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Exploring *Spirulina*'s potential as a functional food ingredient: a comprehensive study on nutritional, techno-functional and physical aspects

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Thesis submitted to the Postgraduate Program in Food Science, Center for Agricultural Sciences, Federal University of Santa Catarina, to obtain the title of Doctor in Food Science.

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Mariana Demarco Gomes

Exploring *Spirulina*'s potential as a functional food ingredient: a comprehensive study on nutritional, techno-functional and physical aspects

O presente trabalho em nível de Doutorado foi avaliado e aprovado em 29 de julho de 2024 pelos membros da banca examinadora:

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Prof. Dr. Giustino Tribuzi

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ABSTRACT

As the global population grows, the Food and Agriculture Organization (FAO) predicts a doubling of meat production by 2050. This projection emphasizes the urgent need to seek alternative and sustainable food sources to address the worldwide demand of food, particularly alternative proteins. In addition, environmental impact and low conversion efficiency associated with animal-based protein systems are under concern. Microalgae, notably *Spirulina*, have emerged as a promising option to meet the increasing global demand for protein. This original research delves into the various aspects of *Spirulin*a's potential as a food ingredient, encompassing its nutritional, functional, and technological applications. An introduction, general and specific objectives are given in the first chapter. In the second chapter, a detailed review on the bioavailability of *Spirulina* and other algae, including microalgae and macroalgae/seaweed components, is presented. Evaluation of the nutritional (protein, lipids, ash, minerals, digestibility), functional (phycocyanin), and technological (water and oil holding capacity, solubility, particle size, zeta potential, color, microscopy) characteristics of multiple commercial *Spirulina* samples from three different countries (Brazil, China, and United States) are discussed in detail, and can be found in the third chapter. In the fourth chapter, the investigation of ultrasound pre-treatment effect on *Spirulina* biomass was evaluated, with focus on *in vitro* protein digestibility, volatile compounds, color and phycocyanin content. This study underscores the potential of *Spirulina* as a valuable food ingredient and offers insights into optimizing its use as a food product. The application of ultrasound pre-treatment was investigated as a means to enhance *Spirulina*'s sensory qualities and functionality. Overall, this research contributes to a better understanding of *Spirulina*'s biomass as one of the food alternatives to future protein scarcity and its application as a functional and nutritious food ingredient.

Keywords: Alternative protein, *Spirulina*, Ingredient, Digestibility, Microalgae, Ultrasound.

RESUMO EXPANDIDO

Introdução

À medida que a população global cresce, a Organização das Nações Unidas para a Alimentação e a Agricultura (FAO) prevê que a produção de carne dobrará até 2050. Essa projeção destaca a necessidade urgente de buscar fontes alternativas e sustentáveis de alimentos para lidar com a demanda mundial por alimentos, especialmente o consumo de proteínas. Além disso, a preocupação com o impacto ecológico e a baixa eficiência de conversão associados aos sistemas de proteína de origem animal estão em foco. Microalgas, especialmente a *Spirulina*, surgiram como uma opção promissora para atender à crescente demanda global por proteínas. Esta pesquisa explora os vários aspectos do potencial da *Spirulina* como ingrediente alimentar, abrangendo suas aplicações nutricionais, funcionais e tecnológicas. A tese está estruturada da seguinte forma: o primeiro capítulo apresenta a introdução e os objetivos gerais e específicos; o segundo capítulo oferece uma revisão detalhada sobre a biodisponibilidade de componentes da Spirulina e outras algas; o terceiro capítulo discute a avaliação das características nutricionais, funcionais e tecnológicas de amostras comerciais de *Spirulina* de três países (Brasil, China e Estados Unidos); e o quarto capítulo investiga o efeito do pré-tratamento por ultrassom na biomassa de *Spirulina*, focando na digestibilidade proteica *in vitro*, perfil de compostos voláteis, cor e ficocianina.

Objetivos

Esta pesquisa de doutorado teve como objetivo avaliar o potencial nutricional, funcional e tecnológico da biomassa de *Spirulina* como ingrediente alimentar. A investigação envolveu a análise comparativa de amostras de Spirulina provenientes de três países, incluindo dois grandes produtores (China e Estados Unidos) e o Brasil, onde foram avaliadas a composição nutricional, digestibilidade, cor, perfil de ácidos graxos, voláteis e minerais, além de ficocianina e parâmetros físicos das amostras estudadas. Também foi determinado o perfil de ácidos graxos e compostos voláteis das amostras comerciais de *Spirulina*. Além disso, buscou-se explorar o impacto das técnicas de ultrassom e secagem a vácuo na redução de odores e compostos voláteis nas amostras de Spirulina, analisado o impacto do método de pré-tratamento por ultrassom, aplicando 500 W, 25 ºC, em diferentes tempos (5, 10, 20 e 40 minutos) nos compostos voláteis de uma amostra comercial de *Spirulina.*

Metodologias

No primeiro estudo o teor de umidade foi determinado secando a amostra em estufa a 105°C até peso constante, enquanto o teor de cinzas foi determinado aquecendo as amostras a 550°C em forno mufla. Os conteúdos de sódio (Na) e potássio (K) foram medidos por fotometria de chama. A análise de proteínas foi realizada pelo método de Kjeldahl após digestão ácida, e os lipídios foram extraídos com éter de petróleo pelo método Soxhlet. O perfil de ácidos graxos foi determinado por cromatografia gasosa (GC-2014, Shimadzu). O complexo pigmento-proteína ficocianina foi extraído com base no protocolo descrito por Doke Jr (2005) e determinado por espectrofotometria. A distribuição do tamanho de partículas do pó foi analisada por difração a laser, e a densidade real foi determinada por picnometria a gás hélio. A caracterização da cor dos pós foi medida conforme o método de Cárdenas-Pérez et al. (2017). A microscopia foi realizada com um microscópio eletrônico de varredura (SEM) e as capacidades de retenção de água (WHC) e de óleo (OHC) foram medidas segundo os protocolos de Bencini (1986) com modificações. A técnica multienzimática para avaliação da digestibilidade proteica seguiu o método de Hsu et al. (1977). Compostos orgânicos voláteis (VOCs) foram determinados conforme os protocolos de Romeo et al. (2007), utilizando microextração em fase sólida por headspace e identificados por cromatografia gasosa acoplada a espectrometria de massa (GC-MS).

No segundo estudo as amostras de Spirulina foram pré-tratadas com uma sonda ultrassônica, sendo diluídas em água destilada na proporção de 1:5 para alcançar a consistência adequada à aplicação de ultrassom. Foram processados 80 mL das amostras diluídas utilizando um equipamento de ultrassom com potência de 500 W, variando o tempo de processamento entre 5, 10, 20 e 40 minutos, mantendo a temperatura constante em 25°C para evitar degradação térmica. As amostras de Spirulina, tanto as controle quanto as pré-tratadas, foram secas em um forno a vácuo, utilizando a técnica de secagem multi-flash condutiva (KMFD). As análises de umidade, digestibilidade proteica *in vitro*, teor de ficocianina, cor e perfil de compostos voláteis das amostras foram realizados conforme descrito acima.

Resultados e Discussão

Como resultados do primeiro estudo com as amostras comerciais do Brasil, China e Estados Unidos, bioquimicamente, as amostras chinesas apresentaram o maior teor de proteínas (~70,54 g/100g), ácidos graxos saturados como o ácido palmítico (~68,83%) e ficocianina (3,66 g/100g). Duas amostras brasileiras exibiram alto teor de ácidos graxos poli-insaturados ômega-6 (35,22%). Uma amostra brasileira mostrou o maior nível de umidade (15,44 g/100g), o que pode favorecer o crescimento de microrganismos. Esta amostra também

apresentou alta atividade de água (0,66), o que favorece a produção de compostos voláteis, como as pirazinas, devido à reação de Maillard. Ao analisar as características físicas, uma amostra brasileira destacou-se pela cor mais brilhante (L* = 20,08), maior tamanho de partícula (67,79 μm), maior densidade real (1,40 g/cm³), maior porosidade (0,632 g/cm³) e o tempo de dispersão mais rápido (6,75 min), o que é ideal para pós usados em alimentos instantâneos com alta dispersibilidade. Além disso, a mesma amostra apresentou propriedades tecno funcionais notáveis, como alta solubilidade (67,79%), capacidade de formação de espuma (60,00%) e digestibilidade proteica in vitro (85,70%), sendo uma das amostras de Spirulina mais eficientes investigadas. Amostra dos Estados Unidos demonstrou excelente capacidade de retenção de água (3,29 g/g) e óleo (1,25 g/g), além de concentrar compostos orgânicos voláteis notáveis, como hidrocarbonetos, cetonas, aldeídos e furanos.

Como resultados do segundo estudo, foi possível observar que a aplicação do ultrassom reduziu significativamente o tempo de secagem. O pré-tratamento com ultrassom não mostrou correlação com um aumento na digestibilidade *in vitro* das amostras de *Spirulina*. Embora não estatisticamente significativo, houve um leve aumento no teor de ficocianina das amostras tratadas com ultrassom em comparação com a amostra controle. O ultrassom demonstrou a capacidade de eliminar compostos específicos das amostras, como decano, dodecano, 2,6,10-trimetil-, e hexadecano, 2,6,11,15-tetrametil-. No entanto, mesmo com o pré-tratamento por ultrassom, D-Limoneno, ácido acético e pentadecano continuaram sendo os três principais compostos presentes em todas as amostras.

Considerações Finais

Este estudo destaca o potencial da *Spirulina* como um valioso ingrediente alimentar e oferece percepções para otimizar seu uso como produto alimentício. A aplicação do pré-tratamento por ultrassom é investigada como meio de aprimorar as qualidades sensoriais e a funcionalidade da *Spirulina*. No geral, esta pesquisa contribui para uma melhor compreensão do papel da biomassa de *Spirulina* como uma das alternativas para a escassez futura de proteínas e sua aplicação como ingrediente alimentar funcional e nutritivo.

Palavras-chave: Proteína alternativa, *Spirulina*, Ingrediente, Digestibilidade, Microalga, Ultrassom.

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SUMMARY

CHAPTER I

1 INTRODUCTION

The Food and Agriculture Organization (FAO) predicts that global meat production will double by 2050 (FAO, 2009). This projection has spurred a pressing need to explore new food sources as viable alternatives. The sustainability of the growing demand for animal-based protein sources, such as dairy and meat, must be carefully evaluated due to their significant ecological impact and low conversion efficiency, making them less sustainable (Neo et al., 2023). Many types of algae produce biomass much more efficiently than terrestrial plants and can be grown effectively without antibiotics or pesticides in either fresh or saltwater (Ullmann & Grimm, 2021). Microalgae have a higher rate of photosynthesis compared to larger plants, resulting in greater biomass productivity. Furthermore, they can be cultivated in areas where traditional agriculture is not feasible, which reduces conflicts related to food security (Chisti, 2013; Savage & Hestekin, 2013).

As protein is an essential nutrient for human metabolism, its potential future scarcity necessitates exploring alternative protein sources and innovative manufacturing food methods (Dopelt, Radon, & Davidovitch, 2019; Geada et al., 2021). To address this concern, algae have emerged as promising protein sources due to their unique attributes compared to traditional animal-based options. Microalgae have emerged as a promising protein source that has been extensively researched for years as a potential food ingredient. Both macroalgae and microalgae are recognized as effective and significant sources of plant-based protein for meat substitutes due to their full content of amino acids (Fu et al., 2021; Chew et al., 2017).

Microalgae, a term used in applied phycology to refer to microscopic algae, are microorganisms found in various forms - unicellular, colonial, filamentous, or siphonaceous (Richmond, 2004). They are characterized by the presence of chlorophyll and other photosynthetic pigments (β-carotene, xanthophylls, and phycobiliproteins) that enable oxygenic photosynthesis (Begum et al., 2015). The consumption of microalgae has historically been uncommon. The only well-known examples is *Spirulina*, a type of cyanobacteria, by the Aztec people in the valley of Mexico and the Kanembu people near Lake Chad (Ullmann & Grimm, 2021; Ahsan et al., 2008). Microalgae can be utilized in food processing to enhance nutritional value, while promoting health status. Foods enriched with microalgae supplementation offer functional attributes (antioxidants, phytochemicals, fatty

acids etc.) and color properties, thus combining health benefits with sensory appeal to consumers (Gouveia et al., 2008; Wells et al., 2017).

Microalgae offer a variety of functional components, including peptides, carbohydrates, lipids, pigments, vitamins, and minerals, offering consumers a wide array of health benefits. Additionally, microalgae boast a rapid growth rate and adaptability to diverse environmental conditions, surpassing that of traditional terrestrial crops. Overall, microalgae show great promise as ingredients for meat alternatives due to their nutritional richness and efficient growth capabilities (Fu et al., 2021).

The functional properties of proteins found in microalgae species like *Arthrospira platensis* indicate a significant potential for their use as additives in various food and non-food products, including cosmetics (Matos, 2017).

Spirulina biomass finds application in foods due to its GRAS (Generally Recognized As Safe) granted by the FDA. The protein content within Spirulina ranges from 50% to 70% by weight, and this variability can be attributed to factors such as strains, cultivation practices and environmental conditions. Parameters such as nitrogen and mineral concentrations, light intensity, and climatic conditions can influence or be manipulated to affect protein synthesis in Spirulina (Matos, 2019; Lourenço, 2006).

The protein content is an important factor in the nutritional value of microalgae (Oliveira et al., 1999). Evaluation of protein content in various species based on biological value and digestibility demonstrate that the amino acid profile of microalgae (content, proportion, and availability) is comparable to other protein sources according to FAO guidelines, classifying microalgae as an alternative source of high-value proteins (Chacón-Lee; González Mariño, 2010). Besides their nutritional function, proteins also contribute to texture, viscosity, gelation, emulsification, and foam formation when microalgae are incorporated as ingredients in food products (Draaisma et al., 2013). Moreover, many of the bioactive properties of microalgae, such as antioxidant, anti-hypertensive, immunomodulatory, anticancer, hepatoprotective, and anticoagulant activities, are associated with the whole protein content and peptides synthesized via fermentative and enzymatic processes (Buono et al., 2014).

The application of new technologies for obtaining functional foods and/or functionalizing agents is a spotlight area of research. The use of microalgae, particularly *Spirulina*, has gained prominence due to high content of proteins and essential amino acids, vitamins, minerals, fatty acids, and pigments (chlorophylls, phycocyanin, and carotenoids). In

fact, it is considered a potential ingredient to enhance the nutritional value of food products (Koyande, et al., 2019).

The sensory and physical qualities of foods are determined by their ingredients, with food processing directly impacting these attributes. Specifically, different processing methods can influence the functional properties of food components, such as bioactivity and bioaccessibility, affecting their potential health benefits. The more extensive the food processing and the longer the storage and transportation, the greater the loss of food bioactives (Galanakis, 2017).

Many conventional techniques like mechanical pressing, wet milling, membrane filtration, ultrafiltration, isoelectric precipitation, and alcohol precipitation are widely used in the food industry, despite encountering numerous challenges that often limit their applicability and result in practical issues. Thermal treatment of food can lead to the degradation of food components due to oxidation and isomerization reactions, ultimately reducing food quality and shelf-life (Galanakis; Jafari; Galanakis, 2014; Mujumdar; Law, 2010). Additionally, other conventional non-thermal processes requiring high pressures can significantly increase energy demands and operational costs. Emerging technologies have garnered interest in both research and the food industry by promising reduced heating and residence times, improved energetic yield, enhanced product quality, control over Maillard reactions, and protection from environmental stresses (Galanakis, 2021). Ultrasound, as another non-thermal technology, is recognized for accelerating mass transfer through the generation of cavitations within food matrices when applied (Mason; Lorimer, 1988).

The pretreatment of algae biomass is frequently employed as an extraction method. These pretreatments play a vital role in extracting compounds and facilitating bioconversion processes (Michalak & Chojnacka, 2014). Various forms of biomass pretreatment, encompassing physical, chemical, and biological approaches, as well as the application of emerging technologies, have been shown to augment the accessibility of target compounds during bioactive extraction by disrupting the cell matrix and aiding mass transfer (Ummat et al., 2021).

During the ultrasonic process, microalgal cells undergo disruption via shock waves generated by cavitation bubbles, thereby improving the release of valuable compounds (Ma et al., 2014). This application of ultrasound, also termed sonication, involves utilizing cavitation to disrupt the cell wall of microalgae suspended in water. Cavitation encompasses the formation, expansion, and sudden collapse of minuscule bubbles within the liquid, induced by fluctuations in bulk pressure caused by ultrasound waves. Micro-turbulence contributes to

vigorous mixing, while shockwaves lead to the disruption of the cell wall (Ranjan; Patil; Moholkar, 2010).

Incorporating innovative technologies like ultrasound, microwave, and pulse electric field as a preliminary step before algae drying has shown promising results in improving process efficiency. For instance, ultrasound-assisted drying of *Ascophyllum nodosum* has proven to decrease drying duration, boost energy efficiency, and maintain color quality (Kadam; Tiwari; O'Donnell, 2015). Similarly, a study on *Phaseolus vulgaris* indicated that ultrasound treatment under vacuum (USV) could accelerate the dehydration process (Tekin; Başlar; Karasu; Kılıçlı, 2017).

In this research, the technological, nutritional and functional potential of the dried *Spirulina* biomass were investigated. The first chapter of this thesis addresses literature review about the potential bioavailability of *Spirulina* and other algae components. The second chapter deals with the nutritional, functional and technological research study of 8 commercial *Spirulina* samples from three different marketing countries. In the third chapter, *Spirulina* samples with and without ultrasound pretreatment were compared regarding functional aspects and volatile compounds profile, which may affect microalgae aroma, scent, flavor and smell. The last chapter provides the main conclusions and future directions crystallized by the student's doctorate research.

1.1 Objectives

1.1.1 General objective

This doctoral research aimed to assess the nutritional, functional, and technological potential of *Spirulina* biomass as a food ingredient. The investigation involved the examination of *Spirulina* samples from three countries, including two major producers (China and The United States) and Brazil, for comparative analysis. Additionally, the study aimed to explore the impact of ultrasound and vacuum drying techniques in reducing odors and volatile compounds in *Spirulina* samples.

1.1.2. Specific objectives

1. Evaluating the composition of commercial samples of *Spirulina* from different countries (Brazil, China and USA);

2. Evaluating and comparing the nutritional composition, digestibility, color, fatty acid, volatile and mineral profile, phycocyanin and physical parameters of the studied samples;
3. Determining the profile of fatty acids and volatile compounds of commercial samples of *Spirulina*;
4. Analysing the impact of ultrasound pre-treatment method (500 W, 25 ºC, in different times - 5, 10, 20 and 40 minutes) on the volatile compounds of a commercial sample of *Spirulina*.

