Turrax under the same conditions described previously (10000 rpm for 2 min). After complete dispersion, they were transferred to test tubes and kept in static condition at room temperature (20 ± 4 °C) for 1 h for structuring and stabilization. Finally, the samples were stored at 4 °C until application.

5.3.2.3. Production of chocolate spreads

chocolate spreads were produced using hazelnuts (22 %), sugar (40 %), milk powder (10 %), cocoa powder (7 %), palm oil (20 %), soy lecithin (1 %) and chocolate flavor extract (1 mL). First, the sugar was caramelized at 100 °C / 2 min. Consequently, hazelnuts were added and homogenized with caramelized sugar until they reached room temperature. Subsequently, the hazelnuts with caramelized sugar were ground using a food processor (14-Cup food processor, Cuisinart) for 10 min. Then, the ingredients were added (in the following sequence: palm oil, milk powder, cocoa powder, lecithin, and chocolate flavor) and homogenized for 5 min. Finally, the obtained mixture was added to a chocolate refiner (chocolate melanger ECGC-12SLTA, Cocoatown) and

processed for 20 min to obtain the chocolate spread control (CSC). Another control sample used 50 % palm oil and 50 % BNO (w/w) as replacement (CSC50).

5.3.2.4. Production of reduced-fat chocolate spreads (RFCS)

To produce RFCS, replacements of 25, 40 and 50 % w/w of palm oil by EGs were developed (CSE25, CSE40, and CSE50, respectively). Thus, the production process mentioned above was reproduced. However, due to the chocolate refiner stones changed the texture of the chocolate in concentrations above 50 % BEG in previous tests, the proportions of EGs were only added after the chocolate refining stage. Thus, the BEG was added and mixed for 10 min using a food processor (14-Cup food processor, Cuisinart). The samples obtained were stored in glass vials at 4 °C until analysis.

5.3.3. Characterization of chocolate spreads

5.3.3.1. Macroscopic appearance analysis

First, samples (5 g) were dispersed on the surface of crackers to obtain a first visual impression. After that, the macroscopic appearance analysis was developed according to Wang et al. (2024), with some adaptations: 5 g of samples were used and, after added in a refrigerator at 4 °C for 24 h, the samples were placed at 25 °C for 2 h.

5.3.3.2. Polarized light microscopy observation (PLM)

chocolate spreads microstructures were analyzed using an Olympus BX51 (Japan), attached to a QImaging digital camera (Media Cybernetics, USA), which transmitted live images to a computer using the software Image-Pro Plus 7.0 (Media Cybernetics, USA) to determine optical homogeneity and/or crystalline morphology of samples studied. An aliquot sample was placed on a glass slide with a coverslip gently pressed on top. The samples were analyzed using a 10 × magnification. Images were recorded isothermally at 20 ºC and during heating at 40 °C, 60 ºC and 80 °C at 5 °C/min using a Linkam thermal system (Linkam Scientific Instruments Ltd., Redhill, UK) attached to the microscope stage (Valoppi et al., 2023). Each sample was measured using Image Pro-Plus software version 7.0 for Windows.

#### 5.3.3.3. Color measurement

Color measurement (L*a*b*) was conducted according to Oyom et al. (2024) and the WI values were calculated as mentioned by Li et al. (2021). These parameters were measured at three random surface areas of each sample.

#### 5.3.3.4. Oil loss (OL) and water activity ($a_w$)

The oil loss of the samples was determined by accelerated stability tests using the method mentioned by (Ferdaus; Blount; Silva, 2022). Water activity of samples was quantified using a LabMaster activity meter (Novasina, Lachen, Switzerland) according to Oyom et al. (2025).

#### 5.3.3.5. Thermal behavior

The thermal behavior of samples was analyzed using a differential scanning calorimeter (DSC; TA Instruments, New Castle, DE, USA). Approximately 8–12 mg of each sample was accurately weighed and hermetically sealed in an aluminum pan, with a sealed empty pan as the reference. Prior to analysis, the samples were equilibrated at 20 °C for 1 minute to ensure stabilization within the DSC chamber. The samples were then cooled to −10 °C to facilitate complete crystallization. After holding at −10 °C for 10 minutes, the samples were subjected to a heating cycle, melting from −10 °C to 250 °C at a controlled rate of 5 °C/min under a continuous nitrogen gas flow (50 mL/min). Key thermal parameters, including onset temperature (°C), endset temperature (°C), peak height (W/g), peak temperature (°C), and melting enthalpy (ΔH, J/g), were determined from the melting curves. All measurements were performed in triplicate.

#### 5.3.3.6. Rheological measurements

To evaluate the viscoelastic properties of the samples, dynamic oscillatory tests (strain sweep) were determined using a rheometer (Discovery HR-10, TA Instruments, New Castle, DE, USA). The upper fixture was a parallel plate (25 mm), and about 4 g of dough was put into the rheometer. Trimmed to remove the excess dough outside of the fixture when the gap was 2050 µm. The strain sweep test was performed in the strain range 0.1–100 % (at a constant frequency

of 1 Hz and a temperature of 25 °C) to determine the following parameters: 1. Linear viscoelastic range (LVR or γ LVR); 2. Crosspoint of storage modulus (G') and loss modulus (G") (flow point, G'=G").

5.3.3.7. Texture analysis

The spreadability, firmness, and adhesivity of the chocolate spreads were evaluated using a texture analyzer TA-XT2 from Texture Technologies Corp. (Hamilton, MA, USA) equipped with a 5000 g load cell and a TTC Spreadability Rig (HDP/SR). This rig consists of a precisely matched male (positive) and female (negative) acrylic 90° cone. The instrument was calibrated for a load of 5000 g and a penetration depth of 25 mm. Measurements were performed at a test speed of 1.0 mm/s, with the probe penetrating 25 mm into the sample. Each sample was analyzed five times (quintuplicate) at room temperature to ensure reproducibility. Data acquisition and analysis were conducted using Stable Micro Systems software. The method was adapted from Ferdaus et al. (2022), with modifications.

5.3.3.8. Sensory analysis

The sensory evaluation was conducted under controlled room conditions to ensure accurate and reliable results. The evaluation focused on multiple attributes, including color, aroma, texture, spreadability, mouthfeel, taste, and overall acceptability. A 9-point hedonic descriptive scale was employed for scoring, ranging from 1 (dislike extremely) to 9 (like extremely), allowing panelists (n = 63) to provide precise and detailed feedback. The panelists were non-vegetarian and had no known allergies to any raw material used. The age range of the panelists was between 21 and 50 years, ensuring a representative demographic. Before the sensory test, the selected panelists underwent a preparatory session to provide them with a clear understanding of how to assess and rate the parameters of the samples. The room conditions during the sensory evaluation were carefully controlled to create a standardized testing environment. Additionally, the samples were presented to the panelists in a randomized order to avoid any systematic effects using a link to a survey presented on Compusense software (Compusense Inc., Guelph, Ontario, Canada) and asked to complete the survey on their personal devices. This study was reviewed and approved by the Institutional Review Board (IRB) at North Carolina A&T State

University, ensuring adherence to ethical standards for research involving human subjects.