CHAPTER II

2 LITERATURE REVIEW

The literature on the algae digestibility and bioaccessibility is presented in this chapter. In this review, an outline of current research on the digestibility and bioavailability of compounds derived from algae is summarized, along with *in vivo* studies that investigate the bioaccessibility of microalgal biomass as a feed supplement for animals. The influence of techniques used to disrupt cell walls on the bioaccessibility of bioactive compounds in algae is also explored, as well as the *in vitro* bioaccessibility of nutrients in foods enriched with algal biomass. This article aims to present the potential of algae as a food ingredient, in order to increase our knowledge about algal food-based.

This literature review was published in Trends in Food Science & Technology (v. 121, p. 114-128, 2022) with the title "*Digestibility, bioaccessibility, and bioactivity of compounds from algae*", and can be found in https://www.sciencedirect.com/science/article/abs/pii/S0924224422000528 (Demarco et al., 2022).

Abstract

Algae are aquatic organisms that contain a variety of beneficial biocompounds such as proteins, amino acids, carbohydrates, lipids, pigments, vitamins, minerals, and polyphenols. Although these compounds are present in high concentrations in raw algal biomass, some may not be available for biological function due to the composition of the algae cell wall.

This review provides an overview of ongoing studies on the digestibility and bioavailability of algae-derived compounds, as well as *in vivo* studies examining the bioaccessibility of microalgal biomass as feed supplementation for animals. The influence of cell wall disruption techniques on the bioaccessibility of algal bioactive compounds is also discussed, including *in vitro* bioaccessible nutrients of foods enriched with algal biomass.

Studies have shown that the digestibility and bioaccessibility of major compounds vary greatly between algal species. Polysaccharides and fibers are undigestible by humans and can inhibit carbohydrate assimilation. However, combined disruption methods, such as bead milling or high-pressure homogenization with enzymatic pretreatment, can increase the

extractability and bioavailability of lipid and fatty acids, pigments, minerals, and protein and amino acids absorption.

In vivo studies on dietary supplementation of algae in animals (sheep, chicken, mice, and fish) have shown good acceptability and digestibility of proteins and lipids. Additionally, traditional foods enriched with up to 5% algal biomass can extend the bioaccessibility of natural bioactive compounds of algae, highlighting the positive impact of consuming algae-based food for human health promotion.

Keywords: Seaweed, Microalgae, In vitro studies, Bioactive compounds, Algal-based food.

2.1. Introduction

The pursuit of sustainable and nutritious food sources has been driven by concerns about food security, climate change, and the challenges posed by a growing global population (Godfray et al., 2010). Humans have consumed seaweed and microalgae for centuries, and their popularity has increased worldwide due to their nutritional value (Dillehay et al., 2008; Torres-Tiji et al., 2020). Algal foods, including raw seaweed and microalgae, as well as processed algal foods, contain a wide range of nutrients such as protein, carbohydrates, dietary fibers, lipids, polyunsaturated fatty acids, vitamins, and minerals, which have numerous health benefits (Caporgno & Mathys, 2018; Matos, 2017; Tiwari & Troy, 2015). However, for these nutrients and compounds to be effective, they must be digestible and capable of being absorbed by the human body, which is regulated by factors such as bioaccessibility and bioavailability (Minekus et al., 2014; Wells et al., 2017).

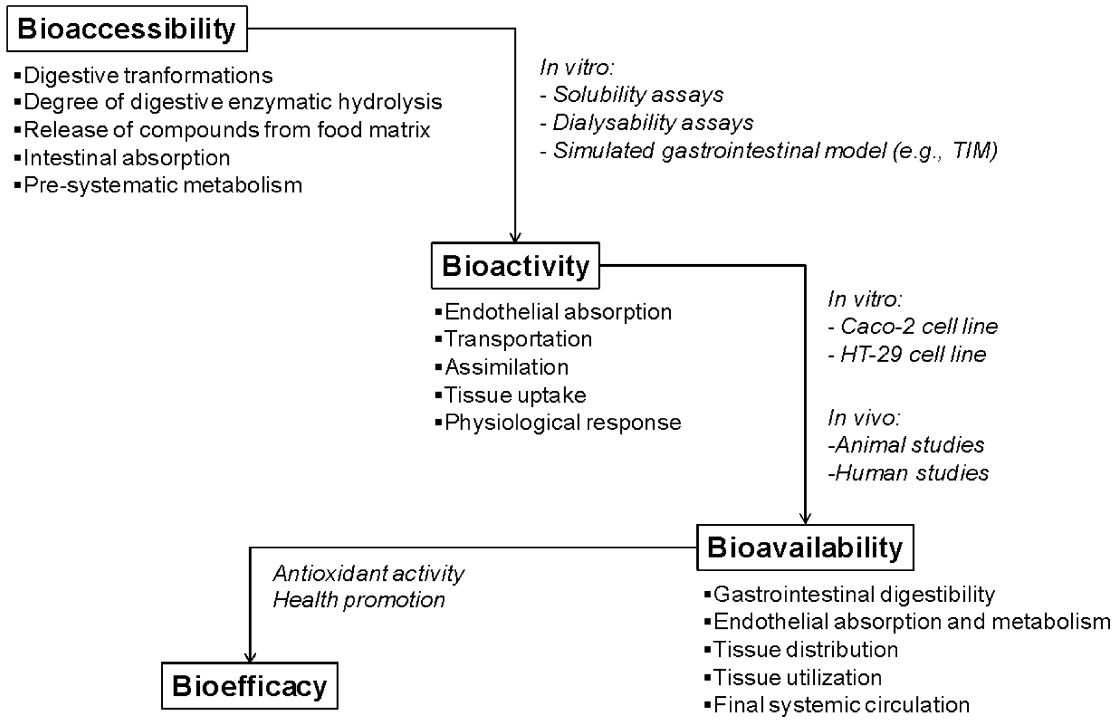
To understand the dynamics of food metabolism, it is important to define the terms digestibility, bioaccessibility, and bioavailability. Digestibility refers to the amount of nutrients absorbed by an individual and is typically calculated by subtracting the amount of nutrients retained in the feces from the amount consumed (Watts et al., 2013). Traditional foods like milk and eggs have high true digestibility values of around 97%, whereas seaweed Ulva sp. and blue-green microalgae *Arthrospira platensis* have an apparent digestibility of approximately 86% (Kazir et al., 2019; Niccolai et al., 2019). Bioaccessibility refers to the amount of nutrients or components that are available for absorption across the intestinal epithelia (Rein et al., 2013), while bioavailability is defined by the FDA as the rate and extent

to which an active ingredient is absorbed and becomes available at the site of action (FDA, 2003).

In vitro and *in vivo* digestibility/bioaccessibility/bioavailability studies are critical to understanding the interactions between nutrients and food components, as well as the effects of pH and enzymes on absorbability (Dima et al., 2020). Therefore, evaluating the apparent digestibility and bioaccessibility of algal compounds is essential to unlocking the potential of algal food products/co-products.

Research on algal digestibility is particularly important given the complex polysaccharide cell wall structure of some algae, which can impede the action of digestive enzymes and limit the bioaccessibility or bioavailability of nutrients and algae components (Niccolai et al., 2019). Techniques such as enzymatic hydrolysis, and chemical, mechanical, and/or physical methods can promote cell disruption, thereby improving the digestibility and bioaccessibility of algal nutrients (Maehre et al., 2016).

Figure 1: The journey of compounds to bioefficacy, with a schematic representation of methods that can be used to determine bioaccessibility, bioactivity, and bioavailability of algae compounds.



Adapted from Bleakley and Hayes (2017).

Numerous reports in the literature discuss the biochemical composition and nutritional value of algae (Batista et al., 2013; Matos et al., 2016). While most literature on digestibility pertains to seaweed, there are a few *in vitro* studies available on microalgae. This review presents examples of digestibility and bioaccessibility of major components (such as protein, carbohydrates, fibers, and lipids) and minor components (like carotenoids, vitamins, and minerals) from both seaweed and microalgae. Additionally, it provides up-to-date information on the bioavailability of algal nutrients that have a biological impact on the human body. The review also emphasizes the impact of cell wall disruption methods on the accessibility of compounds found in algae.

2.1.1. Characteristics of seaweed

Seaweeds, also known as macroalgae, have been utilized as a food source since ancient times, particularly in Asian communities, and have become a staple part of daily diets. These seaweeds, such as *Saccharina japonica*, *Ascophyllum spp.*, *Undaria pinnatifida*, *Sargassum fusiforme, Porphyra spp., Eucheuma spp., Kappaphycus alvarezii, Gracilaria spp., Palmaria spp., Ulva clatharata, Monostroma nitidum,* and *Caulerpa spp.*, are considered to be excellent sources of fiber, protein, and minerals, and have strong commercial potential for human consumption. The global commercial seaweed market size was USD 14.11 billion in 2020, according to Fortune Business Insights.

Seaweed polysaccharides, such as hydrocolloids (e.g., agar, carrageenan, and alginate), are successful byproducts of the food industry, used as stabilizers, gelling agents, thickeners, and texturing agents in food and beverage products. From a nutritional and functional perspective, seaweeds are low in calories and contribute to the soluble dietary fiber fraction in humans. Recent research has focused on emerging polysaccharide extraction techniques and studies on the digestibility and bioavailability of these compounds, particularly by food specialists.

2.1.2. Characteristic of microalgae

In the field of psychology, the term microalgae refers to the collection of microscopic algae that have a eukaryotic structure. This group includes the prokaryotic cyanobacteria. Large-scale commercial cultivation of microalgae for biomass and bioproducts began in the 1960s with the green microalgae *Chlorella vulgaris*, followed by *Arthrospira platensis* (Spirulina) in the 1970s and *Dunaliella salina* (Chlorophyta) in the 1980s for β-carotene

production. In the late 1990s, commercial cultivation of the freshwater green alga *Haematococcus lacustris* began in the United States, Israel, and China.

Microalgae can synthesize a wide range of biocompounds such as single-cell protein, carotenoids, phycobilin pigments, and polyunsaturated fatty acids, which have been commercialized as dietary supplements, nutraceuticals, and functional foods. Developing novel foods based on microalgal biomass is an exciting prospect for providing nutritional supplements with biologically active compounds linked to human health promotion.

However, one of the challenges associated with using microalgae as a food source is the robust cell wall of certain algal strains, which can limit the accessibility of digestive enzymes and affect digestibility. Some species, such as *Arthrospira platensis*, have a fragile cell wall, making their biomass easily digestible. Other species, like Chlorella vulgaris and *Haematococcus lacustris,* have small-size microorganisms with rigid cell walls, which require previous cell wall disruption to enhance the digestibility and bioaccessibility of microalgal compounds.

Therefore, studies on the digestibility and bioaccessibility/bioavailability of raw algal biomass, algal food, and feedstuff are essential for advancing research in this area. This will be further discussed in the following section.

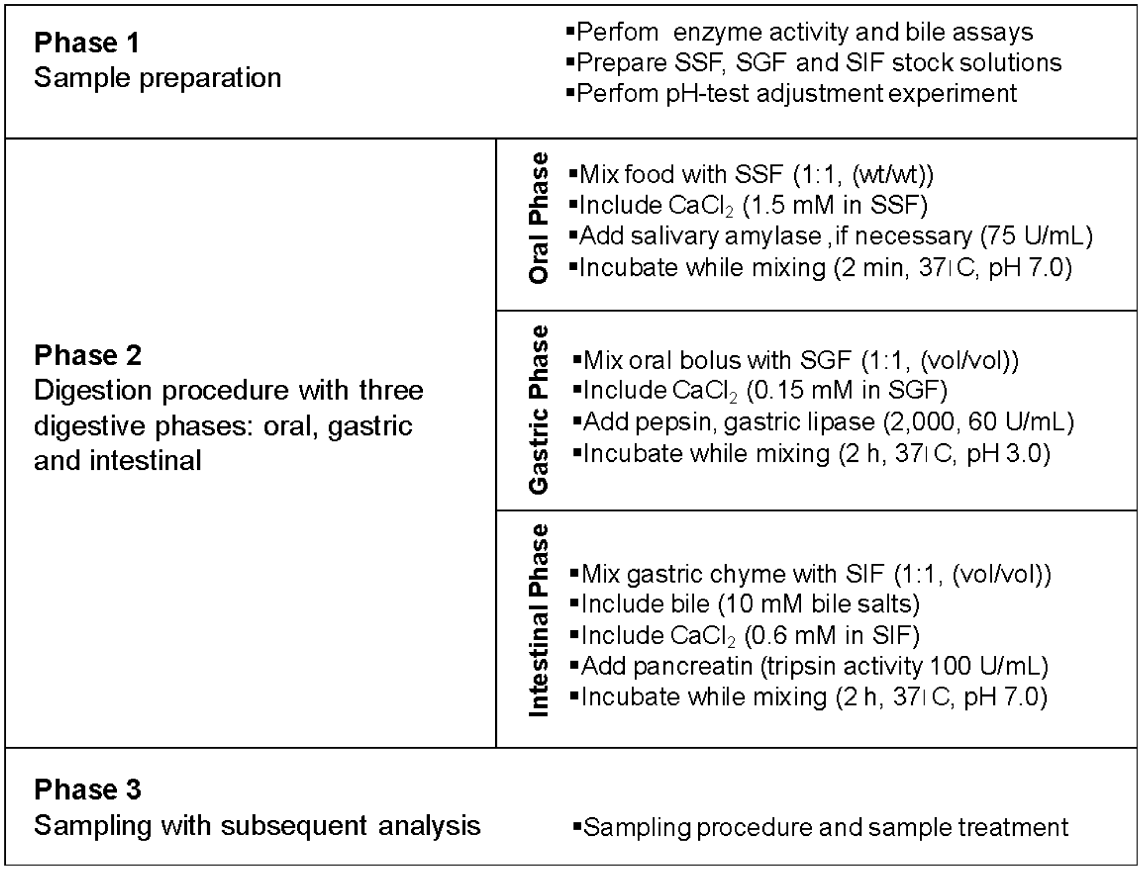
2.2. Bioaccessibility of major compounds from seaweed and microalgae

The bioaccessibility of a compound refers to the maximum proportion that is released from the food matrix into the various parts of the digestive system, including the mouth, stomach, small and large intestines (Brandon et al., 2006). To assess the effective consumption of algal biomass, it is necessary to determine the bioaccessibility of algae, which requires the use of an appropriate *in vitro* digestion model that accurately simulates the human digestive process (Bonfanti et al., 2018).

One of the most reliable and standardized protocols for analyzing the bioaccessibility of food samples is the INFOGEST static *in vitro* simulation of gastrointestinal food digestion, which involves subjecting the sample to sequential oral, gastric, and intestinal digestion. The parameters used in this method, such as electrolytes, enzymes, bile, dilution, pH, and digestion time, are based on available physiological data. This method can be used to analyze digestion products, such as peptides/amino acids, fatty acids, carotenoids, and simple sugars,

and to evaluate the release of micronutrients from the food matrix (Figure 2). Detailed information on this method can be found in Brodkorb et al. (2019).

Figure 2: Schematic process of INFOGEST *in vitro* digestion method that simulates gastrointestinal human digestive system, which can be applied for testing the digestibility and bioavailability of algal biomass. SSF = simulated salivary fluid, SGF = simulated gastric fluid, SIF = simulated intestinal fluid.



Brodkorb, et al. (2019).

2.2.1. Digestibility of dietary fibers, carbohydrates, and polysaccharides

Dietary fiber refers to the portion of food derived from algae that cannot be fully broken down by human digestive enzymes. These fibers vary in chemical composition and can be categorized based on their solubility, viscosity, and fermentability, all of which affect how they are processed in the human body. Examples of dietary fiber found in algae include polysaccharides, oligosaccharides, lignin, cellulose, and hemicellulose (de Jesus Raposo, De Morais, & De Morais, 2016). When consumed, dietary fiber promotes physiological benefits

such as improved laxation and reduced blood cholesterol and glucose levels (Raninen, Lappi, Mykkänen, & Poutanen, 2011). Polysaccharides found in algae also have antioxidant, anti-inflammatory, and cardioprotective activities that contribute to human health (Mayakrishnan, Kannappan, Abdullah, & Ahmed, 2013).

Seaweed polysaccharides and fibers, which make up between 33-62% of the dry weight of algae, are largely indigestible by the human digestive system and therefore act as prebiotic food (Zheng, Chen, & Cheong, 2020). Soluble fibers, which are found in greater amounts in algae, are known for their water retention capacity and hydrocolloid characteristics. Hydrocolloids are functional ingredients used in food formulation to improve consistency, thickness, stability, and emulsification properties (Qin, 2018).

The composition of cell walls in algae, particularly green algae, influences the apparent digestibility analysis and quantification of polysaccharides. Green algae typically have a cell wall made up of cellulose, hemicellulose, pectin compounds, and glycoproteins, which make them resistant to the action of digestive enzymes (Niccolai, et al., 2019).

Studies have evaluated the carbohydrate and dietary fiber content of different microalgae, with *Chlorella vulgaris* and *Arthrospira platensis* found to contain the lowest dietary fiber values among the microalgae studied, making them more easily digestible (Matos et al., 2016). *In vitro*, digestion studies have shown that *Chlorella pyrenoidosa* and *Arthrospira platensis* dietary fibers are around 62% and 82% digestible, respectively (MišurCoVá, KráčMar, KLeJduS and VaCeK, 2010).

The prebiotic potential of marine algae polysaccharides (MAPs) is determined by their fermentation behavior in the digestive tract. MAPs are not digested by saliva enzymes in the oral phase but are mixed with digestive acid juices and subjected to mechanical forces in the stomach phase. In the small intestine, they are mixed with enzymes, minerals, and other substances and can regulate digestion by promoting peristalsis, encouraging satiety, and retarding the rate of gastric emptying (Zhang, et al., 2020; Di, et al., 2018). MAPs have also been shown to have prebiotic effects on gut microbiota, reshaping the structure of the gut microflora by promoting the growth of probiotic populations and inhibiting the growth of harmful bacteria (Guo, et al., 2020).

In vitro digestibility studies of carbohydrates and fibers from algae have been conducted, with results suggesting that different types of algae have varying levels of digestibility (Xu, et al., 2019). Table 1 provides further examples of such studies.

Table 1: *In vitro* studies of carbohydrates, dietary fiber, and polysaccharides in algae.

Algae	Types of carbohydrates	Method	Major findings	Reference
Brown seaweed *Ascophyllum nodosum* (AnPs)	Sulphated polysaccharides	Simulated saliva and gastrointestinal digestion followed by fermentation of AnPs biomass *in vitro*	Sugars from AnPs was reduced after fermentation and decomposed by the human fecal microbiota, indicating potential action as a functional ingredient to promote gut health	(L. Chen, et al., 2018)
Brown seaweed (*Laminaria japonica*)	Dietary fiber	Samples digested with pepsin + pancreatin	Algae showed high content of dietary fiber (10.5%) with *in vitro* digestibility of 60%.	(MišurCoVá, et al., 2010)
Red seaweed (*Porphyra haitanensis*)	Polysaccharides	*In vitro* fermentation by intestinal microbial	Polysaccharides (PHP) yield was 4.10%. PHP was gradually depolymerized and consumed by intestinal flora with potential prebiotic effects	(Xu, et al., 2019)
Brown seaweed (*Fucus vesiculosus*)	Extract containing polyphenols and phlorotannins (PHTs)	*In vivo* absorption of carbohydrates using α-amilase/α-glicosidase activities assays	PHT extract inhibited digestive enzymes *in vitro* and slowing carbohydrate absorption *in vivo*, reducing the glycemic response starch ingestion	(Roy, et al., 2011)

Blue-green (*Arthrospira platensis*)	Dietary fiber	Samples digested with pepsin + pancreatin	Algae showed low content of dietary fiber (0.94%) with *in vitro* digestibility of 82%.	(MišurCoVá, et al., 2010)

2.2.2. Bioaccessibility of lipids

Algae cells contain lipids that serve as energy stores, structural components of cell membranes, and signaling molecules (Goold, Beisson, Peltier, & LiBeisson, 2015). Seaweeds generally have a low lipid content (<5%), while microalgae can have a lipid content ranging from 1.0 to 40.0% (dry weight), depending on the species and growth conditions (Moheimani, McHenry, De Boer, & Bahri, 2015). Many algal oils contain long-chain polyunsaturated fatty acids (PUFAs) that have nutraceutical, pharmaceutical, and therapeutic applications and are associated with preventing cardiovascular, diabetes, and hypertension diseases (Koyande et al., 2019). *Isochrysis galbana, Nannochloropsis sp., Crypthecodinium cohnii,* and *Phaeodactylum tricornutum* are exceptional PUFA producers, with I. galbana being particularly high in eicosapentaenoic acid, making it a good candidate for nutraceutical human nutrition (Batista et al., 2013).

An *in vitro* digestion model was used to determine the lipid/fatty acid bioaccessibility of freeze-dried *I. galbana* biomass, and it was found that the percentage of lipid and fatty acid ω-3 PUFA bioaccessibility was very low (between 7 and 15%) during digestion, compared to other bioaccessibility studies on fish lipids (bioaccessibility = 50% in salmon fish) using similar *in vitro* methods (Bonfanti et al., 2018, Table 2). While *I. galbana* is easily assimilated biomass, the low degree of lipolysis suggests inhibition of the digestive model lipases (Bonfanti et al., 2018).

Cavonius, Albers, and Undeland (2016) found that shifting pH from 7.0 to 10.0 during lipolysis metabolism can improve the bioaccessibility of lipid and fatty acids of *Nannochloropsis oculata*, possibly due to a conformational change of protein-lipid complexes, resulting in better access by lipases. Francisco et al. (2020) investigated the lipid and fatty acid contents of brown seaweed *Fucus spiralis* before and after *in vitro* simulation of the human digestive process. *F. spiralis* had a low lipid content of 3.5% (dry weight) with a lipid bioaccessibility of around 12.1% (Table 2). The major ω-3 fatty acid was eicosapentaenoic acid, with a bioaccessibility percentage of 13.0%. The low lipid bioaccessibility can be attributed to the fact that most intracellular lipids of seaweeds are in the form of phospholipids and glycolipids, which are associated with cell membranes. Seaweeds do not suffer substantial physical disintegration during digestion, possibly due to the high fiber content in their cell wall. The human gastrointestinal tract does not produce the required degradation enzymes to metabolize these polysaccharides, resulting in poor lipid release (Francisco et al., 2020). Therefore, cell wall treatment using mechanical disruption

methods combined with enzymatic cellullases before lipid extraction may increase both the lipid content and lipid/fatty acid bioaccessibility (Bernaerts et al., 2020).

Table 2: Summary and comparison of case studies on bioaccessibility of lipids and fatty acids in algae.

Algae	Digestion method	Bioaccessibility	Major findings	Reference
Isochrysis galbana	*In vitro* digestion model that enabled the simulation of gastrointestinal tract	Lipid/fatty acid (between 7 and 15%)	Low bioaccessibility that is related with the low degree of lipolysis inhibited by digestive lipases	(Bonfanti, et al., 2018)
Schizochytrium aggregatum	*In vitro* simulated gastrointestinal digestion	Lipid (79.4%)	Good *in vitro* lipid bioaccessibliity mainly because docosahexaenoic content	(Lv, et al., 2015)
Schizochytrium sp.	*In vitro* digestion simulated gastrointestinal tract	Lipid (42%) DHA (71%)	Emulsified DHA oil enhanced the solubilization of free fatty acids, especially in the intestinal portion	(Gayoso, Ansorena, & Astiasarán, 2019)
Chlorella vulgaris	An infant *in vitro* digestion model	Lipid (2.5%)	Poor lipid bioaccessibility, which could be due to the lower concentration of digestive enzymes in infant digestion	(Canelli, et al., 2020)
Nannochloropsis oculata	Infogest *in vitro* digestion model	Fatty acids (34%)	Moderate fatty acid bioaccessibility after shifting pH from 7.0 to 10.0	(Cavonius, et al., 2016)
Fucus spiralis	*In vitro* digestion model	Lipid (7.5%) Fatty acid (13.0%)	Low lipid/fatty acid bioaccessibility as this algae contain low lipid yield and high quantity of polysaccharides	(Francisco, et al., 2020)

2.2.3. Bioaccessibility of proteins

The human body needs a constant supply of good quality protein through feeding as it is incapable of maintaining protein reserves. Thus, protein quality is a crucial criterion for providing adequate nutrition (Boye, Wijesinha-Bettoni, & Burlingame, 2012). The nutritional quality of protein is defined by the content of essential amino acids, as well as its digestibility and bioavailability, which correspond to established standards and patterns required by the human body (Joint & Organization, 2007). Digestion and absorption are associated with factors such as the dietary requirement, which is the amount of protein or its constituent amino acids necessary to satisfy the metabolic demand and achieve nitrogen equilibrium. These factors also impact the cellular bioavailability of absorbed amino acids in relation to needs, which ultimately affects the biological value (Joint & Organization, 2007).

According to Becker (2004), the protein quality of various microalgae can be evaluated based on their amino acid content, proportion, and availability and compared to traditional protein sources using the WHO/FAO standards. This classification enables microalgae to be considered as alternative sources of protein. Matos (2019) notes that proteins derived from microalgae, such as *Chlorella* and *Arthrospira* (formerly Spirulina), meet the WHO/FAO guidelines for essential amino acids necessary for human consumption.

Estimating protein availability relies on determining protein digestibility, which is an important factor. For instance, Niccolai et al. (2019) demonstrated that Arthrospira platensis and Chlorella vulgaris have protein digestibility values of 81% and 76%, respectively, indicating that these microalgae are excellent sources of proteins and essential amino acids according to the WHO/FAO standards. However, the *in vitro* bioavailability of the protein in the marine diatom *Phaeodactylum tricornutum* was found to be only 35% after proteolytic enzymatic hydrolysis (Tibbetts, Milley, & Lall, 2015). This low bioavailability is mainly due to the rigid cell wall composed of silicates and ceramides, which requires pretreatment cell disruption to increase protein content and digestibility.

The amount of protein in seaweed varies widely, ranging from 3% to 47% of the dry weight, depending on factors such as species, geographic origin, seasonal variation, and climate conditions. Red seaweed species typically have high protein levels, while green and brown seaweed species generally have moderate and low protein levels, respectively (Harnedy & FitzGerald, 2011).

Algal protein concentrates extracted from *Ulva* (Chlorophyta) and *Gracilaria* (Rhodophyta) species with a protein content range of 70-86% intended for human consumption were investigated by Kazir et al. (2019). Simulated *in vitro* gastrointestinal

digestion demonstrated that approximately 90% of the protein from *Ulva sp.* and 100% of the protein from *Gracilaria* were hydrolyzed by the end of the simulated intestinal phase. These findings suggest that seaweed proteins are readily hydrolyzed by digestive enzymes, indicating their bioavailability for human absorption.

Table 3 presents examples of the digestibility and bioaccessibility of protein from various macroalgae and microalgae. However, *Gracilariopsis longissima* (formerly *Gracilaria verrucosa*) (Rhodophyta) has low in vitro protein digestibility (30%) due to its high soluble fiber content, which limits the protein digestibility and reduces the accessibility of proteolytic enzymes (Marrion et al., 2005). The authors suggested heating the seaweed sample before human ingestion as a solution to increase the bioaccessibility of protein/amino acids (Maehre et al., 2016).

Table 3: Summary and comparison of case studies on digestibility of proteins in algae.

Seaweed	Digestion method	Protein digestibility	Highlights	Reference
Laminaria japonica	Samples digested with pepsin + pancreatin	60%	Brown seaweed have a high content of dietary fiber, rendering low protein digestibility	(MišurCoVá, et al., 2010)
Undaria Pinnatifida	Nitrogen ingestion and nitrogen excretion in feces	85%	The level of glycosylation of the protein fraction and the presence of antinutritional factors may influence the digestibility of algal proteins	(Taboada, Millán, & Miguez, 2013)
Ulva and *Gracilaria* spp.	Simulated gastrointestinal digestion	89%	Algal protein extracted was hydrolyzed by digestive enzymes with excellent enzymatic proteolysis	(Kazir, et al., 2019)
Alaria esculenta	*In vitro* protein digestibility with multienzyme hydrolysis	79%	High levels of soluble polysaccharides fibre that can entrap protein in the cellular matrix	(Tibbetts, Milley, & Lall, 2016)
Palmaria palmata	Simulated gastrointestinal digestion	75%	*In vitro* bioaccessibility of protein/amino acids increased after heat treatment	(Maehre, et al., 2016)
Gracilaria verrucosa	*In vitro* digestibility assays	30%	The high soluble fibre contents limit the protein digestibility, reducing the accessibility of the proteolytic enzymes	(Marrion, et al., 2005)
Microalgae				

Nannochloropsis oculata	Infogest *in vitro* digestion model	50%	Good accessibility of protein/amino acids occurred after pH-shift processing from 7.0 to 10.0	(Cavonius, et al., 2016)
Galdieria sulphuraria	Simulated gastrointestinal digestion	79%	The cell wall of *G. sulphuraria* contains low amounts of cellulose and is rich in proteins	(Massa, et al., 2019)
Arthrospira platensis	Samples digested with pepsin + pancreatin	82%	High content of protein and amino acids and low content of dietary fiber	(MišurCoVá, et al., 2010)
Chlorella vulgaris	Estimation of protein digestibility in pigs	84%	Cell-disrupted increased protein digestibility	(Wild, Steingaß, & Rodehutscord, 2018)
Arthrospira platensis, Nostoc sphaeroides	*In vitro* digestibility based on gastrointestinal tract	76-82%	Both microalgae have fragile cell wall, enhancing the digestibility of algal biomass	(Niccolai, et al., 2019)
Phaeodactylum tricornutum	*In vitro* proteolytic enzymatic hydrolysis	35%	Diatom species with rigid cell wall composed by silicates and ceramides, rendering less bioavailability to enzymatic hydrolysis	(Tibbetts, et al., 2015)

2.2.4. Bioaccessibility of pigments

Algae possess three major categories of photosynthetic pigments: chlorophylls, carotenoids, and phycobilins. These pigments are crucial for maximizing light absorption, carbon dioxide fixation, and protection against damage caused by excessive illumination (Pangestuti & Kim, 2011). Chlorophylls, which are lipid-soluble pigments, give the typical green coloration to green algae (Chlorophyta). Carotenoids, including carotenes and xanthophylls, are also lipophilic compounds, and their colors can range from pale yellow to deep red, with commercially successful carotenoids such as β-carotene, lutein, and astaxanthin (Takaichi, 2011). Phycobilins, on the other hand, are distinctive pigments that bind to specific water-soluble proteins called phycobiliproteins (such as phycocyanin and phycoerythrin) found in cyanobacteria and in the chloroplasts of red algae (Eriksen, 2008).

The natural color and bioactive properties of algal pigments have made them highly desirable to various industries, including food, feed, and pharmaceuticals. These pigments have been shown to possess numerous biological functions and health benefits, such as antioxidant activity (Feller et al., 2018), positive effects on the immune system (Cerezuela, Guardiola, Meseguer, & Esteban, 2012), and the ability to prevent cardiovascular disease and nonalcoholic fatty liver disease (Ku, Yang, Park, & Lee, 2013).

Fernandes et al. (2021) investigated the bioaccessibility of chlorophyll pigments from *Tetradesmus obliquus* (previously known as *Scenedesmus obliquus*) (Chlorophyta) and their subsequent uptake by Caco-2 human intestinal cells (Table 4). In their study, three different products, namely isolated chlorophyll extract, wet ultrasonicated biomass, and whole dried biomass, were subjected to an in vitro digestion model based on the Infogest protocol (Fig. 2). The bioaccessibility of chlorophylls varied depending on the mode of ingestion, and from a nutritional standpoint, chlorophylls in extract form exhibited greater bioaccessibility than wet and dried biomass. These findings suggest that wet and dried biomass could be utilized as an ingredient in developing functional foods based on chlorophyll.

Edible seaweeds, which are highly valued as a delicacy, are known to be rich in chlorophyll. In a study by Chen & Roca (2019), the bioaccessibility of chlorophyll pigments from cooked seaweed samples, including *Porphyra umbilicalis* (Nori, red alga), *Laminaria ochroleuca* (Kombu, brown alga), and *Ulva sp*. (Sea Lettuce, green alga), were investigated following in vitro digestion and micellarization. The results showed that the cooking process had a negative impact on the micellarization rate of chlorophyll derivatives in Nori and Kombu, while it did not affect the micellarization in Sea Lettuce.

Algal carotenoids are a type of pigments that have significant commercial applications. Since humans and animals cannot synthesize carotenoids, they must obtain them from their diet. However, the absorption and metabolism of carotenoids can be influenced by various factors, such as the morphological structure of algae, which directly impacts the digestibility and bioavailability of carotenoids (Wade et al., 2017). For instance, Gille, Trautmann, Posten, and Briviba (2015) investigated the bioaccessibility (micellization) of lutein and β-carotene by subjecting raw *Chlorella vulgaris* and *Chlamydomonas reinhardtii* biomass and sonicated ones to an in vitro digestion model. The results showed that carotenoids from *C. vulgaris* were not bioaccessible without prior sonication, whereas carotenoids from *C. reinhardtii* had minimal effect on bioaccessibility.

Rao et al. (2013) conducted an *in vivo* study on the bioavailability of β-carotene, astaxanthin, and lutein from *Arthrospira platensis* (Cyanobacteria), *Haematococcus lacustris* and *Botryococcus braunii* (Chlorophyta) biomass in rats. The study monitored the post-prandial response of plasma, liver, and eye over 9 hours after administration. The results showed that astaxanthin from *H. lacustris* had better bioavailability and antioxidant properties than other carotenoids. However, microalgal biomass rich in carotenoids could still prevent lipid peroxidation by scavenging free radicals in living cells and restoring enzyme activity such as catalase, superoxidase dismutase, and peroxidase. Lipid peroxidation products have been reported to exert various biological functions *in vivo*, such as regulating gene expression, signaling, activating receptors, and adaptive responses (Niki, 2009). Therefore, daily consumption of carotenoids by humans may help prevent cellular damage to molecules containing lipids in conditions of oxidative stress.

Muszynska and colleagues (2018) investigated the bioaccessibility of phenolic compounds, lutein, and bioelements (zinc, iron, and magnesium) in commercial preparations of *Chlorella vulgaris* powder and tablets using artificial digestive juices. The results showed good bioaccessibility of phenolic compounds and lutein in preparations containing *C. vulgaris* biomass, but bioelements were found to be negligible after digestion. The authors also noted in a previous study that C. vulgaris preparations in the form of powder and tablets may not be a good source of iron, magnesium, and zinc alone (Muszyńska et al., 2016).

2.2.5. Bioaccessibility of minerals

In nutrition, a mineral is a chemical element that is essential for an organism to carry out vital functions necessary for life. These minerals are mainly obtained by consuming plants, animals, or water. The human body requires five major minerals, namely calcium

(Ca), phosphorus (P), potassium (K), sodium (Na), and magnesium (Mg). Additionally, there are trace elements such as sulfur (S), iron (Fe), chloride (Cl), cobalt (Co), copper (Cu), zinc (Zn), manganese (Mn), molybdenum (Mo), iodine (I), and selenium (Se) that have specific biochemical functions in the human body. Algae, such as seaweed or microalgae, are excellent sources of selected minerals. Table 4 highlights some examples of the bioaccessibility of minerals from algae.

Moreda-Pineiro et al. (2012) created an in vitro digestion method using dialyzability to measure the bioavailability ratios of ten trace elements (Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, V, and Zn) from six different edible seaweeds, including *Himanthalia elongata, Saccorhiza polyschides, Laminaria ochroleuca, Undaria pinnatifida* (Ochrophyta, Phaeophyceae), *Ulva rigida* (Chlorophyta), and *Palmaria palmata* (Rhodophyta). The authors established the accuracy of the in vitro bioavailability method by performing a mass balance study and found high dialyzability percentages (30.0-74.7%) in the edible seaweeds. Multivariate analysis revealed a positive correlation between the bioavailability of some metals and the carbohydrate and dietary fiber content, suggesting that samples with higher carbohydrate or dietary fiber contents have higher metal bioavailability ratios. The authors hypothesized that carbohydrates and polysaccharides from seaweed may form micelles, which can increase the partition of hydrophobic molecules in aqueous solution and enhance metal absorption (Yu et al., 2010). The study also found negative correlations between the bioavailability percentages of certain metals, proteins, and lipids. The authors attributed this to the hydrolysis of proteins to amino acids during in vitro digestion, which can increase the ionic strength in the aqueous phase, resulting in lower metal solubility (Kramer, Shende, Motl, Pace, & Scholtz, 2012). Additionally, metals are not emulsified by bile extracts during in vitro digestion, which can limit their accessibility for uptake (Moreda-Pineiro et al., 2012).

Several seaweed species that are undervalued and not well-known require further investigation, given their increasing importance as a food source and/or bioactive substances. In a study by Afonso et al. (2021), three sun-dried and steamed Azorean seaweed species, *Petalonia binghamiae, Halopteris scoparia* (Ochrophyta, Phaeophyceae), and *Osmundea pinnatifida* (Rhodophyta), were evaluated for their elemental composition (As, Br, Cd, Co, I, Li, Mn, Ni, Sr) and in vitro bioaccessibility. Of particular interest was the iodine (I) content in *P. binghamiae*, which is important for synthesizing thyroid hormones in humans. The study found that *P. binghamiae* contained approximately 81 $\mu g.kg.dw^{-1}$ of iodine, with iodine bioaccessibility ranging from 57% to 69%. In Japan, the daily dietary reference intake (DRI)

of iodine is estimated to be within 1000–3000 μg.day^{-1} (Zava & Zava, 2011). Therefore, according to Afonso et al. (2021), consuming 2.7 g of sun-dried *P. binghamiae* seaweed daily can meet the daily iodine intake recommendations.