#### 5.3.3.9. Statistical analysis and visualization

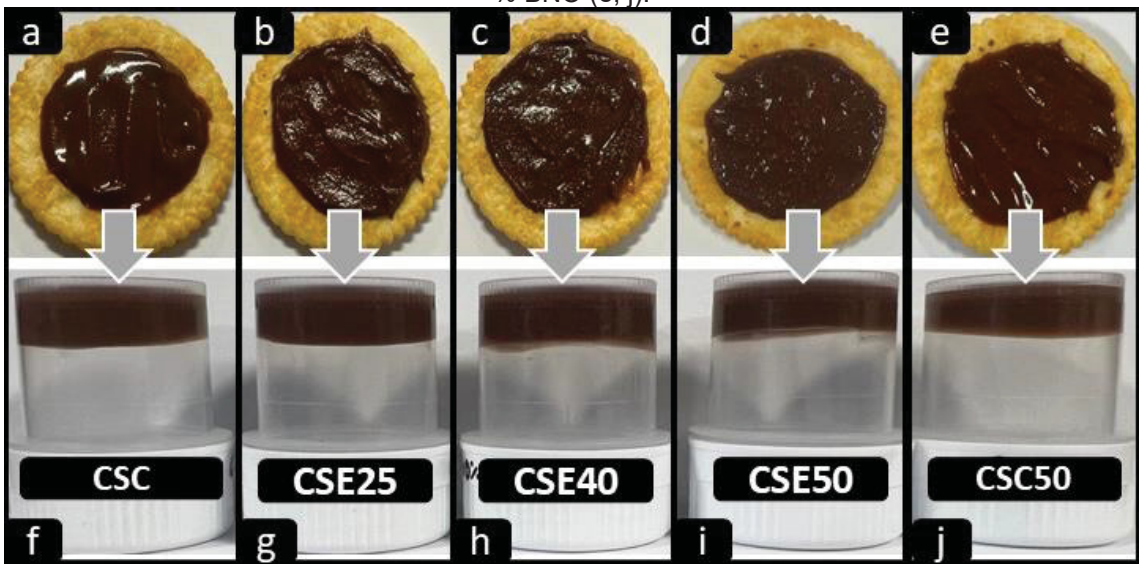
Results were expressed as mean ± confidence interval at a 95 % significance level (at least two measurements from three experimental replicates) and statistically analyzed using one-way analysis of variance (ANOVA) using Statistica 7.0 software (Statsoft Inc., Tulsa, OK, USA). Differences among treatments were identified using Tukey's test.

## 5.4. Results and discussion

### 5.4.1. Macroscopic appearance analysis

FIGURE 5.1 presents the visual test and macroscopic appearance analysis of chocolate spreads. All samples had uniform texture without graininess or separation, no visible bubbles, and a slight sheen, indicating proper fat crystallization and emulsification. In contrast, by increasing the BEG concentration, the RFCS had a darker brown general color and better textural integrity, differing from the findings of Tirgarian et al. (2023), who reported that incorporating water-OG emulsions reduced these visual characteristics in chocolate spreads. Regarding the macroscopic appearance analysis, it can be seen that BEG was efficient in stabilizing the structure of the chocolate spread after the inversion (FIGURE 5.1.f-j) and worked as a continuous phase (substitute of solid fat, e.g., cocoa butter, palm oil and other). As a continuous phase, BEG performs a fundamental role in the product structure, providing a crystalline network for dispersing solid particles (cocoa powder, sugar, milk, and others).

FIGURE 5.1 – VISUAL TEST (a-e) AND MACROSCOPIC APPEARANCE ANALYSIS (f-j) OF CHOCOLATE SPREADS WITH BRAZIL NUT EMULSION GEL (BEG). SAMPLES: CSC WITH 100 % PO (a, f); CSE25 WITH 75 % PO AND 25 % EG (b, g); CSE40 WITH 60 % PO AND 40 % EG (c, h); CSE50 WITH 50 % PO AND 50 % EG (d, i); AND CSC50 WITH 50 % PO AND 50 % BNO (e, j).