Selenium (Se) is a vital micronutrient that plays a crucial role in the functioning of antioxidant enzymes such as glutathione peroxidase in the human body. Good sources of selenium include Brazil nuts, seafood, dairy products, organ meats, and eggs. Recently, researchers investigated the in vitro bioaccessibility of selenium combined with *in vivo* bioavailability and bioactivity of Se-enriched microalga *Chlorella sorokiniana* to be used as a functional food (Gomez-Jacinto et al., 2020). The selenized microalga exhibited a high in vitro gastrointestinal digestion rate of 81%, while *in vivo* mice supplemented with Se-enriched *C. sorokiniana* showed a high concentration of selenium in the kidney, indicating a potential excretion mechanism via urine. Therefore, Se-enriched *C. sorokiniana* can be used not only as an ingredient in the formulation of nutraceuticals but also as a dietary supplement for humans seeking selenium supplementation (Gojkovic et al., 2014; Saurav et al., 2019).

2.2.6. Bioaccessibility of vitamins

Vitamins are vital organic molecules that an organism requires in small amounts for proper metabolic function, making them essential micronutrients. In general, these micronutrients are obtained from the diet. However, the Food Fortification Initiative (www.ffinetwork.org) has compiled a list of countries with mandatory fortification programs for folic acid, niacin, and vitamins A, B1, B2, and B12.

Both seaweed and microalgae, collectively known as algae, are rich sources of vitamins. The Chlorophyta phylum, which includes *Chlorella vulgaris*, is particularly abundant in multivitamins such as provitamin A, vitamins B1, B2, B6, B12, C, and E, as well as folic acid, niacin, and pantothenic acid, as noted by Liu and Hu in 2013.

Cobalt is an element found in the active center of cobalamin, a group of co-enzymes also known as vitamin B12, which is an essential vitamin for all animals. The United States' Dietary Reference Intakes (DRI) recommends a daily intake of 2.0 μg of vitamin B12, and it is generally assumed that healthy adults absorb 50% of dietary vitamin B12 (Watanabe, 2007). A study was conducted on the bioaccessibility of vitamin B12 from *Neopyropia yezoensis,* formerly known as *Porphyra yezoensis,* in rats fed a diet supplemented with dried *N. yezoensis* seaweed (10 μg vitamin B12/kg diet). The findings revealed that *N. yezoensis* contains approximately 52 μg of vitamin B12, and a significant amount (80%) of the vitamin

B12 compound was bioaccessible in the intestinal tracts of rats (Table 4) (Watanabe et al., 2000).

Table 4: Summary and comparison of case studies on bioaccessibility of pigments, vitamins, phenolic compounds and minerals in algae.

Seaweed	Type of compound	Digestion method	Bioaccessibility	Reference
Porphyra umbilicalis, *Laminaria ochrolueca*, *Ulva* sp.	Chlorophyll	*In vitro* digestion protocol, samples were cooked or microwaved	4-10% boiled 6-12% microwaved	(K. Chen & Roca, 2019)
Five edible seaweed	Toxic elements (Al, As, Ni, Cd, Pb)	Simulation of gastrointestinal tract	5-15% aluminium and lead 40-55% arsenic and nickel 76-90% cadmium	(Desideri, Roselli, Feduzi, Ugolini, & Meli, 2018)
Petalonia binghamiae	Minerals (Li, Mn, Co, Ni, As, Br, Sr, Cd, I)	*In vitro* digestion model	32-77% depending upon mineral	(Afonso, et al., 2021)
Hawaiian seaweeds	Iron (Fe)	*In vitro* digestion/Caco-2 model	7%	(Flores, Dobbs, & Dunn, 2015)
Sargassum sp.	Iron (Fe)	*In vivo* study in 90 human	20%	(Maria N García-Casal, Pereira, Leets, Ramírez, & Quiroga, 2007)
Microalgae				

Scenedesmus obliquus	Chlorophyll	Infogest protocol, Caco-2 human intestinal cells	33.5% isolated chlorophyll extract	(Fernandes, et al., 2021)
Chlorella vulgaris, Chlamydomonas reinhardtii	Lutein, β-carotene	*In vitro* digestion model	6-20% lutein sonicated 7-12% β-carotene sonicated	(Gille, et al., 2015)
Chlorella sorokiniana	Selenium	*In vitro* simulated gastrointestinal digestion	81% selenium	(Gómez-Jacinto, et al., 2020)
Chlorella vulgaris	Lutein, phenolic compounds, minerals	Artificial digestive saliva, gastric, intestinal juices	4-7% lutein 1-2% phenolic compounds (p-coumaric acid, cinnamic acid, kaempferol 7-rhamnoside) <0.02% Zn, Fe, Mg	(B. Muszyńska, et al., 2018)
Arthrospira platensis *Haematococcus pluvialis* *Botryococcus braunii*	β-carotene Astaxanthin Lutein	*In vivo* studies in rat model (plasma, liver, eyes)	1.4% β-carotene 2.8% astaxanthin 2.5% lutein	(Ranga Rao, Raghunath Reddy, Baskaran, Sarada, & Ravishankar, 2010)
Porphyra yezoensis	Vitamin B_{12}	*In vivo* studies in rat fed with B_{12} supplementation	80%	(Watanabe, et al., 2000)

Certain species of cyanobacteria, such as *Arthrospira platensis, Aphanizomenon flosaquae,* and *Nostoc commune*, are exceptional producers of vitamin B12. Commercially available tablets of *A. platensis*, commonly known as Spirulina, contain 127-244 μg of vitamin B12 per 100 g weight (Watanabe, 2007). However, some studies suggest that vitamin B12 from Spirulina tablets may not be bioavailable in mammals due to the identification of the main corrinoid as pseudovitamin B12 (83%), with only 17% confirmed as actual vitamin B12 (Watanabe et al., 1999).

2.3. Digestibility and bioaccessibility of algae in animal feed

Numerous nutritional and toxicological articles in the literature have demonstrated the potential of algal biomass as a valuable animal feed and aquaculture supplement (Shields & Lupatsch, 2012). Approximately 30% of the global algal production is estimated to be used for animal feed applications (Becker, 2004). Moreover, algae can be an excellent alternative protein source for the animal nutrition sector, as it contains a range of natural bioactive compounds, pigments, and polyunsaturated fatty acids (Angell, Angell, de Nys, & Paul, 2016).

Algae have traditionally been utilized as a protein supplement in animal feeds or as fertilizers (Becker, 2007). In fact, during the First World War on the French Atlantic coast, algae were fed to animals as a substitute for oats and forage due to their scarcity. From 1960 to 1980, significant amounts of brown algae from the *Fucus* and *Sargassum genus* were added to animal feed. The initial studies on supplementing animal diets with algae (swine, poultry, and horses) intended for human consumption demonstrated that algae are well accepted and have good digestibility and assimilation. Furthermore, studies have shown the beneficial effects of adding algae to the feed at levels of 5%–10% (Coffey, Dawson, Ferket, & Connolly, 2016; Fleurence et al., 2012).

When considering all types of algae, many of them contain cellulosic cell wall material that could pose digestibility challenges. Additionally, the effects of algal supplementation must be analyzed in two distinct animal groups: ruminants and non-ruminants (monogastric vertebrates). Ruminants such as cattle, bovines, and sheep are capable of digesting cellulosic material, whereas, for monogastric vertebrates, the algal

biomass must undergo proper processing before it can be deemed suitable as feed (Becker, 2004).

Numerous studies have assessed the digestibility and bioaccessibility of microalgal biomass as a feed supplement in various animals, including sheep, chickens, mice, and rats. Other studies have focused on determining the bioavailability of microalgal compounds in aquatic animals, such as rainbow trout, Atlantic salmon, Nile tilapia, and Pacific white shrimp. Table 5 presents these studies along with the primary digestion method utilized and the major findings.

In light of the continual advancements in poultry nutrition, there is currently a particular emphasis on developing optimal amino acid concentration profiles in diets. Tavernari et al. (2018) studied the apparent metabolizable energy and amino acid digestibility of the microalgae *Arthrospira platensis* as an ingredient in broiler chicken diets. The results demonstrated that substituting soybean and corn meal with up to 7.5–10% *Arthrospira* biomass in the poultry diet during the first three weeks of life increased bird weight and improved food efficiency (Austic, Mustafa, Jung, Gatrell, & Lei, 2013).

Sean Tibbetts and colleagues from the National Research Council of Canada have conducted several specialized studies on the digestibility of microalgal biomass in animal feed, producing high-quality research. Their investigations include: i) analyzing the chemical composition and nutritional properties of freshwater and marine microalgal biomass cultured in photobioreactors (Tibbetts et al., 2015); ii) studying the ruminal in vitro gas production, dry matter digestibility, methane abatement potential, and fatty acid biohydrogenation of six microalgae (Tibbetts, MacPherson, McGinn, & Fredeen, 2016; Anele, Yang, McGinn, Tibbetts, & McAllister, 2016); iii) assessing the simulated ruminal fermentation and in vitro monogastric digestibility of the microalga Scenedesmus sp. (Chlorophyta) for animal feed (Sean & Alan, 2017); iv) examining the nutritional composition, total phenolic compound, and in vitro digestibility of some wild seaweeds (Tibbetts, Milley, & Lall, 2016); v) predicting the in vitro digestible protein content of marine microalgae *Nannochloropsis granulata* (Ochrophyta, Eustigmatophyceae) meals for Pacific white shrimp (*Litopenaeus vannamei*) and rainbow trout (*Oncorhynchus mykiss*) (Tibbetts, Yasumaru, & Lemos, 2017), and vi) evaluating the apparent digestibility of essential amino acids and fatty acids of juvenile Atlantic salmon (*Salmo salar L.*) diets containing *Chlorella vulgaris* meals (Tibbetts, Mann, & Dumas, 2017). All the results of these studies are summarized in Table 5.

The apparent digestibility coefficients (ADCs) of microalgal biomass in aquatic animals are also being investigated by several authors. For example, Sarker et al. (2016)

studied the ADC of Nile tilapia juveniles fed diets containing *Arthrospira* biomass and found that the tilapia juveniles showed good digestibility of protein and amino acids from the *Arthrospira* biomass. These examples illustrate that research on the digestibility and bioaccessibility of algae in animal feed is a rapidly evolving field.

Table 5: Summary and comparison of case studies on digestibility and bioaccessibility of microalgal biomass in feed supplementation.

Animal studied	Microalgae	Digestion method highlight	Major findings	Reference
Ruminant animal	Several microalgae biomass	*In vitro* dry matter digestibility with fermentation	Microalgae biomass is easily digested and utilized by ruminants, with greater digestibility of short chain fatty acids	(Anele, Yang, McGinn, Tibbetts, & McAllister, 2016)
Sheep	Combo of microalgae	*In vivo* trials in lambs, blood analysis	Lambs had an average 70% dry matter and 60% nitrogen digestibility	(Stokes, Van Emon, Loy, & Hansen, 2015)
Monogastric animal	*Scenedesmus* sp.	Simulated ruminal fermentation, *in vitro* monogastric digestibility	Protein and energy digestibility were high (80%), while whole algal cell resulted in moderate digestibility (55%)	(M Tibbetts & H Fredeen, 2017)
Broiler chicken	*Spirulina platensis*	*In vivo* trials in male broilers, excreta collected for analysis	Algal amino acids in poultry diet revealed an increase chicken weight with good AA digestibility	(Tavernari, et al., 2018)
Mice	*Chlorella sorokiniana* (selenium-enriched microalga)	*In vivo* experiments in male mice (*Mus musculus*), blood test	Selenium (Se) bioavailability and bioactivity was 76-85% in serum from these mice animals	(Gómez-Jacinto, et al., 2020)
Rat	*Dunaliella bardawill*	*In vivo* female weanling rats, blood analysis	Animals accumulated large quantity of phytoene-rich algae in the liver organ	(Werman, Mokady, & Ben-Amotz, 2002)

Rainbow trout	*Schizochytrium* sp.	Apparent nutrient digestibility	Digestibility of lipid, fatty acids and DHA in rainbow trout maintained at 8 and 15ºC were similar.	(Bélanger, Sarker, Bureau, Chouinard, & Vandenberg, 2021)
European seabass juveniles	Three types of microalgae	Apparent digestibility coefficients (ADCs)	Digestibility of protein, lipid and energy in non-disrupted algal cell wall were reduced during digestion	(S. Batista, et al., 2020)
Atlantic salmon	*Nannochloropsis oceanica*	Apparent digestibility coefficients (ADCs)	Algal inclusion in extruded feeds led to higher ADCs of dry matter and gross energy in salmon fish	(Gong, Guterres, Huntley, Sørensen, & Kiron, 2018)
Atlantic salmon	*Schizochytrium* sp.	Apparent digestibility coefficients (ADCs)	Algal biomass offers a highly digestible source of DHA and protein without any previous algal cell disruption	(Hart, et al., 2021)
Nile tilapia juveniles	*Schizochytrium* and *Spirulina*	Apparent digestibility protocol, faecal collection	Tilapia showed high digestibility of lipid/fatty acid from *Schizochytrium*, and good digestibility of protein/amino acid from freshwater *Spirulina* sp.	(Sarker, et al., 2016)
Pacific White shrimp and rainbow trout	*Nannochlorospsis granulata*	Species-specific *in vitro* pH-Stat determination of protein degree of hydrolysis	Predicted protein apparent digestibility coefficient of algal meals was moderate for shrimp (69-78%) and high for rainbow trout (79-88%)	(Sean M. Tibbetts, et al., 2017)

2.4. Digestibility and bioaccessibility of algae in food matrix

Seaweed has a long history of consumption worldwide, especially in East Asia, including Japan, China, Korea, Taiwan, and Southeast Asia. The food industry commonly uses seaweed polysaccharides as a functional ingredient in various products, including frozen foods, ice cream, jams, jellies, and beverages. In addition, numerous commercial food products made from seaweed are available in the market under different brand names, such as potato chips with seaweed, nori snack with wasabi, rolling crispy, grilled thins seaweed, and raw unroasted seaweed (Pandey, Chauhan, & Semwal, 2020).

Despite the well-established market for seaweed-based food products, there is limited research on the digestibility and bioaccessibility of these products. Some examples in the literature include gluten-free bread enriched with *Ascophylumm nodosum* (Rózyło, Hameed Hassoon, Gawlik-Dziki, Siastała, & Dziki, 2017), maize bread (arepa) enriched with *Ulva, Sargassum,* or *Porphyra* (García-Casal, Ramírez, Leets, Pereira, & Quiroga, 2009), and yogurt containing extracts of brown algae (O'sullivan et al., 2016).

Researchers have recognized the potential of microalgae as an innovative and healthy food source and have explored the creation of new food products enriched with various types of microalgae biomass. Examples include pasta incorporating *Chlorella vulgaris* and *Limnospira maxima* (formerly *Spirulina maxima*) (Cyanobacteria) (Fradique et al., 2010), macaroni enriched with *Isochrysis galbana* and *Diacronema vlkianum* (Haptophyta, Pavlovophyceae) as a source of PUFA's (Fradique et al., 2013), biscuits with *Isochrysis galbana* PUFA-ω3 (Gouveia et al., 2008), butter cookies with *Chlorella vulgaris* biomass (Gouveia, Batista, Miranda, Empis, & Raymundo, 2007), and gelled dessert enriched with *Limnospira maxima* and *Haematococcus lacustris* (Batista et al., 2012). Studies on microalgal-based foods have investigated their bioactivity and their impact on the digestibility and bioavailability of major compounds in different food matrices (Batista et al., 2017; Mjaurcova, Kramar, Klejdus, & Vacek, 2018). Table 6 summarizes selected reports on the digestibility of algal biomass as a food ingredient, highlighting the diversity of their applications in different food matrices.

Researchers have conducted in vitro digestibility tests on microalgae-based food products in different food matrices. One study investigated the sensory, physical, chemical properties, antioxidant activity, and in vitro digestibility of cookies enriched with microalgae biomass of *Arthrospira platensis, Chlorella vulgaris, Tetraselmis suecica,* and

Phaeodactylum tricornutum (Batista et al., 2017). The study tested two levels of biomass (2% and 6% w/w) and compared them to a control. The addition of microalgal biomass resulted in cookies with varying appearances, ranging from blueish-green (*A. platensis*) to brownish-green (*P. tricornutum*). Cookies prepared with *A. platensis* showed the highest sensory score, as well as high protein and phenolic contents. However, in terms of in vitro digestibility (IVD) analysis that reproduced the chemical-enzymatic catalysis, no significant difference in IVD between microalgae cookies and the control (IDV 87–95%) was found (Batista et al., 2017; Morais, Miranda, & Costa, 2009).

Another study investigated the in vitro bioaccessibility of minerals like Ca, Fe, K, Mg, P, Se, and Zn in cookies enriched with 1.5 or 2.0% of *Chlorella* or *Arthrospira* added with functional mineral (Uribe-Wandurraga, Igual, García-Segovia, & Martínez-Monzo, 2020). In vitro digestion of functional cookies made from three flours (wheat, barley, and oat) and 15% or 10%, or 5% of *Haematococcus lacustris* microalgae rich in astaxanthin resulted in lower glucose release when astaxanthin increased in the formulation (Hossain et al., 2017). These studies emphasized the potential for creating new foods, such as cookies, enriched with microalgal biomass that provides natural bioactive compounds.

Dairy products, including yogurt, cheese, and butter, are consumed globally. To introduce innovation in the dairy industry, a new yogurt enriched with extracts (0.25% and 0.50% (w/w)) from two seaweeds, *Ascophyllum nodosum* and *Fucus vesiculosus*, was created. The yogurt was subjected to in vitro antioxidant assays to determine its total phenolic content (TPC) and DPPH scavenging activities. The results showed that the ferrous-ion chelating activity of the seaweed-enriched yogurt was stable after digestion, but the DPPH radical scavenging activity was not stable (O'Sullivan et al., 2016). Previous research has found that the presence of milk proteins can reduce polyphenol compounds, such as those found in seaweed extracts, due to the formation of polyphenol-milk protein complexes, which bind the sites of antioxidant activity (O'Sullivan et al., 2014; Xiao et al., 2011).

Several studies have examined the potential of seaweed as a mineral bioaccessibility enhancer in traditional food. One study evaluated the addition of red seaweed *Pyropia columbina* (formerly *Porphyra columbina*) to extruded maize snacks. The product was tested for mineral bioaccessibility (Na, P, Ca, and Mg), angiotensin-converting enzyme (ACE) inhibitors, and antioxidant activity (Cian, Caballero, Sabbag, Gonzalez & Drago, 2014). The results showed that extruded maize snacks with added red seaweed had higher dialyzability of ACE inhibitor compounds, total phenolic content, and antioxidant capacity (measured by DPPH radical scavenging assay) compared to extruded maize alone (Cian, et al., 2014).