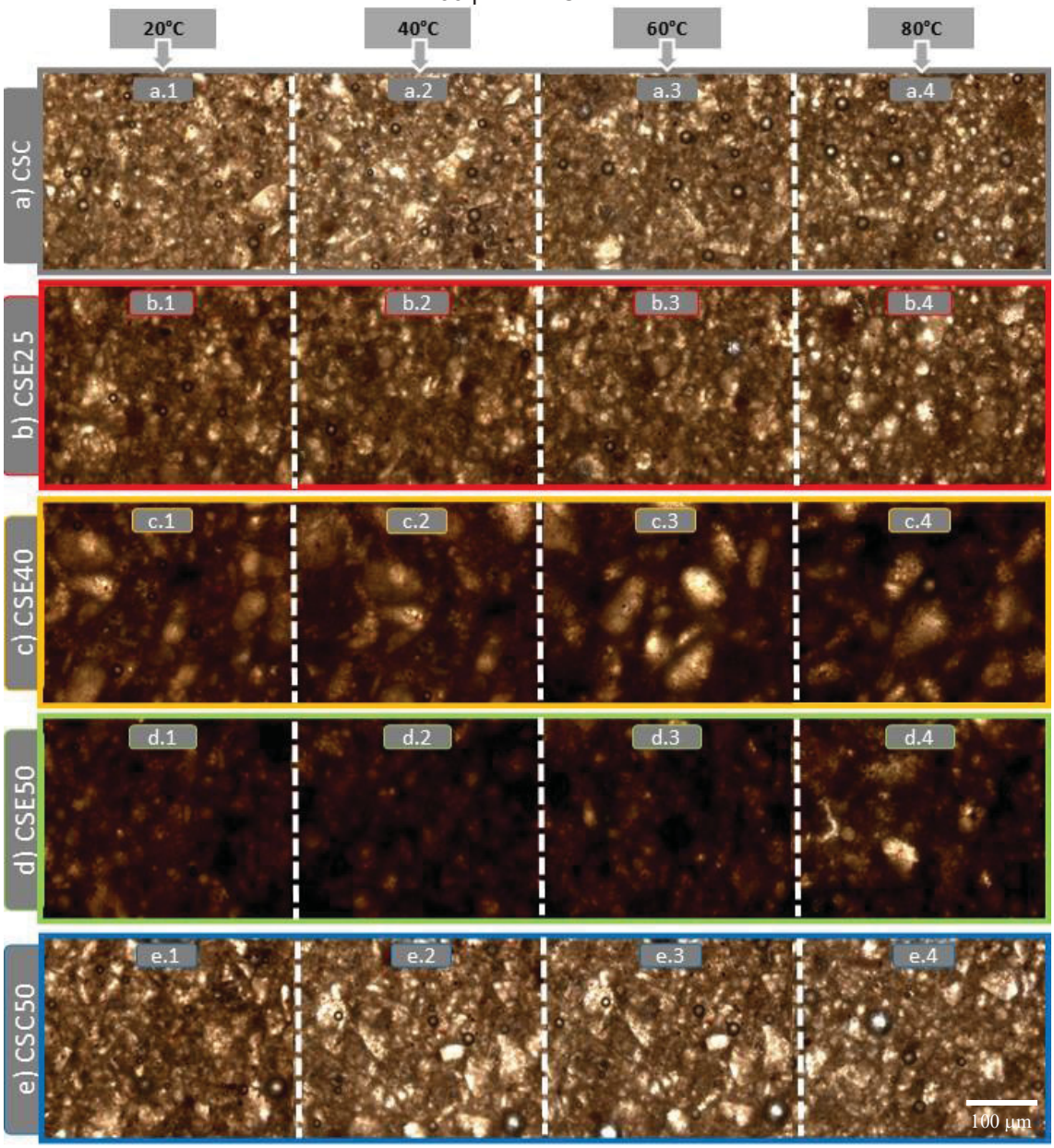

5.4.2. Polarized light microscopy observation (PLM)

PLM was used to reveal the microstructure morphology of RFCSs made with different proportions of BEG (FIGURE 5.2). Cocoa powder (brown color) and sugar (bright part) presented no polarization for all samples (FIGURE 5.2.a1-e1), and brown color was more intense with increasing of BEG. Besides, emulsion droplets could be identified for all samples, demonstrating the successful formation of the emulsion structure. When the spreads were made with a mixture of palm oil and BNO (CSC50), their appearance was more similar to the CSC than those made with BEG due to the control samples having a homogeneous droplet-droplet interactions and, consequently, crystalline network dispersed with a large specific surface area. As mentioned by Afoakwa et al. (2009), a higher fat content generates less dense sugar crystal networks and reduced particle-particle interactions, providing more open structures and empty spaces between crystals.

In contrast, the incorporation and gradual increase of BEG concentration in the spreads (CSE25, CSE40, and CSE50) led to structural changes compared to the control samples (CSC and CSC50), primarily due to two factors: a higher water-surfactant ratio results in a larger interfacial area that needs to be stabilized (Prosapio; Norton, 2019); and BEG can also led to the replacement of fat-fat

interactions with gelatin-fat and gelatin-sugar interactions. As consequence, higher water fraction and gelatin-fat interactions can generate a wide range of particle sizes, with smaller droplets forming a tightly packed network that enhances droplet–droplet interactions by occupying the voids among the large ones (FIGURE 5.2c and 2d). Besides, compared with the effects of amphiphilic compounds in another study (Bascuas et al., 2020), gelatin improves the coalescence with the formation of a hard and thick interface layer, limiting the mobility of the globules inside of the matrix. As result, these interactions could limit the mobility of the chocolate matrix, leading to higher textural, viscosity, and thicker chocolate spread with a dense agglomeration of structured fat droplets (FIGURE 5.2.b-d) (Tirgarian et al., 2023). These effects align with the results aforementioned at visual analysis.

FIGURE 5.2 – POLARIZED LIGHT MICROSCOPIC IMAGES OF CHOCOLATE SPREADS MADE WITH BRAZIL NUT EMULSION GEL (BEG) AT DIFFERENT TEMPERATURES. SAMPLES: a) CSC WITH 100 % PO); b) CSE25 WITH 75 % PO AND 25 % EG; c) CSE40 WITH 60 % PO AND 40 % EG; d) CSE50 WITH 50 % PO AND %) % EG; e) AND CSC50 WITH 50 % PO AND 50 % BNO. THE NUMBERS FROM LEFT TO RIGHT INDICATE THE INCREASE IN TEMPERATURE: 1. 20 °C; 2. 40 °C; 3. 60 °C; AND 4. 80 °C). IMAGES SHOW 100 µm IN SCALE.




## 5.4.3. Color measurement

Color plays a critical role in the development of chocolate spread, influencing consumer perception, product quality, and shelf stability. A lighter (higher L*) color and more smooth appearance are often associated with high-quality products and provide greater consumer acceptance, while deviations may

reflect variations in formulation, fat content, or oxidative stability (Sözeri Atik et al., 2020). TABLE 5.1 presents chromatic characteristics such as L*, WI, a*, and b* (chromaticity coordinates). With the incorporation of BEG in the chocolate spread, a* and b* decreased with significant variation ($p < 0.05$) from 3.75 ± 0.47 and 3.63 ± 0.30 (CSC) to 1.74 ± 0.32 and 2.30 ± 0.32 (CSE50), respectively.

As previously demonstrated, the addition of BEG resulted in a darker chocolate spread. This is supported by the significant reduction ($p < 0.05$) in both L* and WI values, with L* decreasing from 20.54 ± 0.06 and 20.37 ± 0.04 in the control sample (CSC) to 12.49 ± 2.74 and 13.66 ± 2.75 in CSE50, respectively. This result is consistent with the findings of Almeida and Lannes (2017), who reported similar effects upon incorporating chicken by-product gelatin into chocolate spread. Despite gelatin being colorless, the gel network formed can modify how light interacts with the spread due to the structure modification related to the increased interactions between particles (Oba; Yıldırım, 2024), stabilizing fat (SFA) and water interactions, giving the impression of a deeper, richer color. Besides, enhanced interactions in chocolate spread can create high light scattering, generating a dark contrast in chocolate spread (Afoakwa et al., 2009). Additionally, the brown color may be more intense with friction between the ingredients and the mixing blades, generating additional heat that could elevate the temperature sufficiently to promote the Maillard reaction, leading to potential darkening of the final product (Kchaou et al., 2018).

TABLE 5.1 – COLOR PARAMETERS RESULT FOR CHOCOLATE SPREADS.

| | Color | | | |
|---|---|---|---|---|
| **Samples** | $L^*$ | $a^*$ | $b^*$ | Whiteness index (WI) |
| CSC | 20.54 ± 0.06$^{a}$ | 3.75 ± 0.47$^{a}$ | 3.63 ± 0.30$^{a}$ | 20.37 ± 0.04$^{a}$ |
| CSE25 | 17.47 ± 3.19$^{bc}$ | 2.43 ± 0.35$^{b}$ | 2.42 ± 0.39$^{b}$ | 17.72 ± 3.18$^{bc}$ |
| CSE40 | 15.34 ± 0.16$^{cd}$ | 2.15 ± 0.51$^{bc}$ | 2.42 ± 0.36$^{b}$ | 15.30 ± 0.16$^{cd}$ |
| CSE50 | 12.49 ± 2.74$^{d}$ | 1.74 ± 0.32$^{c}$ | 2.30 ± 0.32$^{b}$ | 13.66 ± 2.75$^{d}$ |
| CSC50 | 17.77 ± 3.35$^{ab}$ | 3.40 ± 0.37$^{a}$ | 2.34 ± 0.07$^{b}$ | 18.67 ± 3.33$^{ab}$ |

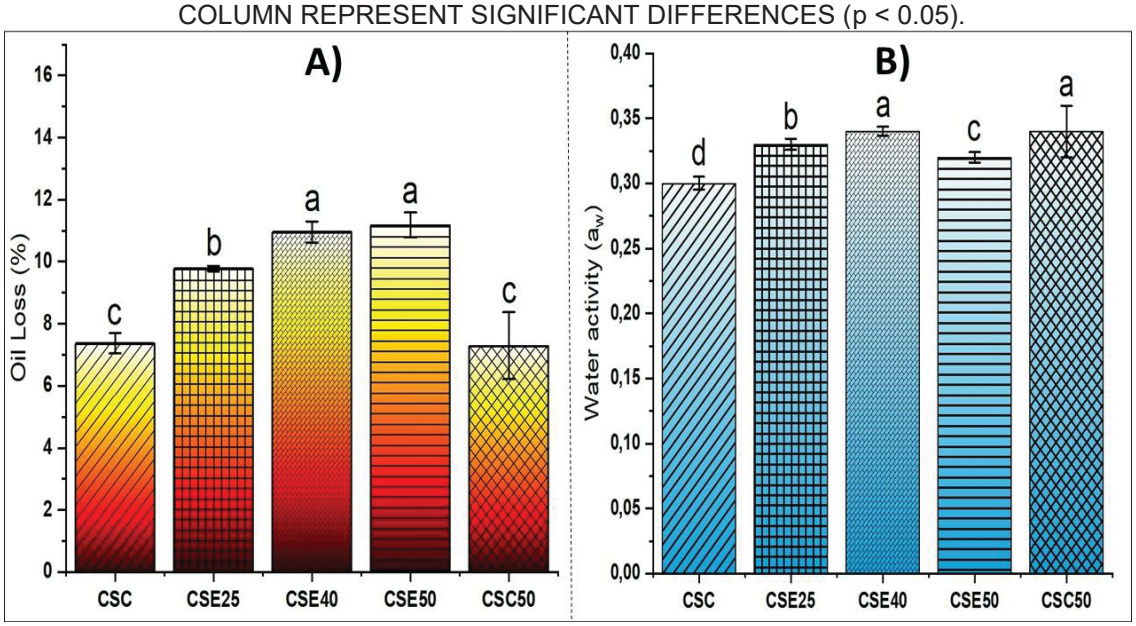
*Values are mean ± confidence interval at a 95 % level of significance; Different letters superscripted within the same column represent significant differences ($p<0.05$). Samples: CSC with 100 % PO; CSE25 with 75 % PO and 25 % EG; CSE40 with 60 % PO and 40 % EG; CSE50 with 50 % PO and %) % EG; and CSC50 with 50 % PO and 50 % BNO. L* = color intensity, L* = 100 for lightness, and L* = 0 for darkness; +a* = increasing red, -a* = increasing green; +b* = increasing yellow, -b* = increasing blue.

### 5.4.4. Oil loss (OL)

OL is a critical factor in the development of chocolate spread because it directly affects the product's texture, stability, and shelf-life. According Manzocco et al. (2014), chocolate spread can be considered physically stable when oil release upon centrifugation is below 10 %. The OL results are shown in FIGURE 5.3, CSC (7.37 ± 0.33 %) and CSC50 (7.29 ± 1.08 %) exhibited the lowest values, meeting this stability criterion without significant differences ($p < 0.05$). Among the formulations with gelatin addition, only CSE25 (9.78 ± 0.09 %) remained below the 10 % threshold. For formulations with 40 % and 50 % palm oil substitution, although CSE40 (10.96 ± 0.34 %) and CSE50 (11.18 ± 0.40 %) slightly exceeded this limit, both showed significant differences ($p < 0.05$) compared to the other formulations. Increasing the fluidity of chocolate spread by replacing SFAs (solid) with UFAs (liquid) generates a greater tendency to release oil after centrifugation, even with the structuring of BNO with gelatin (Cozentino et al., 2022; Manzocco et al., 2014). Moreover, this phenomenon can also occur due to the formation of distinct agglomerates of structured fat droplets (different density), which, when subjected to centripetal force, led to the separation of particulate material and oil (UFA), resulting in increased OL in the chocolate spread. Conversely, Prakansamut et al. (2024) incorporated different types of OGs in chocolate spread and identified higher values (from 11.19 to 15.44 %)

than those mentioned in the present study (FIGURE 5.3), which demonstrates the excellent potential for BEG application in chocolate spread.

FIGURE 5.3 – A) OIL LOSS (OL), AND B) WATER ACTIVITY (aw) RESULTS FOR CHOCOLATE SPREADS. VALUES ARE MEAN ± CONFIDENCE INTERVAL AT A 95 % LEVEL OF SIGNIFICANCE. DIFFERENT LETTERS SUPERSCRIPTED WITHIN THE SAME COLUMN REPRESENT SIGNIFICANT DIFFERENCES (p < 0.05).