However, seaweed is also rich in dietary fiber, polyphenolic compounds, and phytic acid, which may impact mineral bioavailability (Nissar, Ahad, Naik, & Hussain, 2017). For example, fiber may form insoluble complexes with minerals, while phytic acid can bind to other minerals to create phytates, which may interfere with nutrient bioavailability and digestibility (Baye, Guyot, & Mouquet-Rivier, 2017; Gharibzahedi & Jafari, 2017;Lopez, Leenhardt, Coudray, & Remesy, 2002).

Iron deficiency is a widespread nutritional deficiency globally (Nag, Ray, & Rakshit, 2020). The U.S. Institute of Medicine (IOM) recommends a daily iron intake of 15.0 mg (Milman, 2019). Iron is often added to fortified foods such as breakfast cereals or enriched wheat flour. Seaweeds are a good source of iron, and if it is bioavailable, their consumption could help combat iron deficiency and anemia. Therefore, in a study conducted by García-Casal et al. (2009), *in vivo* iron bioaccessibility was examined in eighty-three adult volunteers who were administered with radioactive Fe. The volunteers received maize- or wheat-based meals containing marine seaweed (*Ulva, Sargassum,* and *Porphyra* species) in varying proportions (2.5, 5.0, and 7.5 g) added to the water used to prepare the dough. *Sargassum sp.* was found to have the highest iron intake due to its high iron content, and the bread containing 7.5 g *Sargassum sp.* met daily iron requirements. Furthermore, *Sargassum sp.* had a high polyphenol content with excellent antioxidant power and did not affect iron absorption (García-Casal, Pereira, Leets, Ramírez, & Quiroga, 2007). This study also emphasized that combining marine seaweed with maize bread (arepa) is a good example of a Fe-fortified food product that could be provided in social programs to increase iron intake.

Recent scientific studies have focused on analyzing the inclusion of edible brown seaweed combined with gluten-free bread and pasta prepared from maize, rice, and tapioca flours due to the increasing demand for gluten-free products (Alvarenga et al., 2011; Fradinho et al., 2019). In vitro digestion studies have been conducted to investigate the impact of adding different amounts (2-10%) of brown seaweed (*Ascophyllum nodosum*) powder to gluten-free bread (Rozyło et al., 2017). Additionally, the active compounds bioaccessibility (ACB) index and antioxidant bioaccessibility (BAC) index were determined to better understand the relationships between active compounds and their potential bioaccessibility (Gawlik-Dziki et al., 2013). The seaweed addition significantly increased the antioxidant potential of gluten-free bread and was found to be highly accessible in vitro. However, the study concluded that a maximum of 2-4% seaweed could be added to achieve acceptable gluten-free bread due to an unpleasant taste associated with over-addition.

Table 6: Summary and comparison of case studies on digestibility and bioaccessibility of algal biomass in different food matrices.

Type of food product	Microalgae	Methods/tests/assays	Outcome	Reference
Pills of astaxanthin carotenoid	*Haematococcus pluvialis*	*In vivo* 32 healthy male, blood sampling	Glycerolmono- and dioleate and polysorbate 80 lipid formulation enhanced astaxanthin bioavailability	(Odeberg, Lignell, Pettersson, & Höglund, 2003)
Cookies	*A. platensis, C. vulgaris, T. suecica, P. tricornutum*	*In vitro* digestibility (IVD) tests	87-95% IVD microalgal cookies	(A. P. Batista, et al., 2017)
Cookies	*A. platensis, C. vulgaris*	*In vitro* digestibility test with Infogest	Excellent accessibility of minerals (P, K, Ca, Mg, Fe, Zn and Se)	(Uribe-Wandurraga, et al., 2020)
Cookies	*Haematococcus pluvialis* (astaxanthin)	*In vitro* carbohydrate digestion (Glycaemic glucose equivalent - GGE)	Lower glucose release when astaxanthin increased in the formulation	(Hossain, et al., 2017)
Chocolate biscuit	*Spirulina* biomass	*In vitro* protein digestibility	86% protein digestibility of biscuits containing 1.0% of *Spirulina*	(Morais, et al., 2009)

Snack	*Spirulina* biomass	*In vitro* protein digestibility	89% protein digestibility of snacks enriched with 2.6% of *Spirulina*	(Lucas, de Morais, Santos, & Costa, 2018)
Bread wheat pasta	*Spirulina* biomass	*In vitro* protein digestibility	Protein digested increased slightly with the incorporation of microalgae in pasta formulation	(De Marco, Steffolani, Martínez, & León, 2014)
Yogurt	*Schizochytrium* sp. (nanoemulsion DHA oil)	*In vivo* 11 (men and women) humans, blood testing	The nanoemulsions enriched yogurt gave rapid increases in DHA levels, which peaked 2h after ingestion	(Lane, et al., 2014)
Emulsions with soy lecithin-stabilized	*Schizochytrium* sp. (DHA oil)	Simulated *in vitro* digestion gastrointestinal tract	Bioaccessibility of DHA from soy-lecithin improved slightly the algal oil emulsion	(Lin, et al., 2014)
Type of food product	Seaweed			
Gluten-free bread	Brown algae (*Ascophyllum nodosum*)	Simulated *in vitro* digestion gastrointestinal tract	Adding 2-4% of algal biomass greatly increased the antiradical potential of gluten-free bread	(Różyło, et al., 2017)
Maize bread (arepa)	*Ulva*, *Sargassum*, *Porphyra*	*In vivo* iron (Fe) bioavailability in 83 adult humans	Maize bread with algal inclusion increased greatly the iron absorption	(M. N. García-Casal, et al., 2009)

Snack (extruded maize)	*Porphyra columbina*	*In vitro* digestion using multi-enzimatic method	Increased biosorption of protein, minerals (Na, P, Ca and Mg) and phenolic compounds.	(Raúl E. Cian, et al., 2014)
Yogurt	*Ascophylumm nodosum, Fucus vesiculosus* extracts	*In vitro* digestion and antioxidant assays	Yogurt containing algae extracts (0.25 and 0.50% (w/w)) indicated that Fe-chelating activity was stable, while DPPH was not stable.	(A. O'sullivan, et al., 2016)

2.5. Influence of pretreatment methods on the accessibility of algae compounds

The composition, thickness, and rigidity of algal cell walls can significantly affect their digestibility. The cellular apparatus of algae plays a crucial role in determining the bioaccessibility and bioavailability of intracellular compounds. Extraction of intracellular metabolites from microalgae cells can be challenging due to their small size, relatively thick cell walls, and the presence of products in globules or bound to cell membranes. However, pretreatment methods such as cell disruption can be applied to enhance the digestibility and bioavailability of algae without compromising their bioactivity. Studies by Phong et al. (2018) and Ummat et al. (2021) have demonstrated the effectiveness of such methods.

Cell disruption or microalgal cellular disintegration can be achieved through various methods that can be classified into two groups: mechanical and non-mechanical methods. Mechanical methods involve the use of mechanical forces such as bead mills and high-speed homogenization, liquid-shear forces such as high-pressure homogenization and microfluidization, energy transfer through waves such as ultrasonication and microwave, and currents such as pulsed electric field and non-thermal plasma, or heat treatments such as thermolysis and autoclaving. Non-mechanical methods, on the other hand, involve cell lysis with chemical agents, enzymes, or osmotic shock. These pretreatment methods have been tested on microalgal suspensions and have been shown to enhance the yield of product recovery. A review by Günerken et al. (2015) provides a comprehensive overview of the advantages and disadvantages of these pretreatment methods applied to microalgae cells. Further studies by Matos et al. (2020), Sierra et al. (2017), and Yap et al. (2015) have also explored the effectiveness of these methods.

Numerous studies have investigated the effects of various pretreatment methods on the digestibility and bioaccessibility of microalgae and seaweed compounds. Table 7 provides an overview of these case studies, including the primary pretreatment methods and conditions employed, as well as their effects on the digestibility and bioaccessibility of major compounds extracted from the algae cells in question.

Laboratory-scale cell disruption of microalgae commonly employs mechanical methods such as bead milling, high pressure homogenization, high-speed homogenization, and ultrasonication (Halim, Harun, Danquah, & Webley, 2012). The high-pressure homogenization (HPH) technique has been used to enhance lipid recovery and digestibility,

as well as improve the bioaccessibility of carotenoids and eicosapentaenoic acid in *Nannochloropsis sp.* and *Chlorella vulgaris* suspensions in vitro (Bernaerts et al., 2020; Tibbetts et al., 2017). *Nannochloropsis* species are particularly challenging to lyse due to their small size (1-2 μm) and rigid cell wall, but the use of HPH allowed for an increase in lipid recovery and digestibility (40-62%) and enhanced bioaccessibility of carotenoids (1-6%) and eicosapentaenoic acid (13-29%) (Bernaerts et al., 2020).

Laboratory-scale experiments have demonstrated that the bead milling method can effectively disrupt the cell walls of microalgae such as *Microchloropsis gaditana, Arthrospira, Chlorella, Nannochloropsis,* and *Phaeodactylum*, leading to increased digestibility of protein and lipid, and higher crude protein digestibility values ranging from 78-84% (Teuling et al., 2019; Wild et al., 2018). Therefore, the bead milling technique not only breaks down the cell wall but also enhances the accessibility and bioavailability of essential compounds from microalgae. These findings are summarized in Table 7.

Ultrasonication is a method for disrupting microalgal cells, using ultrasonic disruptors operating at 20 and 40 kHz, as proposed by Gerde et al. (2012). The impact of sonication on the bioaccessibility of carotenoids from *Chlorella vulgaris* and *Chlamydomonas reinhardtii* was evaluated by Gille et al. (2015), and the results showed that sonication increased the bioaccessibility of C. vulgaris carotenoids to a level comparable to *C. reinhardtii*, with β-carotene and lutein reaching levels of at least 10% and 15%, respectively.

Enzymatic methods for cell lysis modify the cell wall or membrane components, allowing products to leach out selectively (Sierra et al., 2017). Carbohydrases, a group of enzymes that catalyze the breakdown of carbohydrates into simple sugars, are commonly used for this purpose. Seaweed cells have a rigid cell wall made up of complex polysaccharides, with a small amount of embedded proteins (Deniaud-Bou ̈et, Hardouin, Potin, Kloareg, & Hervé, 2017). Enzymatic pretreatment with solutions containing 10-100 U of xylanase and cellulase in 2 mL sodium acetate buffer has been tested for protein extraction from Rhodophyta seaweed *Palmaria palmata* before a simulated in vitro gastrointestinal digestion model. This pretreatment increased the availability of amino acids for extraction three-fold, along with increased bioaccessibility of protein and amino acids for intestinal absorption (Mæhre, Jensen, & Eilertsen, 2016). In summary, studies have shown that cell disruption methods not only enhance the extractability of intracellular components from algae but also increase the bioaccessibility and bioavailability of algal compounds in in vitro digestion assays.

Table 7: Summary and comparison of case studies on digestibility and bioaccessibility of algal compounds.

Pretretament method	Summary technique	Algae	Outcome	Reference
High pressure homogenization (HPH) before *in vitro* digestion	Biomass suspension: 100 Pa (4 passes) with an ice batch after each pass of HPH	*Nannochloropsis*	↑ digestibility of lipid (40 to 62%) ↑ bioaccessibility of carotenoid (1 to 6%) and EPA (13 to 29%)	(Bernaerts, et al., 2020)
Bead milling before *in vivo* digestion	Algae suspension was treated 3 times: 0.5 mm yttria-stabilized zirconia grinding beads; pump speed: 20 L/h; milling speed: 14 m/s	*Nannochloropsis gaditana*	↑ apparent digestibility of protein (62 to 78%), lipid (50 to 82%)	(Teuling, et al., 2019)
pH-shift process before an *in vitro* digestion model with added gastric lipase	pH 7.0/10.0 solubilization of protein and lipid pellets	*Nannochloropsis oculata*	↑ bioaccessibility of proteins (32 to 47%) and lipid (43 to 52%)	(Cavonius, et al., 2016)
Sonication before *in vitro* digestion	5 cycles/min, 20 kHz, 15 min., 25 °C	*Chlorella vulgaris*	↑ bioaccessibility of lutein (7.0 to 18.0%) and β-carotene (0 to 12.5%)	(Gille, et al., 2015)

High pressure homogenization before *in vivo* digestion	Microfluidizer (M-110P): 1724 bar; flow rate: 75 mL/min	*Chlorella vulgaris*	↑ apparent digestibility of proteins (77 to 87%), lipids (66 to 87%), carbohydrates (38 to 81%)	(Sean M Tibbetts, et al., 2017)
Stirred ball mill before *in vitro* digestion	Microalgae suspension was pumped thought a stirred ball mill at a pumping capacity of ~50 mL/min, 3200 rpm	*Arthrospira*, *Chlorella*, *Nannochloropsis*, *Phaeodactylum*	↑ crude protein digestibility of all microalgae species (78-84%)	(Wild, et al., 2018)
Proteolytic enzymes (pancreatic hydrolysis) before *in vitr*o protein digestibility	E/S ratio at 8% level, heat treatment (85 ºC, 20 min), centrifuge 3000×g (10 min, 4 ºC), supernatant collect (protein hydrolysates)	*Arthrospira platensis, Chlorella vulgaris*	↑ digestibility 50% to 97% ↑ digestibility 35% to 70%	(Kose, Ozen, Elibol, & Oncel, 2017)
Enrichment process before *in vitro* digestion	For mineralization: microwave accelerated reaction system model	*Chlorella sorokiniana*	81.0% of Selenium extracted after *in vitro* digestion process	(Gómez-Jacinto, et al., 2020)
Emulsified systems (whey protein isolate - WPI and xanthan gum - XG) before *in vitro* digestion	WPI stabilized emulsion and WPI-XG stabilized emulsion passed through a high-pressure homogenizer (3 times at 500 bars); pH 7.0	*Haematococcus pluvialis* - Astaxanthin oleoresin	WPI-XG demonstrated lower lipid digestibility; Higher digestibility of astaxanthin in WPI emulsion system (46.2%) than WPI-XG emulsion system (12.6%)	(Boonlao, Shrestha, Sadiq, & Anal, 2020)

Carbohydrases before *in vitro* digestibility	Xylanase and cellulase in 2 mL NaO_2CCH_3 buffer and incubation for 18h at 40°C under agitation	*Palmaria palmata*	↑ digestibility of total amino acids to 3-fold than control treatment	(Mæhre, et al., 2016)
Blend of alginate with non-gelatinized and gelatinized corn starch before *in vitro* digestion	Non-gelatinized starch suspensions: powders mixed with distilled water; Gelatinized starch solutions: mix with distilled water, autoclave treatment (120 ºC, 40 min). Blends: dialyzed in membranes against $CaCl_2$ solution (0.5 L, 150 mM, 3 days)	Brown macroalgae	Gels with gelatinized starch solutions: ↑ digestibility; Gels with high amylopectin: more degraded microstructure after digestibility	(Feltre, Almeida, Sato, Dacanal, & Hubinger, 2020)

2.6. Final considerations of the chapter

This chapter discusses research on the digestibility, bioactivity, and bioavailability of various compounds found in algae, including seaweed and microalgae. The composition of algae, particularly its thickness and rigidity, can impact how easily it is digested and absorbed by the human body. In vitro studies have shown that carbohydrates and polysaccharides in algae are only moderately digestible due to the high fiber content. Meanwhile, lipids and fatty acids are not highly bioaccessible because the human digestive tract does not efficiently break them down. However, in vitro studies have demonstrated that protein and amino acids from algae are highly bioaccessible during gastrointestinal digestion.

Dietary supplementation with algal biomass has been shown to improve the health of animals. *In vitro* studies of functional foods enriched with algal biomass have demonstrated good digestibility and bioaccessibility of natural bioactive compounds, supporting the idea that consuming algae can promote human health.

It is important to note that certain conditions, such as temperature, sonication, pressure, and enzymatic treatments, directly affect the digestibility and bioaccessibility percentages of algal biomass or compounds. These treatments can often double these numbers, making it possible to select the best conditions for cell disruption methods to obtain algae products with higher values accessible for absorption by the human body.

Studies such as these are necessary not only to improve the applicability of algae as food and ingredients in the human diet but also to extend the nutritional value of algae for human health.

CHAPTER III

3 COMMERCIAL SAMPLE CHARACTERIZATION

Using microalgae to confection plant-based products with well-balanced composition and good performance is a feasible alternative to complement plant-based products. As such, it is important to evaluate the microalgae biomass characteristics in a way that provides better knowledge about chemical composition and physical properties. This chapter aims to characterize commercial *Spirulina* samples, determining the chemical composition, physical characterizations and *in vitro* protein digestibility (IVPD) of commercially available *Spirulina* samples.

This chapter is based on the article "***Evaluating the biochemical composition, physical characteristics, and technofunctional properties of eight commercially Spirulina powders for food applications*** " submitted in May, 2024.

Abstract

The biochemical constituents, physical characteristics, techno functional properties, and volatile organic compounds (VOCs) of eight commercial *Spirulina* powders purchased online from Brazil (S-BR1, S-BR2, S-BR3, S-BR4), China (S-CH1 and S-CH2), and the United States (S-US1 and S-US2) were analyzed. Biochemically, the Chinese samples showed the highest content of proteins (~70.54 g/100g), saturated fatty acids like palmitic acid (~68.83%), and phycocyanin (3.66 g/100g). Two Brazilian samples (S-BR1 and S-BR3) exhibited high content of omega-6 polyunsaturated fatty acids (35.22%). Powder of S-BR4 showed the highest level of moisture (15.44 g/100g), potentially susceptible to microorganisms' growth. It also had elevated water activity (0.66), rendering the production of pyrazines volatile compounds due to Maillard reaction. By analyzing physical characteristics, the S-BR1 sample had the brightest color (L* = 20.08), the largest particle size (67.79 μm), the heaviest real density (1.40 g/cm3), the maximum porosity (0.632 g /cm3), and the fastest dispersion time (6.75 min), which is required for powders utilized in instant foods with high dispersibility. S-BR1 also displayed notable technofunctional properties, such as high solubility (67.79%), foaming capacity (60.00%), and in vitro protein digestibility (85.70%) among all *Spirulina* powders investigated. Sample from the United States (S-US2) demonstrated great water (3.29 g/g) and oil (1.25 g/g) holding capacity, with

notable concentrations of volatile organic compounds such as hydrocarbons, ketones, aldehyde, and furans. The present data evidenced *Spirulina* biomass is rich in valuable nutrients, but physical and technofunctional properties of powders differ strongly within the same species, suggesting that food quality parameters of commercially available *Spirulina* biomass is still not standardized.

Keywords: *Spirulina* powder, protein, *in vitro* digestibility, physical and functional properties, volatile organic compounds.

3.1. Introduction

Microalgae are a group of photosynthetic organisms able to accumulate proteins, carbohydrates, lipids, fatty acids, pigments, vitamins, and minerals by converting inorganic substances (C, N, P, S, Fe) and trace elements into organic matter (Matos, 2021). The large quantities of macronutrients found in microalgal biomass make it a valuable source of bioactive compounds, which can be used to enhance the nutritional value of foods (Gouveia et al., 2007; Matos, 2017). Considering the diversity of microalgae species, they must undergo a comprehensive physicochemical characterization before being used as an ingredient for food formulation (Batista et al., 2013; Matos et al., 2016).

Incorporation of microalgal biomass and/or extracts into conventional food products can render textural and rheological modifications, which are categorized as product and process attributes (de Farias Neves et al., 2019; Matos et al., 2023). Product attributes can be appearance, texture, smell, safety, post-harvest life, and convenience (Francezon et al., 2021). Detailed analysis of physical characteristics and technofunctional properties of microalgal biomass are also important factors to be considered when selecting microalgae species for food product development (Munialo et al., 2022; Robertson et al., 2016). Basic information about physical and technofunctional properties of different foods such as gluten-free bread (Khemiri et al., 2020), wheat bread dough (Qazi et al., 2021), soy protein isolated hydrogel (Wang et al., 2023), yogurts (Lane et al., 2014), and biscuits (Gouveia et al., 2008) enriched with a few species of microalgal biomass or its derivatives (oil, extracts, proteins, pigments) can be found in the literature, which have expanded the scientific knowledge towards the discovery of novel foods based on microalgal biomass (Hellwig et al., 2022).

The sensory appeals of a food product play a crucial role in influencing consumer acceptance, rejection, and purchase intention (Maehle and Skjeret, 2022; Onwezen et al., 2021). The palatability of microalgal biomass influenced by aroma, flavor, color, textural mouthfeel, and taste (salty, bitter, sweet, sour, and umami) makes its use in products challenging (Moons et al., 2018; Weinrich and Elshiewy, 2019). For instance, research on volatile organic compounds (VOCs) from microalgae biomass is in the spotlight due to its distinct odors affecting the food products' sensorial properties (Coleman et al., 2023b; Van Durme et al., 2013). Alcohols, aldehydes, alkenes, ketones, terpenes, furans, and pyrazines are the major discriminant chemical classes already identified in various microalgae species (Coleman et al., 2022; Moran et al., 2022), including the most biotechnologically cultivated microalgae *Arthrospira platensis* (Magpusao et al., 2022).

Owing to its historical use and GRAS (generally recognized as safe) status by the Food and Drug Administration (FDA) in the US, the *A. platensis*, usually denoted as *Spirulina* in the market, is one of the top trends microalgae in food industry (Lafarga et al., 2020; Nunes et al., 2023). It is the most dietetically relevant microalgae for human consumption because of its high protein content (55-70 g/100g), full spectrum of essential amino acids associated with high digestibility (up to 85%) (Demarco et al., 2022; Matos, 2019). *Spirulina* is also rich in γ-linolenic acid (C18:3ω6), and natural bioactive compounds, notably phycocyanin, carotenoids, and vitamin B12, enlightening the positive impact of consuming *Spirulina*-based food products for human health promotion (Buecker et al., 2024; Ferreira de Oliveira and Bragotto, 2022).

Most of *Spirulina* biomass is being consumed as a nutritional supplement labeled as a "superfood" and is markedly sold as dried powder, flakes or capsules (Lafarga et al., 2020). Taking into consideration that *Spirulina* biomass can be incorporated into food matrices to enhance nutritional and technological values, the several biomasses available in the market can have variations in their nutrient profile, reflecting on the food application (Sandgruber et al., 2021). As *Spirulina* biomass is gaining even more popularity among consumers in Brazil, more research on the chemical composition, physical and technofunctional properties, as well as VOCs attributes of commercially powders of *Spirulina* is needed (Hellwig et al., 2022; Matos, 2021). This will advance our knowledge of choosing the appropriate biomass for developing a specific food product carrying on natural bioactive compounds from *Spirulina*.

This study aims at evaluating the chemical composition in terms of moisture, ash, protein, lipid, fatty acids, phycocyanin, sodium and potassium of eight commercial powders of *Spirulina* biomass purchased online from three countries (Brazil, China, and the United States). To discuss the potential use of *Spirulina* for food applications, a meticulous analysis on the physical characteristics (particle size, surface charge, real density, apparent density, porosity, dispersion time, water activity, color, and microstructural images), technofunctional properties (water holding capacity, oil holding capacity, solubility, foaming ability and in vitro protein digestibility), and major VOCs (hydrocarbons, ketones, aldehydes, alcohols, terpenes, furans, pyrazines, and esters) on *Spirulina* powders using a wide range of analytical methodologies and techniques was conducted.

3.2. Material and methods

3.2.1. Spirulina powders

Eight commercially available *Arthrospira* (*Spirulina*) samples of different origin were analyzed. *Spirulina* samples as a dried powder from different dealers, i.e., Brazil (BR code), the Republic of China (CH code), and the United States (US code) were purchased online. Samples were identified with codes as seen below. Detailed information on the culture medium, cultivation mode, harvesting and dry processes were mostly unknown. Samples were stored in the refrigerator under darkness at 5 °C for later use.

Identification	S-BR1	S-BR2	S-BR3	S-BR4	S-CH1	S-CH2	S-US1	S-US2
Origin location	Brazil	Brazil	Brazil	Brazil	China	China	USA	USA

3.2.2. Biochemical composition of powders

3.2.2.1. Proximate composition

Moisture content was determined by drying the sample in an oven at 105°C for 3-4 h until constant weight (Horwitz, 2010). Ash content was determined by heating the samples to 550ºC for 5 h using a muffle furnace (Lutz, 2008). Sodium (Na) and potassium (K) contents were determined by flame photometric method (AOAC 969.23) (Horwitz, 2010).

Protein analysis was determined by the Kjeldahl method after acid digestion (AOAC 991.20), ammonium addition, steam distillation and titration with 0.1 N HCl. A nitrogen-to-protein conversion factor of N x 6.25 was used to calculate protein content (Horwitz, 2010). Lipids were extracted with petroleum ether by the Soxhlet method (AOAC 963.15) after acid digestion with 4.0 N HCl for 6 h, concentrated in a rotary evaporator, dried in an oven and weighed (Horwitz, 2010).

3.2.2.2. Fatty acid analysis

After converting the fatty acids (FAs) to their corresponding fatty acids methyl esters, FAs were determined by gas chromatograph using a GC-2014 (Shimadzu, Kyoto, Japan), equipped with split-injection port, flame-ionization detector and Restek capillary column (105 m-long, coated with 0,25 μm of 90% biscyanopropylsiloxane and 10% cyanopropylphenyl). The injector and detector temperatures were both 260 °C. The oven temperature was initially set at 140 °C for 5 min, programmed to increase at 2.5 °C/min, and held at 260 ºC for 30 min. The injection volume was 1μL, and the split ratio was 10:1. Nitrogen was used as the carrier gas at a flow rate of 2.2 mL/min and at a constant pressure of 130.3 pKa. Fatty acid methyl esters were identified by comparison with the retention time of individual standards (Sigma, St. Louis, USA), and expressed as mass percentage (LUTZ-IAL, 2005).

3.2.2.3. Phycocyanin determination

The blue phycocyanin pigment-protein complex was extracted from *Spirulina* biomass based on the protocol described by Doke Jr (2005). Samples were suspended in 5.0 mL of 20 mM sodium phosphate buffer at pH 7.0, and frozen and thawed in 4 cycles, i.e., -18 °C for 21 h and 25 °C for 3 h. The mixture containing the biomass and the extracted solution were centrifuged (K14-4000, KASVI, Brazil) at 6000 rpm for 20 min to recover the supernatant (blue-colour phase). Phycocyanin concentration was measured spectrophotometrically (UV-Vis, Thermo Fischer Scientific, USA) at 620 nm and 652 nm wavelengths. Phycocyanin concentration in percentage (%) was calculated according to Bennett and Bogorad (1973) following Equation 1.

$$Phycocyanin\ (\%) = Abs_{620nm} - \frac{0{,}475 \times Abs_{652nm}}{5{,}34} \quad (1)$$

3.2.3. Physical characterization of powders

3.2.3.1. Particle size and surface charge

Particle size distribution of powder was analyzed by laser diffraction using Zetasizer-Nano ZS equipment (Malvern Instruments Ltd., Malvern, UK). Sample was dispersed (0.5% (w/v)) in deionized water and stirred with a vortex mixer (Kasvi, K45-2820, Brazil). A refractive index of 1.4683 and an absorption index of 0.01 were used (Martínez-Sanz et al., 2020).

Surface charge was evaluated by electrophoretic mobility measurements (zeta potential or ζ-potential) using the same equipment apparatus and conditions set for the particle size analysis. The surface charge was measured in coulombs per square meter (C/m2).

3.2.3.2. Real density

Real density was determined by a helium gas pycnometer (Micromeritics, AccuPyc II 1340, USA). Ten volume readings of each sample were taken. The analysis consists of displacing helium gas into the sample, measuring the transferred volume. The ratio between the powder mass and the gas volume represents the particles' actual density, expressed as g/cm3.

3.2.3.3. Apparent density

Apparent density was determined by pouring approximately 5 g of powder into a graduated cylinder (25 mL), which was repeatedly tapped on a flat surface until there was no further change in the height of the bed of particles between 100 successive taps. Then, the apparent specific density (g/cm3) by observing the volume occupied by the powder in the cylinder was calculated.

3.2.3.4. Porosity

Porosity of the particle bed (θ) was calculated according to Equation 2.

$$\theta = \left(\frac{\rho\, real - \rho\, ap.}{\rho\, real}\right) \qquad (2)$$

where θ is the powder porosity and ρ $real$ and ρ $ap.$ are the real and apparent specific masses of the particles, respectively.

3.2.3.5. Dispersion time

The device scheme for dispersion time is like that reported by Dacanal and Menegalli (2010). The measurement of dispersion time begins when the *Spirulina* powder sample and the testing liquid (80 mL of distilled water at 25 °C) are brought into contact by quickly removing the slider that separates the powder and liquid sections. About 1 g of the powder sample was placed on the slide. Then, with the help of a rubber band, the slide was moved, and the time that all particles completely submerged in the water was measured.

3.2.3.6. Water activity

Water activity (aw) was measured at 25 °C by a dew point hygrometer Aqualab® series 3 TE (Decagon Devices, Inc., Pullman, USA). The analyses were performed in triplicate.

3.2.3.7. Color measurements

Color characterization of the powders was measured according to the method described by Cárdenas-Pérez et al. (2017), with adaptations. Images were acquired with a Nikon D5500 (Nikon Corporation, Japan) charge-coupled device (CCD) color camera. The color parameters were as carried out using ImageJ v.1.6.0 software (National Institutes of Health, Bethesda, MD, USA). A color converting plug-in was used to convert the RGB system colors to the CIELa*b* system with a reference to illuminate D65 and a visual angle of 10 °. L* represents lightness and darkness, a* indicates chromaticity on a green (-a*) to red (+a*) axis and b* indicates the chromaticity on a blue (-b*) to yellow (+b*) axis as defined by the International Commission on Illumination (CIE, Vienna, Austria). The analyses were performed in triplicate.

3.2.4. Scanning electron microscopy

A scanning electron microscope (SEM) (JEOL, JSM 6390LV, Tokyo, Japan) with a tungsten filament electron gun and accelerating voltage of 15 kV to visualize the microstructure of samples was conducted. Dried *Spirulina* powders were mounted onto SEM stubs by sputtering them on a carbon double-sided adhesive tape. Gold coating was done for 2 min with a metallizer (Leica EM model SCD500, Wetzlar, Germany).

3.2.5. Technofunctional properties of powders

3.2.5.1. Water holding capacity (CRA) and oil holding capacity (CRO)

WHC (g H2O/g) and OHC (g oil/g) were measured based on the protocols of BENCINI (1986), with slight modifications (García-Vaquero et al., 2017). About 1 g of sample in 10 mL of distilled water (for the determination of WHC) or sunflower oil (for the determination of OHC) was placed into a Falcon tube. The mixture was vortex agitated and then centrifuged (K14-5000M, Kasvi, China) at 2200 x g for 30 min. The supernatant was discarded, and the settled sample was weighted. The difference between the sample mass before and after the water/oil addition displayed the quantity of water/oil absorbed. WHC or OHC were calculated using the following Equation 3:

$$WHC\ or\ OHC\ (g/g) = \left(\frac{water/oil\ absorbed(g)}{sample\ initial\ weight\ (g)}\right)\times 100 \quad (3)$$

3.2.5.2. Solubility

Solubility of the powders test consisted of adding 1 g of powder to a vessel containing 100 mL of distilled water under agitation. After 1 min of agitation, the solution was filtered in a Buchner funnel and the filter containing the non-dissolved particles was dried at 105 °C for 24 h under vacuum. The solubility of the powder was calculated from the fraction of non-dissolved material (Dacanal and Menegalli, 2009).

3.2.5.3. Foaming capacity

Foaming capacity was measured as described elsewhere (Poole et al., 1984). Sample suspensions were prepared at a concentration of 1.5% and homogenized using a T-25 digital ULTRA-TURRAX (IKA® Werke Co, KG, Staufen, Germany) at 10,000 rpm for 1 min. The whipped samples were transferred to a 50 mL measuring cylinder. Foaming capacity was calculated using the Equation 4:

$$Foaming\ capacity\ (\%)\left(\frac{V_f - V_0}{V_0}\right)\times 100 \quad (4)$$

where, V_f is the volume before whipping (mL), V_0 is the volume just after the whipping (mL).

3.2.5.4. *In vitro* protein digestibility (IVPD)

The multienzyme technique for evaluating protein digestibility (Hsu et al., 1977) with minor modifications (Tinus et al., 2012) to determine the in vitro protein digestibility (IVPD) was used. Protein suspension (6.25 mg/mL of distilled water) was adjusted to pH 8.0 with 0.1 N NaOH or 0.1 M HCl while stirring at 37 °C. An enzyme mixture from Sigma (St. Louis, MO, USA) consisting of 1.6 mg trypsin (porcine pancreatic trypsin type IX-S, 13.000-20.000 BAEE units/mg protein, product no. T0303), 1.3 mg peptidase (porcine gastric mucosa pepsin, 3.200 – 4.500 units/mg protein, product no. P6887), and 3.1 mg of α-chymotrypsin (bovine pancreatic chymotrypsin type II, ≥ 40 units/mg protein, product no. C4129) per mL was kept in an ice-bath and adjusted to pH 8.0. The enzymatic solution was added to the protein solution (1:10 v/v) and stirred at 37 ºC. A rapid decrease in pH value occurred due to the action of proteolytic enzymes on the amino acid carboxyl groups. The pH mixture was measured after 10 min using a portable pH meter (Testo 205, Testo Instrument Co., Germany). The IVPD was estimated as a percentage of digestible protein according to pH variation after 10 min (ΔpH10min) using Equation 5.

$$IVPD\ (\%) = \left((65.66 + 18.10) \times \Delta pH_{10min}\right) \quad (5)$$

3.2.6. Profile of volatile organic compounds

Volatile organic compounds (VOCs) were determined according to the protocols found in Romeo et al. (2007), using automated headspace solid-phase microextraction (HS-SPME) and identified by gas chromatography coupled to the single quadrupole mass spectrometer (GC-MS, Agilent 7890B and Agilent 7000D). For extraction, 400 mg of the sample was hermetically sealed in 20 mL glass SPME vial. The vial was incubated for 30 min at 40 °C, followed by 5 min extraction at 50 °C using a Supelco 65 µm PDMS/DVB (polydimethylsiloxane/divinylbenzene) fiber (Bellefonte, PA, USA). The loaded fiber was desorbed in splitless mode (250 °C, 30 min). Sample injection was carried out using an automatic PAL-RTC 120 sampler. Compounds were separated on a capillary HP-5MS (5% phenyl-methylpolysiloxane) column (30 m × 250 µm × 0.25 µm) using a helium gas flow rate of 1 mL/min. The oven temperature program started at 45°C for 10 min, raised to 150 to 100 °C at 3 °C/min, and then raised to 150 °C at 5 °C/min, and held for 5 min. The mass spectra in the electron impact ionization (EI) mode were generated at 70 eV with a multiplier voltage of 850 V. Mass spectra was recorded in full scan (40-550 m/z) at 6.34 scans/s utilizing a 5975C inert XL mass spectrometer (Agilent). Identification of the aroma

compounds was based on spectral match compared to NIST14 library (National Institute of Standards and Technology, Gaithersburg, MD, USA).

3.2.7. Statistical analysis

The software Statistica® (v.13.5, Statsoft Inc.) was used to perform the experimental data statistical analysis. One-way ANOVA was applied to compare samples and Tukey test for pairwise comparisons was used for significant values reported, adopting a confidence level of 95%.

3.3. Results and discussion

3.3.1. Biochemical composition of Spirulina powders

Eight samples in which four samples are from Brazil, two samples from China, and two other samples from the United States were analysed. The biochemical compositions of these commercial samples of *Spirulina* are shown in Table 8.

Protein content of *Spirulina* biomass ranged from 44.85 to 70.98 g/100g, followed by moisture (range 7.00-15.44 g/100g), ash (range 8.64-12.36 g/100g) and lipid (range 4.39-6.86 g/100g) contents. Phycocyanin-pigment content ranged from 0.94 to 4.11 g/100g, while small amounts of sodium (range 0.79-1.56 g/100g) and potassium (1.09-1.62 g/100g) in the samples were found.

A remarkable variability on the macro- and micro-nutrient profiles was noted, and statistical differences between samples were obtained (Table 8). Moisture content in all *Spirulina* samples is in accordance with literature data (Matos et al., 2016), with the exception of the Brazilian sample (S-BR4) that showed elevated moisture content (15.44 g/100g), and is not in consonance with the maximum limits stated by the Brazilian National Health Surveillance Agency (Agência Nacional de Vigilância Sanitária, Anvisa) where the dried microalgal biomass with less than 10% of moisture for food application is required (Brasil, 2001). High moisture content in microalgal products can render growth of bacteria, mould and fungi (Becker, 2013). In addition, high water content in microalgal biomass affects the cohesion of powders, resulting in powder aggregation, low flowability and caking (Osorio-Fierros et al., 2017).

Two samples had relatively higher contents of ash (S-US2 = 12.36 g/100g and S-BR2 = 11.98 g/100g) than the others (average of 8.99 g/100g). This may result from the varying micronutrients utilized in the culture medium, especially for *Spirulina* mass cultivation where high bicarbonate waters with elevated pH are required (Matos et al., 2021). For this reason, harvested biomass of *Spirulina* should be previously washed with dilute HCl (~0.01 M) to remove adhering salts and carbonates without breaking the cells (Moheimani et al., 2013).