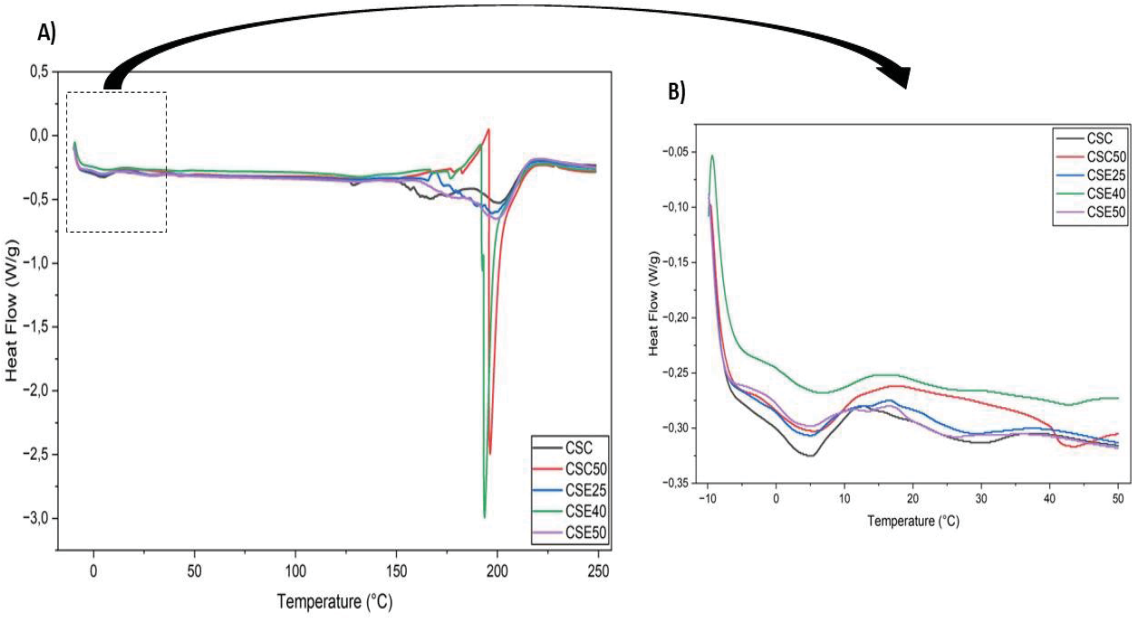

5.4.5. Water activity ($a_w$)

$a_w$ is crucial in chocolate spread development and its control ensures product safety, prevents phase separation, and maintains desirable consistency throughout storage, directly impacting shelf-life, sensory attributes, and overall quality of the final formulation. According to Tirgarian et al. (2023), chocolate spread are products with high $a_w$ content (0.80–0.87), and higher $a_w$ products are susceptible to microbial growth and rancidity. As expected, RFCS presented high free water ($a_w$) (0.34 ± 0.02 % CSE40 and CSC50) compared the control (0.30 ± 0.01 % CSC) due the water concentration in BEG (FIGURE 5.3). Furthermore, despite the samples presenting significant differences (p < 0.05), the results lower than those mentioned in the literature (Acan et al., 2021b; Almeida; Lannes, 2017; Shahamati et al., 2024; Tirgarian et al., 2023; Tolve et al., 2021). These results indicate product stability and extended shelf-life, as lipid oxidation progresses slowly within the $a_w$ range of 0.2-0.4 (Acan et al., 2021b; Tolve et al., 2021).

### 5.4.6. Thermal behavior (DSC)

Given the scarce use of EGs as fat or shortening substitutes in food applications, it is critical to evaluate their melting profiles to ensure that they closely reproduce the thermal behavior of conventional fats, thus preserving the desired sensory and textural properties of the final product (Sejwar et al., 2024). Analyzing the chocolate spread components, lipid sources, such as palm oil and cocoa butter, are primarily responsible for the phase transitions occurring within the oral temperature range (32–34 °C) (Tirgarian et al., 2023). Accordingly, FIGURE 5.4B shows the thermograms amplification of the main effects observed in the control samples and in the presence of EGs occur. It can be observed that the thermal profiles were comparable within the oral temperature range (without peaks), indicating that the addition of up to 50 % EGs can promote similar melting behavior.

FIGURE 5.4 – THERMAL BEHAVIOR RESULTS (DSC) FOR CHOCOLATE SPREADS (CS) WITH BRAZIL NUT EMULSION GELS (CSE) AND BRAZIL NUT OIL (CSC50). A) TEMPERATURE RANGE BETWEEN -10 °C TO 250 °C. B) AREA AMPLIFICATION: FROM -10 °C TO 50 °C).

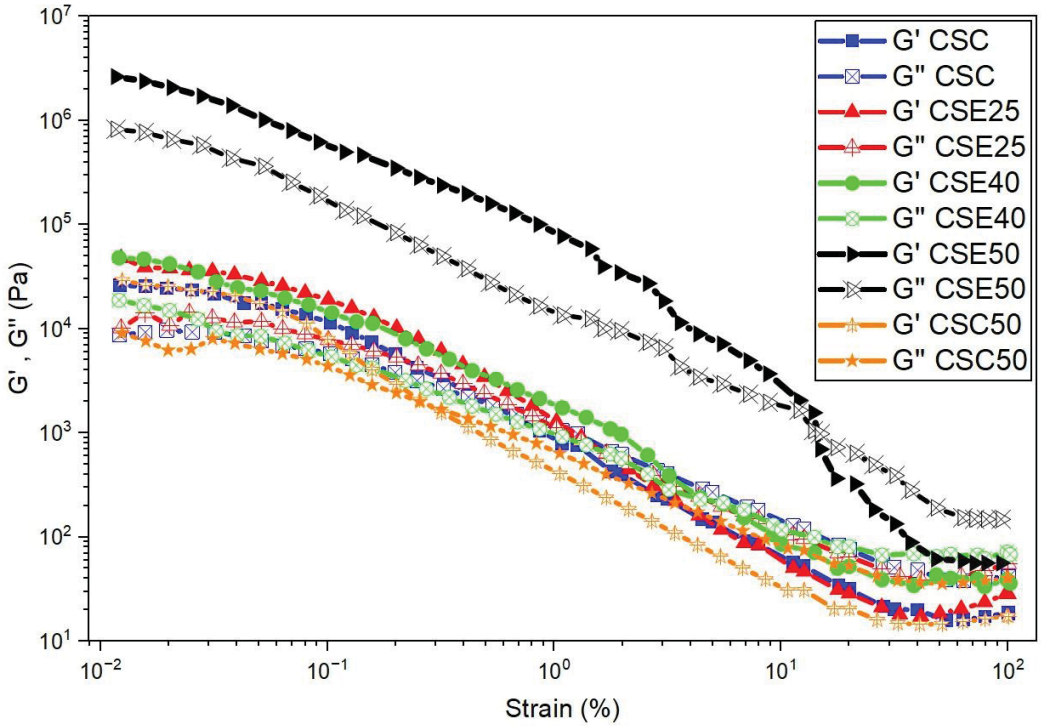



### 5.4.7. Rheological measurements

Rheological measurements play a crucial role in the chocolate spread development and provide important data to analyze the plasticity, stability, structure of fat crystals network, and sensory properties for improved processing, storage, and consumer acceptance. In this sense, the strain sweep was applied

as an initial test to study the behaviors of the storage modulus (G′, which indicates the solid-like nature) and loss modulus (G′′, which indicates the fluid-like nature) and to determine the LVR (the range where the material's structure remains intact under small deformations). As illustrated in FIGURE 5.5, the strain sweeps of all chocolate spread shown G′ > G'' (solid-like behavior and elastic behavior) in a non-linear region (from 0.01 to 100 %). Variations in the crossover point were observed where G' = G'', indicating the transition to a liquid-like state. Hence, lower crossover points (0.2 % and 0.4 %) are associated with CSC50 and CSC, respectively. Conversely, incorporating BEG led to higher crossover strain values (2 % for CSE25), which increased further with higher BEG content in chocolate spread (4 % and 20 % for CSE40 and CSE50, respectively). The crossover point represents the specific strain level at which the material's internal structure collapses, resulting in a transition to liquid-like behavior (Upadhyay; Chen, 2020). Thus, this rheological behavior suggested a progressive enhancement in the internal structural integrity (20 % to 50 % BEG) and rheological attributes of the chocolate spreads as the fat content was substituted with BEG. This result confirms the discussion mentioned before, where the incorporation of BEG change the interactions between the continuous and solid phase, creating heterogeneity (irregular shapes) in the fat crystal network impacting in the structure (dispersion of solid particles). Additionally, Acan et al. (2021a) highlighted the presence of different particle sizes may affect the rheological properties of chocolate spreads.

FIGURE 5.5 – RHEOLOGICAL BEHAVIORS OF CHOCOLATE SPREADS MADE WITH BRAZIL NUT EMULSION GEL (BEG). PLOTS SHOW THE LOG-LOG PLOT OF THE STORAGE MODULUS (G′) AND VISCOUS MODULUS (G″) AS A FUNCTION OF APPLIED STRAIN, RESPECTIVELY. SAMPLES: CSC WITH 100 % PO; CSE25 WITH 75 % PO AND 25 % EG; CSE40 WITH 60 % PO AND 40 % EG; CSE50 WITH 50 % PO AND %) % EG; AND CSC50 WITH 50 % PO AND 50 % BNO.




Regarding the differences between the samples, CSE50 exhibited a distinct rheological behavior compared to the others, with higher G’ and G’’ values (indicating a strong internal structure) followed by a more pronounced decline. According Patel et al. (2014), the increase in the dispersed aqueous phase difficult the formation of small droplets sizes and the reduction in the crystalline phase (the decrease in solid fat content) promote the agglomeration of water droplets. Consequently, the choice of emulsifier is identified by these researchers as a critical and challenging factor in the chocolate spread development with reduced solid fat content (in terms of emulsification and stabilization). These modifications in chocolate spread were also identified by these authors as contributing to structural instability and phase separation, resulting from the 40 % reduction in fat content by shellac (a food-grade resin) emulsion. This may explain preliminary results identified in this work, where it was noted that concentrations above 50 % BEG impacted a change in the chocolate spread structure, changing

from a spread to an elastic gum with a separated oil fraction. Moreover, You et al. (2023) reported that additions of more than 50 % water-oil emulsion (ratios of 4:6 and 3:7, v/v) using arabic gum as surfactant generated fat-reduced chocolate with rough surface and relatively poor shape retention.

### 5.4.8. Texture

The parameters of texture (firmness, spreadability, and adhesiveness) are key factors influencing sensory acceptance of chocolate spread. Firmness refers to the maximum force required for deformation, and the results are presented in TABLE 5.2. The firmness outcomes for CSC (38.78 ± 2.56 N) and CSC50 (25.64 ± 4.96 N) were similar without significant difference ($p < 0.05$). Compared with BEG samples, higher firmness (N) was observed and CSE40 (139.39 ± 18.83) and CSE50 (134.63 ± 19.22) showed similar results ($p < 0.05$). In relation the spreadability (N.sec) – work of shear or the force needed to initiate and maintain flow across a surface – the same patterns (the highest value, $p < 0.05$) were showed with CSE40 (30.22 ± 4.12) and CSE50 (24.26 ± 5.63) compared with CSC (7.60 ± 1.17) and CSC50 (6.29 ± 0.20), impacting . In addition, regarding the adhesiveness data (TABLE 5.2), CSE40 (-2.05 ± 0.35 N.sec) and CSE50 (-1.01 ± 0.09 N.sec) were also the highest values ($p < 0.05$), demonstrating high capability to adhere to surfaces than CSC (-2.66 ± 0.26 N.sec), CSC50 (-2.46 ± 0.19 N.sec), and CSE25 (-3.88 ± 0.81 N.sec). Hence, as the BEG content increased, the chocolate spread became less spreadable, harder structure and more adhesive. Thus, this was in agreement with the aforementioned data, mainly obtained from the rheological assessments (CSE40 and CSE50) due to the incorporation of gelatin and, consequently, alteration in the concentration of solid fat crystals within the continuous oil phase. As a result, there is greater intermolecular interaction among particulate matter (mainly crystalline sugar particles) at a reduced fat concentration (Patel et al., 2014). As mentioned by Manzocco et al. (2014), nonspecific interactions occur between the dry ingredients and the lipid one so that the final firmness of the spread, for example, is mainly the result of the physical properties of the lipid fraction. Considering previous works, Acan et al. (2021a) studied the effect of grape pomace usage in chocolate spread to estimate physical and digestibility properties. They found that

the amount of fiber added increased the spreadability from 2.0 N.mm (control) to 4.24 N.mm (chocolate spread with 15 % of red grape pomace), generating a harder structure.

TABLE 5.2 – TEXTURE PARAMETERS (FIRMNESS, SPREADABILITY, AND ADHESIVENESS) OF CHOCOLATE SPREADS MADE WITH WITH BRAZIL NUT EMULSION GEL (BEG).

| Sample | Texture parameters | | |
|---|---|---|---|
| | Firmness (N) | Spreadability (N.sec) | Adhesiveness (N.sec) |
| CSC | 38.78 ± 2.56$^{bc}$ | 7.60 ± 1.17$^{cd}$ | -2.66 ± 0.26$^{c}$ |
| CSE25 | 41.58 ± 7.41$^{b}$ | 10.58 ± 0.27$^{c}$ | -3.88 ± 0.81$^{d}$ |
| CSE40 | 139.39 ± 18.83$^{a}$ | 30.22 ± 4.12$^{a}$ | -2.05 ± 0.35$^{b}$ |
| CSE50 | 134.63 ± 19.22$^{a}$ | 24.26 ± 5.63$^{b}$ | -1.01 ± 0.09$^{a}$ |
| CSC50 | 25.64 ± 4.96$^{c}$ | 6.29 ± 0.20$^{d}$ | -2.46 ± 0.19$^{bc}$ |

*Values are mean ± confidence interval at a 95 % level of significance; Different letters superscripted within the same column represent significant differences ($p < 0.05$). Samples: CSC – 100 % PO; CSE25 – 75 % PO : 25 % EG; CSE40 – 60 % PO : 40 % EG; CSE50 – 50 % PO : 50 % EG; and CSC50 with 50 % PO : 50 % BNO.