Protein is the most abundant component found in all *Spirulina* biomass studied with a percentage between 60 to 70% of dry matter, similar to literature reports (Severo et al., 2024). The Chinese samples showed the highest protein content (average of 70.54 g/100g), while the Brazilian sample (S-BR2) had low protein content (44.85 g/100g). *Spirulina* strain from S-BR2 sample has particular features. It was originally isolated from Mangueira Lagoon, and is cultivated in the extreme south of the southernmost Brazilian state of Rio Grande do Sul, where the subtropical climate prevail with year-round air temperature ranging from 4 to 44 °C (Morais et al., 2009). So, the harsh environmental conditions may affect protein content.

By nature, *Spirulina* contains low quantities of lipids and values found in all *Spirulina* samples (4.39 to 6.86 g/100g) are comparable to literature data (Matos et al., 2016). On the other hand, *Spirulina* is rich in phycocyanin, a natural blue coloring pigment ample used as functional ingredient in food industry (Matos, 2017), as well as nutraceutical source due to its antioxidant properties with medicinal benefits for humans (Ashaolu et al., 2021). Phycocyanin content found in all *Spirulina* differed greatly between samples with statistical differences being observed. The Brazilian sample (S-BR3) showed the highest content of phycocyanin (4.11 g/100g), followed by the Chinese samples (S-CH1 = 4.05 and S-CH2 = 3.27 g/100g), and the United States (S-US2 = 2.88 g/100g).

Two minerals (Na+ and K+) were also determined in all *Spirulina* samples. S-BR1 sample showed the maximum values for Na (1.56 g/100g) and K (1.62 g/100g), while the S-US1 sample had the lowest ones (Table 8). *Spirulina* biomass is a good source of several minerals like calcium (Ca+2), magnesium (Mg+2), iron (Fe+3), manganese (Mn), nickel (Ni), cupper (Cu), zinc (Zn), molybdenum (Mo), selenium (Se), and iodine (I), which are all essential microelements required by human body to perform vital metabolic activities (Grosshagauer et al., 2020). An important study conducted by Sandgruber et al. (2021) have detected small quantities (mg) of heavy metals like silver (Ag), cadmium (Cd), mercury (Hg), and lead (Pb) in 15 commercially powders of 7 types of microalgae *A. platensis (Spirulina),*

Chlorella pyrenoidosa, Chlorella vulgaris, Dunaliella salina, Haematococcus pluvialis, Tetraselmis chuii, and Aphanizomenon flos-aquae, compromising its intake consumption by humans. Heavy metals have no kwon biological function in mammals, and are toxic even at low concentrations (Bánfalvi, 2011).

Table 8: Chemical composition of *Spirulina* commercial powders.

Sample	Moisture	Ash	Protein content	Lipids	Phycocyanin content	Sodium	Potassium
S-BR1	7.00 ± 0.06^{d}	9.98 ± 0.13^{c}	65.63 ± 0.67^{d}	5.87 ± 0.09^{ab}	0.94 ± 0.08^{a}	1.56 ± 0.70^{d}	1.62 ± 0.22^{d}
S-BR2	7.37 ± 0.01^{ad}	11.98 ± 0.03^{d}	44.85 ± 0.33^{e}	4.39 ± 0.12^{c}	2.55 ± 0.49^{bc}	1.33 ± 0.16^{b}	1.43 ± 0.68^{ac}
S-BR3	7.87 ± 0.03^{abc}	8.64 ± 0.08^{a}	69.25 ± 0.15^{ab}	5.86 ± 0.34^{ab}	4.11 ± 0.38^{c}	1.17 ± 0.94^{ab}	1.37 ± 0.27^{a}
S-BR4	15.44 ± 0.20^{f}	8.80 ± 0.10^{ab}	61.79 ± 0.05^{c}	5.67 ± 0.01^{abc}	1.39 ± 0.13^{a}	1.08 ± 0.35^{a}	1.56 ± 0.68^{cd}
S-CH1	8.20 ± 0.04^{bc}	8.75 ± 0.03^{ab}	70.11 ± 0.11^{ab}	5.13 ± 0.61^{ac}	4.05 ± 0.17^{c}	1.23 ± 0.19^{ab}	1.41 ± 0.28^{a}
S-CH2	8.46 ± 0.52^{c}	8.81 ± 0.17^{ab}	70.98 ± 1.81^{b}	6.86 ± 1.47^{b}	3.27 ± 0.38^{bc}	1.16 ± 0.22^{ab}	1.32 ± 0.11^{a}
S-US1	9.55 ± 0.07^{e}	8.99 ± 0.08^{b}	67.56 ± 0.12^{ad}	5.55 ± 0.03^{abc}	1.73 ± 0.54^{ab}	0.79 ± 0.29^{c}	1.09 ± 0.11^{c}
S-US2	7.84 ± 0.15^{ab}	12.36 ± 0.06^{e}	60.69 ± 0.07^{c}	5.95 ± 0.57^{ab}	2.88 ± 0.15^{b}	1.10 ± 0.95^{a}	1.14 ± 0.77^{b}

Values in the same column with different superscript letters are significantly different ($p < 0.05$).
Data represent the mean g/100g ± standard deviation (SD).

3.3.2. Fatty acid composition

The main fatty acids (FAs) composition as well as the proportion of total saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) ω-3, ω-6, and ω-9 fatty acids in *Spirulina* powders were analysed. Fourteen FAs, ranging from lauric ($C_{12:0}$) to erucic ($C_{22:1\omega-9}$), quantified as percentage of the total fatty acid content were identified (Table 9).

Results showed the predominant FAs in all *Spirulina* samples were palmitic acid ($C_{16:0}$, 42.87-69.99%), linoleic acid ($C_{18:2\omega6}$, 8.16-20.15%), and γ-linolenic acid ($C_{18:3\omega6}$, 2.73-17.42%). FAs present at intermediate levels were oleic acid ($C_{18:1\omega9}$, 1.80-9.03%), palmitoleic acid ($C_{16:1}$, 3.56-4.93%), and stearic acid ($C_{18:0}$, 2.45-0.91%). The FAs lauric ($C_{12:0}$, 0.60-0.71%), tridecanoic ($C_{13:0}$, 0.36-0.62%), myristic ($C_{14:0}$, 0.15-0.41%), heptadecanoic cis-10 ($C_{17:0}$, 0.20-0.52%), heptadecanoic ($C_{17:1}$, 0.15-0.62%), eicosadienoic ($C_{22:2}$, 0.21-0.29%), behenic ($C_{22:0}$, 0.22-0.95%), and eurucic ($C_{22:1\omega9}$, 0.54%) were present at trace levels below to 1.00% of the total FAs. Overall, the main proportion of FAs were saturated (SFAs; 45.47-72-70%), polyunsaturated (PUFAs; 10.98-35.27%), and monounsaturated (MUFAs; 4.22-14.69%) fatty acids.

Discrepant FAs composition and distinct values between *Spirulina* samples were identified. In particular, large variations of palmitic acid were found. For instance, the Chinese samples showed the highest $C_{16:0}$ (69.99-67.58%) and SFAs (72.70-69.83%) values. On the contrary, the Brazilian samples (S-BR1, S-BR2, and S-BR3) and one from the United States (S-US2) presented high levels of $C_{18:2\omega6}$ (19.33-20.15%), $C_{18:3\omega6}$ (14.82-17.42%), and PUFAs (31.80-35.27%). The S-BR2 sample had a well-balanced composition of SFAs (45.47%), PUFAs (31.81%), and MUFAs (14.69%) being unique among all samples studied. As previously mentioned, the S-BR2 sample, with brand commercial name *Spirulina platensis* has unique features because it is cultivated in the southern of Brazil using Zarrouk medium supplemented with bicarbonated waters (0.126 g/L of HCO_3^-) with elevated pH (9.0-10.7) from the Mangueira Laggon, which is an important hydrological resource near to the Atlantic Ocean (de Jesus et al., 2018; Morais et al., 2009).

Table 9: Fatty acid profile in powdered commercial *Spirulina* samples.

Fatty acids		Concentration (%)*							
		S-BR 1	**S-BR 2**	**S-BR 3**	**S-BR 4**	**S-CH 1**	**S-CH 2**	**S-US 1**	**S-US 2**
C12:0	Lauric	0.714	-	-	-	-	-	-	0.608
C13:0	Tridecanoic	0.359	-	-	-	-	-	-	0.623
C14:0	Myristic	0.192	0.324	0.176	0.150	0.322	0.410	0.213	0.245
C16:0	Palmitic	43.593	42.876	49.691	56.692	67.686	69.995	59.591	46.982
C16:1	Palmitoleic	4.023	4.626	4.201	4.486	4.937	4.588	3.797	3.562
C17:0	Heptadecanoic	0.204	0.253	0.338	0.288	0.521	0.482	0.392	-
C17:1	cis-10-Heptadecanoic	0.203	0.508	0.502	0.622	0.362	0.257	0.200	0.159
C18:0	Stearic	1.376	1.080	0.911	1.096	1.319	1.820	2.452	1.569
C18:1n9c	Oleic ω9	3.268	9.036	2.383	4.562	2.135	1.800	3.020	5.321
C18:2n6c	Linoleic ω6	20.157	14.111	19.330	16.027	12.239	8.166	14.882	19.757
C18:3n6	Linolenic acid ω6	14.823	17.420	15.947	7.480	5.816	2.733	9.187	11.765
C20:2	Eicosadienoic	0.212	0.289	-	0.182	-	-	-	0.297
C22:0	Behenic	0.218	0.951	0.223	0.249	-	-	-	0.247
C22:1n9	Erucico ω9	-	0.544	-	-	-	-	-	-
N.I		10.660	7.986	6.300	8.146	4.665	9.748	6.264	8.870
Saturated fat (g/100g)		46.656	45.854	51.339	58.475	69.848	72.707	62.648	50.274
Unsaturated fat (g/100g)	Monounsaturated	7.494	5.134	4.703	9.670	5.299	6.645	3.997	9.042
	Polyunsaturated	35.192	41.400	37.660	23.689	20.190	10.899	27.089	31.819
N.I		10.659	7.986	6.300	8.146	4.665	9.748	6.264	8.870

*Values not inserted were below the identification limit

3.3.3. Physical characteristics of powders

Many of the characteristics that define the quality (e.g., texture, structure, and appearance) and stability (e.g., water activity) of a food product are linked to its physical properties (Berk, 2018). When considering dry ingredients like *Spirulina* biomass, every powder has unique characteristics (composition, particle size, density, surface morphology,

etc.), and the way the powder is handled will have an impact on its food applicability (Sarkar et al., 2022). For these reasons, a careful analysis of physical properties of *Spirulina* powder was carried out.

As shown in Table 8, the chemical composition of *Spirulina* powders varied considerably, as does their physical characteristics like particle size, surface charge, real and apparent densities, porosity, solubility, dispersion time, water activity, and color varied largely among all samples studied with statistical differences ($p < 0.05$) being observed (Table 10).

Analysis of particle size provides quantitative data on the texture of various foods and beverages by acquiring the data of the particle size distribution and their shape (Sarkar et al., 2022). In our study, the particles of *Spirulina* powders varied from 28.16 μm (S-BR4) to a maximum 67.79 μm (S-BR1) in size. Literature reports on particle size between 22.0 to 28.8 μm in *Spirulina* powders used for food and feed supplement (Jin et al., 2020), while particle size of 202 μm in *Spirulina* powder incorporated in food tablets has been described (Adiba et al., 2011). In food manufacturing, the range of particle size of the powder lies between 20 to 80 μm. Dust, non-spherical particles or large, fused grains disturbed the manufacturing process and cause defects in the food component (Servais et al., 2002). Particles of *Spirulina* powders were visualized by scanning electron microscopy (SEM) as a complement of the particle size analysis, showing details of the cell microstructure (Figure 3). SEM images showed *Spirulina* powders are compacted, irregular and/or distorted spherical shapes with rough cell surface. The morphologies of *Spirulina* powders seen in our study are comparable to those of previously reported (Jin et al., 2020).

Surface charge is typically measured by applying an electric field in the liquid followed by observing the migration or distribution of the particles (Feng et al., 2020). In food systems, zeta potential (ζ-potential) is an important and readily measurable indicator of the stability of colloidal dispersions. The magnitude of the ζ-potential has been considered within 0-5, 10-30, 30-40, 40-60, and > 61 mV as rapid coagulation or flocculation, incipient instability, moderate stability, good stability, and excellent stability, respectively (Sherman, 1970). These values are commonly employed in food science and technology, when developing fluid food systems like alcoholic beverages, juices, yogurt and edible films (Cano-Sarmiento et al., 2018). A negative ζ-potential surface charge with values ranging from - 30.00 to - 67.79 mV were obtained in all *Spirulina* powders (Table 10), indicating

moderate to good electrically stabilized while colloids. A similar outlook was observed in *Spirulina* sp. LEB-18 microencapsulated with negative ζ-potential value -33.26 mV, providing good suspension stability when incorporated in chocolate milk (Batista de Oliveira et al., 2021).

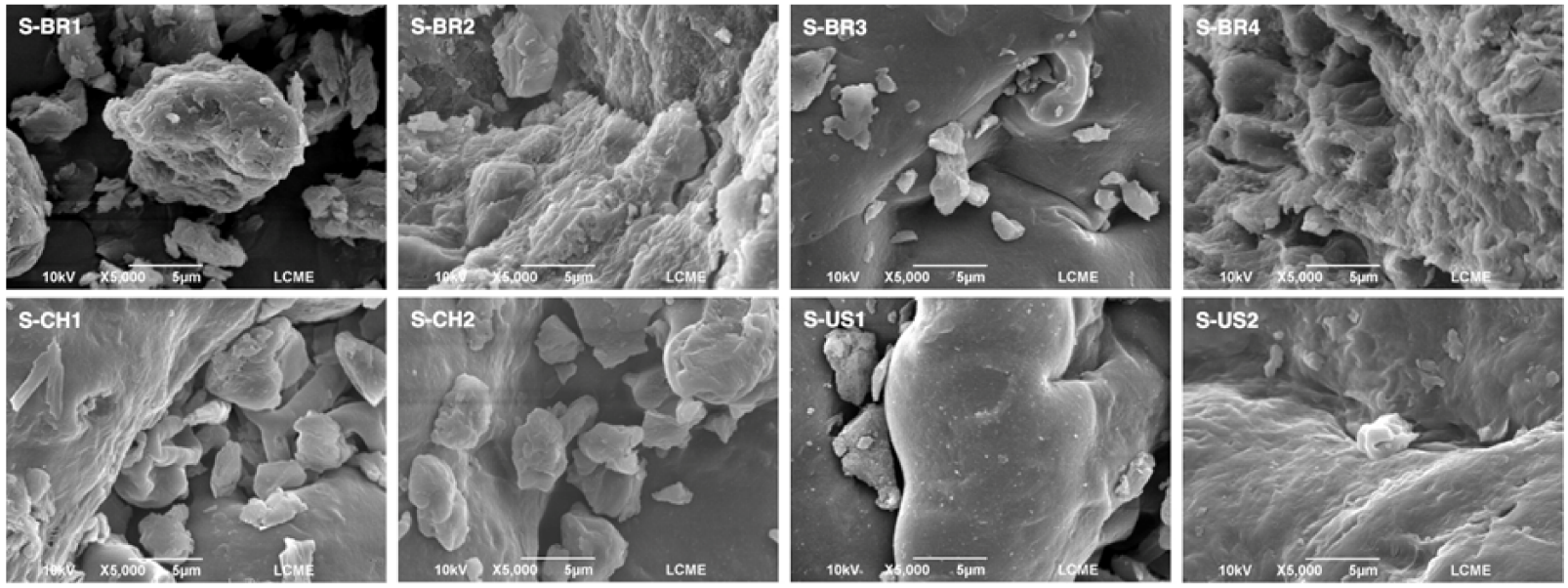
Zeta potential, stability and surface charge of several food matrices including single polymers (gums, pigments, proteins, preparations containing microorganisms), and complex systems (beverages, plant extracts, dairy products, food-related nanoparticles, edible films, food gels, liposomes, and food wastes) have been well-documented and reviewed by Cano-Sarmiento et al. (2018). In the case of microalgae and its extracts, a study conducted by Anarjan et al. (2012) investigated the ideally particle size and surface charge characteristics of astaxanthin powder (>90%) to be used in a food colloidal system. To optimally stability the astaxanthin, a combination of three stabilizing agents (29% sodium caseinate, 65% polysorbate 20, and 6% gum arabic) together with astaxanthin particle size (98.3 nm) and negative ζ-potential (- 28 mV) were required. This optimal colloidal system guaranteed desired physicochemical characteristics for maximum astaxanthin bioavailability.

Table 10: Physical characteristics of *Spirulina* commercial powders.

Sample	Dispersion time (min.)	Particle size (µm)	Surface charge	Real density ($g.cm^{-1}$)	Apparent density ($g.cm^{-1}$)	Porosity ($g.cm^{-1}$)	Water activity	Color		
								L*	a*	b*
S-BR1	6.751 ± 0.024^{a}	67.794 ± 22.630^{a}	-40.433 ± 1.343^{a}	1.404 ± 0.002^{h}	0.516 ± 0.014^{e}	0.632 ± 0.01^{a}	0.32 ± 0.01^{c}	20.08 ± 0.63^{c}	-29.81 ± 0.34^{a}	26.62 ± 0.35^{e}
S-BR2	14.418 ± 2.239^{a}	44.721 ± 31.82^{a}	-46.900 ± 1.400^{c}	1.245 ± 0.001^{c}	0.719 ± 0.003^{b}	0.423 ± 0.02^{c}	0.28 ± 0.01^{b}	34.66 ± 0.04^{d}	-27.02 ± 0.07^{d}	40.61 ± 0.11^{f}
S-BR3	80.32 ± 0.052^{b}	31.185 ± 2.454^{a}	-40.200 ± 0.265^{a}	1.326 ± 0.000^{g}	0.664 ± 0.005^{a}	0.499 ± 0.01^{b}	0.36 ± 0.01^{a}	28.91 ± 0.04^{a}	-37.32 ± 0.04^{c}	36.01 ± 0.06^{a}
S-BR4	17.625 ± 0.506^{a}	28.168 ± 4.501^{a}	-32.633 ± 1.721^{d}	1.311 ± 0.000^{a}	0.708 ± 0.017^{b}	0.460 ± 0.03^{c}	0.66 ± 0.01^{g}	26.99 ± 0.05^{b}	-23.75 ± 0.03^{b}	8.63 ± 0.04^{b}
S-CH1	84.959 ± 8.567^{b}	50.961 ± 15.402^{a}	-41.767 ± 0.757^{ab}	1.277 ± 0.001^{e}	0.641 ± 0.024^{ac}	0.498 ± 0.01^{b}	0.37 ± 0.00^{a}	28.75 ± 0.11^{a}	-30.31 ± 0.06^{a}	36.13 ± 0.10^{a}
S-CH2	187.492 ± 25.373^{c}	45.206 ± 2.378^{a}	-40.867 ± 0.404^{a}	1.274 ± 0.001^{d}	0.657 ± 0.010^{a}	0.485 ± 0.01^{c}	0.46 ± 0.02^{e}	29.35 ± 0.05^{a}	-20.40 ± 0.06^{e}	17.44 ± 0.11^{d}
S-US1	11.043 ± 0.202^{a}	36.969 ± 8.783^{a}	-29.933 ± 0.473^{d}	1.311 ± 0.001^{a}	0.616 ± 0.011^{c}	0.530 ± 0.02^{b}	0.49 ± 0.00^{f}	28.87 ± 0.08^{a}	-30.15 ± 0.12^{a}	36.20 ± 0.06^{a}
S-US2	14.359 ± 2.157^{a}	48.791 ± 32.814^{a}	-42.967 ± 1.266^{ab}	1.286 ± 0.000^{f}	0.706 ± 0.017^{b}	0.451 ± 0.01^{c}	0.43 ± 0.00^{d}	26.93 ± 0.09^{b}	-23.65 ± 0.05^{b}	9.58 ± 0.13^{c}

Values in the same column with different superscript letters are significantly different ($p < 0.05$).
Data represent the mean % ± standard deviation (SD).