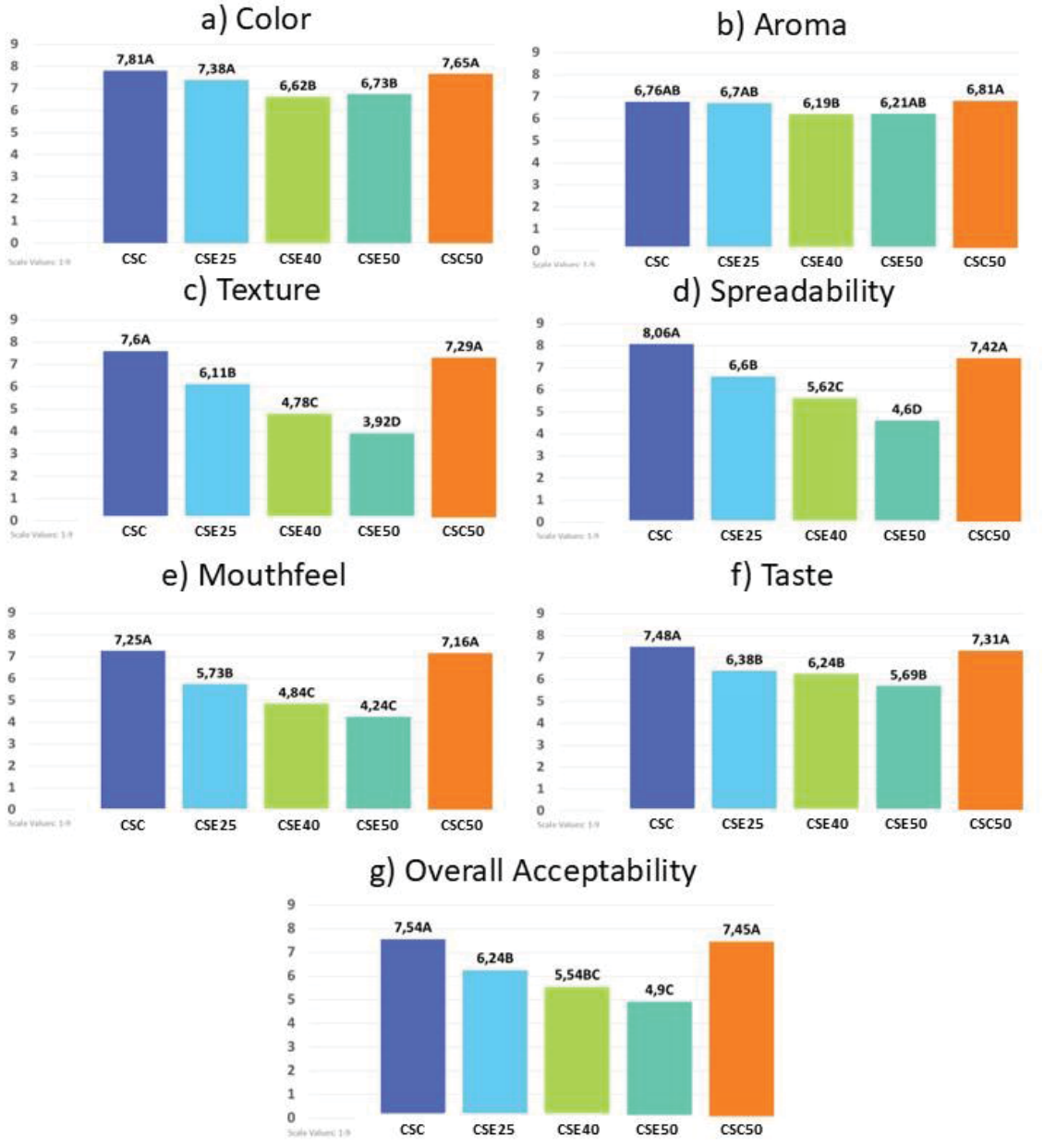
### 5.4.9. Sensory analysis

The hedonic test using a 9-point hedonic scale was conducted to evaluate the sensory attributes of chocolate spread (FIGURE 5.6). The panelists' assessment indicated that sensory properties were significantly affected ($p < 0.05$) by the substitution rate, except for aroma. However, none of the samples achieved the highest rating of "like extremely" (9), with scores ranging from "neither like nor dislike" to "like moderately". For color evaluation, a significant difference ($p < 0.05$) was observed between the samples, with scores ranging from 6.6 (CSE40) to 7.8 (CSC). These results align with findings from macroscopic appearance, PLM, and color measurements (Section 3.1–3), where an increased BEG content resulted in darker samples, thereby affecting the panelists' color perception. Regarding aroma (FIGURE 5.6b), CSC50 received the highest score (6.8), followed by CSC (6.7), with no significant differences ($p < 0.05$) between CSC and CSE25, CSE40, or CSE50.

In terms of texture preference (FIGURE 5.6c), CSC was rated highest (7.6), showing a significant difference ($p < 0.05$) compared to the others. These findings correlate with texture parameters (TABLE 5.2), where RFCSs exhibited

higher firmness and adhesiveness, highlighting the importance of maintaining a soft texture for better consumer acceptance. Spreadability also presented the same behavior, ranging from 4.6 (CSE50) to 8.1 (CSC). These results impacted directly the mouthfeel and taste (FIGURE 5.6e and 5.6f, respectively), where the high spreadability and firmness value (TABLE 5.2) – required greater force for spreading – generated medium panelists preferences (neither like or dislike to like moderately) for CSE25, CSE40, and CSE50 than CSC and CSC50. According Oba and Yıldırım (2024), higher textural and rheological measurements in spreads are commonly associated to negative sensory evaluation (low appreciation by panelists or consumers). For overall acceptability (FIGURE 5.6g), CSC and CSC50 were scored as like moderately ($p < 0.05$) and BEG ranged from neither like or dislike (4.9) to like slightly (6.4) for CSE50 and CSE25, respectively. These results indicate that while the incorporation of BEG influenced sensory perception, particularly in terms of texture and spreadability, moderate substitution levels (e.g., CSE25) still maintained an acceptable sensory profile. On the other hand, we believe that applying a better chocolate spread production process (more specific and optimized equipment) could improve these sensorial attributes, mainly regarding the high BEG proportion (CSE40 and CSE50).

FIGURE 5.6 – RESULTS OF SENSORY PARAMETERS OF CHOCOLATE SPREADS. PO: PALM OIL, BNO: BRAZIL NUT OIL, AND EG: EMULSION GEL. SAMPLES: CSC – 100 % PO; CSE25 – 75 % PO : 25 % EG; CSE40 – 60 % PO : 40 % EG; CSE50 – 50 % PO : 50 % EG; AND CSC50 WITH 50 % PO : 50 % BNO. a) COLOR; b) AROMA; c) TEXTURE; d) SPREADABILITY; e) MOUTHFEEL; f) TASTE; AND g) OVERALL ACCEPTABILITY. VALUES ARE MEAN AND DIFFERENT LETTERS SUPERSCRIPTED IN THE BARS REPRESENT SIGNIFICANT DIFFERENCES ($p < 0.05$). SCALE VALUE: 1 (DISLIKE EXTREMELY); 2 (DISLIKE VERY MUCH); 3 (DISLIKE MODERATELY); 4 (DISLIKE SLIGHTLY); 5 (NEITHER LIKE OR DISLIKE); 6 (LIKE SLIGHTLY); 7 (LIKE MODERATELY); 8 (LIKE VERY MUCH);AND 9 (LIKE EXTREMELY).




## 5.5. Conclusion

This study demonstrated that 50 % of emulsion gel can act as an effective replacement for palm oil in chocolate spread formulations, enabling the production of reduced-fat alternatives while improving structural integrity and

keep sensory attributes, such as aroma and color. Moreover, oil loss and $a_w$ content of chocolate spread with BEG was notably low, which show structure stability and may reduce their susceptibility to microbial growth, respectively. Therefore, the partial replacement of fat with BEG in traditional spreads presents a promising approach for reformulating foods with a healthier nutritional profile. Conversely, further research is needed to increase the level of palm oil substitution and to approximate the texture – particularly firmness and spreadability – of RFCSs with traditional chocolate spread, thereby improving consumer acceptance. Additionally, future studies could explore approaches such as modifying the production process of chocolate spread, combining BEG with oleogel to develop products that more closely mimic traditional chocolate spread or even applying BEG to develop other food products (cake, ice cream, and others).

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# CHAPTER VI

## Contributions and suggestions for future research

This chapter aims to concisely present the main contributions of this thesis, from the author's perspective. These contributions are organized into four key dimensions: social, scientific, technological, and economic, reflecting the multidimensional impact of the work developed. In addition, suggestions for future research are proposed, which may serve as a conceptual, methodological, or applied foundation for researchers interested in further exploring the topics addressed in this study, thereby expanding the frontiers of investigation in the field.

## Contributions and suggestions for future research

### 5.7. Contributions

#### 5.7.1. Social relevance

This research aims to contribute to the reduction of socioeconomic inequalities through the valorization of native oilseeds from the Amazon region, fostering income generation for traditional communities, particularly riverside populations engaged in the cultivation, harvesting, and processing of these raw materials. By encouraging local and sustainable value chains, this work strengthens the regional bioeconomy and promotes social inclusion and environmentally responsible practices.

Additionally, by highlighting the functional and technological potential of these natural resources, the thesis reinforces the socio-environmental importance of Amazon oilseeds, offering an economic alternative to extractive exploration and the continued advancement of deforestation. The sustainable use of these species supports the "standing forest" model, linking environmental conservation to value-added opportunities for local communities.

Furthermore, the application of the OGs and EGs developed in this study in food formulations has the potential to replace SFA and TFA, which are often associated with cardiovascular and metabolic diseases. The introduction of healthier alternatives in the food industry represents a significant step toward improving public health, particularly among nutritionally vulnerable populations, thus contributing to better quality of life and the promotion of healthier eating habits.

#### 5.7.2. Scientific relevance

This research makes a meaningful contribution to scientific advancement by investigating the potential of Amazon oilseeds as alternative sources of functional lipids for use in structured systems such as OGs and EGs. In the context of increasing demand for healthier and more sustainable ingredients, the study expands scientific knowledge on underexplored vegetable oils, addressing critical gaps in the literature regarding their composition, physicochemical properties, and technological performance.

By combining extraction methods, characterization techniques, and practical applications of these oils, this thesis offers valuable insights and generates novel data that support the development of alternative fat systems aimed at reducing SFA and TFA in processed foods. The methodologies proposed may also be applicable to a wide range of plant-based lipid matrices, broadening the scope of research in the field.

Furthermore, this investigation bridges the domains of food science, process engineering, and biodiversity valorization, establishing a connection between fundamental science and industrial application. As such, the work not only strengthens the theoretical foundations of the field but also encourages the emergence of new research pathways related to the bioeconomy, functional food innovation, and the sustainable development of the Amazon value chain.

### 5.7.3. Technological relevance

This thesis presents significant advancements in the development of structured lipid systems by proposing the application of recent and innovative methods, mainly regarding the EGs using Amazon vegetable oils. The work contributes to the refinement of lipid structuring techniques by investigating formulation and processing variables aimed at ensuring the stability, functionality, and industrial feasibility of these materials.

By adapting and optimizing emerging technologies for underutilized lipid matrices, this research expands the available technical-scientific repertoire for the formulation of structured ingredients suitable for food industry applications – particularly in products requiring the replacement of SFAs without compromising texture, stability, or sensory acceptance.

Moreover, the study promotes the integration of materials science and food technology, providing technical foundations that can be transferred to industrial-scale processes. This fosters innovation in the development of healthier food products aligned with sustainability goals and nutritional quality standards. In this sense, the work represents a promising technological platform for the application of functional ingredients derived from Amazon resources.

### 5.7.4. Economic relevance

This research holds significant economic potential by promoting the valorization of Amazon oilseeds through their application in innovative lipid systems for industrial use. By encouraging the demand for regional raw materials and the sustainable use of local biodiversity, the study directly contributes to strengthening the value chain of these species, creating new business opportunities and adding value to Amazon-sourced products.

The development of technologies applicable to the food industry, based on functional ingredients from the rainforest, may stimulate investments in local infrastructure, processing, and commercialization, thus boosting the regional economy. Additionally, the strengthening of these value chains contributes to the economic diversification of the Amazon, reducing dependency on environmentally harmful activities such as deforestation and extractive exploitation.

This research also gains strategic relevance in the context of the global rise in cocoa and cocoa butter prices, driven by climate change, production decline, and increasing consumer demand. In this scenario, the use of OGs and EGs as alternative lipid sources in products such as chocolate spreads represents an economically feasible and technically promising solution. Partial or full substitution of traditional fats may help reduce production costs, improve product competitiveness, and offer healthier, more sustainable alternatives.

As a result, the findings of this study are expected to improve the economic and social conditions of local populations, fostering income generation, productive inclusion, and the appreciation of traditional knowledge – integrating science, technology, and sustainability as pillars of regional economic growth.

## 5.8. Suggestion for future research

- Apply UTAE to other oilseed matrices incorporating viscous and environmentally friendly solvents to avoid sensitive compounds degradation (antioxidants) and preserve the environment, respectively;

- Conduct an economic analysis (energy costs and scalability), and assessing tribological impacts;
- Future studies may focus on the identification and quantification of minor components present in the oils (such as tocopherols, phytosterols, and phenolic compounds), evaluating their functional, antioxidant, and anti-inflammatory properties, as well as their potential for use in food or cosmetic applications.
- Combine UTAE with other advanced technologies for vegetable oil extraction;
- Studies can focus on the selection and optimization of structuring agents;
- Develop bigel using OG and EG to combine the properties;
- Apply OG and EG to produce ice cream, cake, cereals, meat, and other food products;
- Apply BOG, TOG, and TEM to produce chocolate spreads;
- To develop all analysis comparing with margarine and butter, such as cocoa butter;
- Thermodynamic investigations regarding EGs are needed to understand the thermal and structural stability of EG;
- In-depth characterization of OGs, EGs, and chocolate spread, such as FTIR, X-Ray, and rheological behaviors (frequency sweep, temperature sweep, and other);