Figure 3: Scanning electron micrographs (SEM) of *Spirulina* commercial powders.



The author (2023).

Real density and apparent density are two other physical features used for evaluating the granular properties of food powders (Dhanalakshmi et al., 2011). For real density analysis, the pycnometer equipment was filled with *Spirulina* powder and helium fluid was displaced, penetrating the finest pores. The measurement of real density of all *Spirulina* powders varied between 1.24 to 1.40 g/cm3, and are comparable to real density (1.18 g/cm3) found in *Arthrospira sp.* biomass (Desmorieux et al., 2010). In terms of apparent density that ranged from 0.51 to 0.70 g/cm3 (Table 4) was carried out in a graduated cylinder (25 mL) by observing the volume occupied by the *Spirulina* powder. Apparent density values between 0.30-0.85 g/cm3 during convective drying of *A. platensis* powders have been obtained (Dissa et al., 2010).

The term porosity is a way to describe how many air spaces there are in a powder and is directly related to the real density. In our study, the porosity values for *Spirulina* powders ranged from 0.42 to 0.63 g/cm3. The Brazilian sample (S-BR1) showed the highest porosity value (0.63 g/cm3), which in turn favors the fluidity of gas during porosity analysis. Larger porosity as well as particle size (67.74 μm) observed for S-BR1 sample encourage its applicability in instant foods due to rapid water/fluid infiltration within the particles. Additionally, for many dried and processed food matrices, greater porosity ensures less shrinkage and uniform shape change, porous foods enjoy higher customer acceptance due to its crispiness attributes (Desmorieux et al., 2010). Variation of porosity in different foods using different drying processes has been detected, and choosing the right drying technique and condition to ensure expected porosity of the final product is recommended (Joardder et al., 2017).

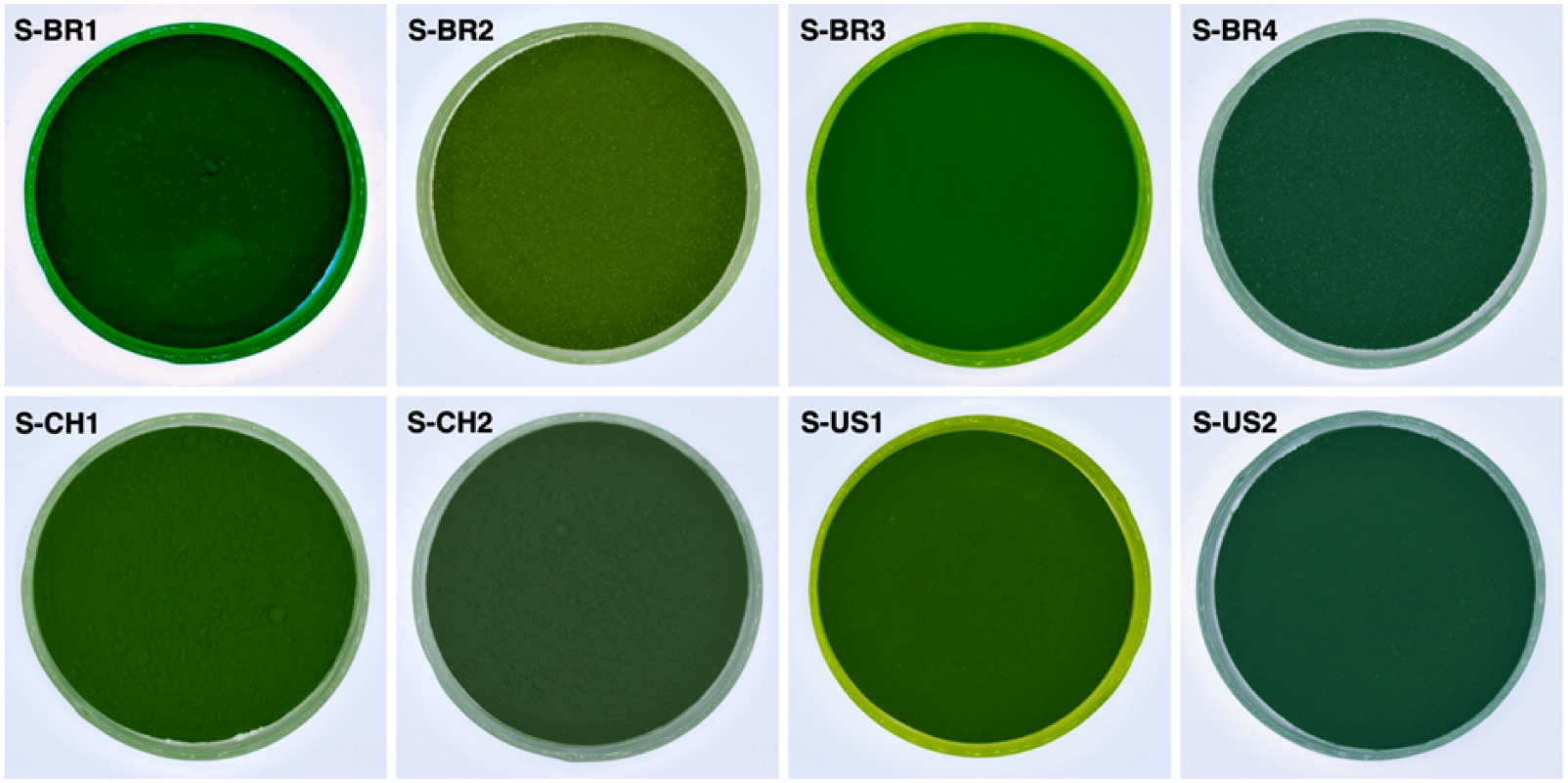
Dispersion time of powder occurs when all particles are completely submerged in the water. The knowledge of this analysis is useful for developing instant foods like instant baby nutrition, instant chocolate beverages, and/or instant sauce/soup, where high dispersibility powders is desirable. The longest full dispersion time in water occurred after 3 h and 19 min for the Chinese sample (S-CH2), while the Brazilian sample (S-BR1) completely dispersed in water after 6 min and 45 sec. We thus conclude that the largest particle size (67.79 μm), the highest real density (1.40 g/cm3), and the maximum porosity (0.632 g/cm3) together with rapid dispersibility observed for S-BR1 sample make it an appropriate powder for instant food utilization.

Water activity (aw), with scale ranging from 0.0 to 1.0, is a measurement of the availability of water for biological reactions, especially microbial spoilage (Berk, 2018). Food powders generally have low a_w = 0.20-0.50 levels. The average a_w found in all *Spirulina* powders was around 0.42. Nevertheless, the Brazilian sample (S-BR4) showed a water activity of 0.66, and it may be susceptible for growth of xerophilic (aw = 0.61-0.80) microorganisms or "dry" molds like *Aspergillus chevalieri, A. candidus, Wallemia sebi* and *Saccharomyces bisporus* (Tapia et al., 2020). High water content in *Spirulina* powders can render physical, chemical, and enzymatic reactions, altering the texture, color, odor, and nutritional value of foods (Bourne, 2017).

Color measurement as a complement of food quality parameters was tested on all *Spirulina* powders (Table 10 and Figure 4). The CIELAB system color method encompassing the range of human color perception and commonly used in food industry was employed. Significant differences between the samples after observing the L*, a*, and b* color values of *Spirulina* powders was obtained. L* is a parameter range from 0 (absolute black) to 100 (absolute white), and the Brazilian sample (S-BR1) was slightly brighter (L* = 20.08) than the others with an average L* value of 28.07. A L* value of 33.50 for *Spirulina platensis* biomass using the same CIELAB color space parameter has been described (Benucci et al., 2023).

The negative values found on a* parameter with values ranging from -20.40 to -37.32 for all *Spirulina* samples (Table 10), suggests powders with green tones. Visualizing b* parameter, all *Spirulina* powders showed positive values from 8.36 to 40.61, exhibiting a tendency towards yellow coloration. The different visual appearance on the colorations of *Spirulina* samples (Figure 4) may be associated with the degradation of compounds during the dehydration process (Demarco, 2020). For instance, the blue light phycocyanin pigment responsible for contributing to the brightness appearance of *Spirulina* biomass and directly affected by heat treatment can be degraded (Antelo et al., 2008). Correlation of water activity and color parameters cannot be ruled out. As an example, the Brazilian sample (S-BR4) presented the highest a_w (0.66) and moisture content (15.44 g/100g, Table 8), exhibiting an opaque blue-green color due to the lowest value of b* = 8.63 together with a small quantity of phycocyanin (1.39 g/100g, Table 8).

Figure 4: Pictures of *Spirulina* commercial powders.



The author (2023).

3.3.4. Technofunctional properties of powders

Emulsification, solubility, water and oil capacity, foam capacity and stability, gelation, and viscosity are among the technofunctional properties vital in food processing (Grossmann et al., 2020). Some of these properties were evaluated on *Spirulina* powders and results are shown in Table 11.

Table 11: Technofunctional properties and IVPD of *Spirulina* commercial powders.

Sample	Water holding capacity (g_{water}/g_{sample})	Oil holding capacity (g_{oil}/g_{sample})	Solubility (%)	Foaming capacity (%)	IVPD (%)
S-BR1	1.750 ± 0.043^{a}	1.099 ± 0.028^{ab}	67.794 ± 22.630^{a}	60.0 ± 3.000^{a}	85.70 ± 1.20^{a}
S-BR2	2.423 ± 0.075^{d}	1.062 ± 0.103^{ab}	44.721 ± 31.82^{a}	55.5 ± 4.950^{ab}	81.20 ± 2.30^{a}
S-BR3	2.832 ± 0.027^{e}	0.813 ± 0.009^{a}	31.185 ± 2.454^{a}	36.0 ± 1.414^{c}	82.00 ± 1.50^{a}
S-BR4	1.784 ± 0.069^{a}	0.972 ± 0.039^{ab}	28.168 ± 4.501^{a}	52.0 ± 3.464^{abd}	82.00 ± 1.50^{a}
S-CH1	2.676 ± 0.051^{b}	0.815 ± 0.017^{a}	50.961 ± 15.402^{a}	42.3 ± 4.041^{cd}	81.00 ± 2.40^{a}
S-CH2	2.611 ± 0.076^{b}	0.801 ± 0.062^{a}	45.206 ± 2.378^{a}	44.0 ± 1.414^{bdc}	85.00 ± 2.20^{a}
S-US1	2.181 ± 0.019^{c}	0.998 ± 0.042^{ab}	36.969 ± 8.783^{a}	37.3 ± 2.517^{c}	82.00 ± 1.00^{a}
S-US2	3.298 ± 0.023^{f}	1.256 ± 0.287^{b}	48.791 ± 32.814^{a}	55.5 ± 3.536^{ab}	79.70 ± 1.40^{a}

Values in the same column with different superscript letters are significantly different ($p < 0.05$).
Data represent the mean % ± standard deviation (SD).

Water holding capacity (WHC) and oil holding capacity (OHC) correspond respectively to the amount of water or oil that a sample/powder can absorb per unit of weight. Both parameters can influence the functional and sensorial properties of foods (Aryee et al., 2018). Incorporating *Spirulina* powders in food products is dependent on their interaction with water and oil (Almeida et al., 2021). In our study, the S-US2 sample had by far the highest WHC and OHC values 3.298 and 1.256 g/g, respectively. The mean value of WHC and OHC of *Spirulina* powders were 2.444 and 0.977 g/g, respectively (Table 11), illustrating that powders tend to absorb more water than oil. This might be attributed to factors like high protein content and conformation, amino acid composition, and surface polarity/hydrophobicity of *Spirulina* (Suresh Kumar et al., 2014).

Isolated proteins from A. platensis biomass showed a WHC of 2.14 g/g and OHC of 2.87 g/g (Taragjini et al., 2022). When comparing WHC and OHC with commercial powders of plant protein, soy protein powder illustrated a WHC = 6.30 g/g, while chickpea, fava bean, mung bean, oat, and wheat tended to have WHC below 2.6 g/g. On the other hand, powders of canola and potato protein exhibited an OHC of 2.8 and 2.1 g/g, respectively (Jakobson et al., 2023).

Solubility is the ability of a powder to dissolve in water, indicating the complete rehydration of powders. Results from the water solubility of *Spirulina* powders showed ample variation from 28.16% to a maximum solubility of 67.79% (Table 11), obtained for S-BR1 sample, which again suggests its application in instant foods. Batista de Oliveira et al. (2021) designed an interesting chocolate milk product by incorporating 8.75% of *Spirulina sp*. LEB-18 microencapsulated. Authors noted the microencapsulated *Spirulina* has higher water solubility (73.16%) than the *Spirulina sp.* LEB-18 powder only (51.88%). The increased solubility was associated with the inclusion of soy lecithin (5%) in the formulation, ensuring the maintenance of solubility due to its emulsifying nature.

Foaming capacity (FC) refers to the ability of certain compounds to form foams on air-water interfaces. Foams are found in foods like whipped cream, mousses, and meringue (Zayas and Zayas, 1997). The FC of the *Spirulina* powders, shown in Table 11, varies from 36.0 to 60.0% with statistical differences being observed ($p < 0.05$). Some reports have attributed high FC (between 150 to 180%) depending upon pH (2.0 to 10.0) when testing isolated proteins of *A. platensis* (*Spirulina*) (Taragjini et al., 2022), probably due to the molecular flexibility of proteins (Zayas and Zayas, 1997). In our study, the sample S-BR1 had the highest FC (60.0%), indicating that this powder indeed has good foaming stability if added in a colloidal system like instant foods.

The *in vitro* digestibility is a parameter to measure the nutritional quality of protein, which is used as an indicator of the bioavailability of amino acids in a protein (Sá et al., 2021). *Spirulina* samples undergone IVPD analysis and results are shown in Table 11. Values for IVPD ranged from 79.7 to 85.0%, with no significant differences ($p < 0.05$) between samples. The IVPD values obtained in our study are consistent with protein digestibility (88.0%) reported in *Spirulina platensis* biomass (Rodríguez De Marco et al., 2014), and are generally higher than other food ingredients and products like red sorghum + cowpea (IVPD = 62.0%), wheat flour (IVPD = 58.0%), durum wheat (IVPD = 40.0%), and pea protein

concentrate (IVPD = 74%) (Orlien et al., 2023). In a review conducted by Demarco et al. (2022), the digestibility, bioaccessibility, and bioactivity of compounds such as proteins, pigments, minerals, vitamins from several types of seaweed and microalgae species with emphasizes to the high digestibility of *A. platensis* (*Spirulina*) has been well documented. Digestibility is an essential factor when considering *Spirulina* biomass as an ingredient in food or protein supplements, and the high IVPD values obtained, emphasize the nutritional quality of *Spirulina* biomass to be used in food formulations (Santos et al., 2016).

Table 12: Volatile organic compounds (VOCs) in *Spirulina* commercial powders. Values are presented in %.

	Compound	Relative content (%) in each sample								Odor
		S-BR 1	S-BR 2	S-BR 3	S-BR 4	S-CH 1	S-CH 2	S-US 1	S-US 2	
Ester	Acetic acid, hexyl ester	NI	NI	NI	1.176	NI	NI	NI	NI	Sweet
Alcohol	1-Octen-3-ol	0.957	NI	1.239	1.005	1.408	NI	NI	1.534	Mushroom, earthy, musty
	1-Hexanol	NI	0.552	0.602	NI	NI	0.583	NI	0.745	Green, fruity, oily
Terpenes	D-Limonene	NI	NI	NI	NI	NI	NI	1.152	NI	Citric
	trans-.beta.-Ionone	NI	NI	3.379	NI	3.829	3.29	NI	NI	Violet, flower, raspberry
Aldehyde	1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-	1.884	2.244	2.443	NI	2.768	2.378	NI	3.026	Tropical, sweet, green, truffled
Ketones	Isophorone	NI	1.527	NI	1.347	NI	1.616	NI	2.055	Camphor, mint, pepper
	Cyclohexanone, 2,2,6-trimethyl-	1.169	1.397	1.516	NI	1.719	1.478	NI	1.88	Tobacco, candy, honey
	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclo-hexen-1-yl)-	2.606	3.103	NI	2.742	NI	NI	2.589	4.185	Woody, dry, violet, fruity
Furans	2(4H)-Benzofuranone, 5,6,7,7a-tetra-hydro-4,4,7a-trimethyl-	2.678	NI	3.473	2.821	NI	3.381	2.661	4.3	Musk, coumarin, licorice
	2(4H)-Benzofuranone, 5,6,7,7a-tetra-hydro-4,4,7a-trimethyl-, (R)-	NI	NI	NI	NI	3.934	NI	NI	NI	Musk, coumarin, licorice
Hydrocarbons	Tetradecane	2.644	2.9	NI	2.781	NI	3.337	2.626	4.244	Alkane
	Dean	NI	1.249	NI	NI	NI	NI	NI	NI	Alkane
	Dodecane	NI	2.195	2.388	NI	NI	NI	NI	NI	Alkane

	Hexadecane	2.835	3.375	3.675	2.981	4.164	3.578	2.816	4.551	Alkane
	8-Heptadecene	3.022	3.599	3.919	3.18	NI	3.815	NI	4.853	Alkane
	Cyclodecene	3	NI	NI	NI	NI	NI	NI	NI	Alkane
	3-Heptadecene, (Z)-	NI	NI	NI	NI	4.44	NI	NI	NI	Alkane
Pyrazines	Pyrazine, tetramethyl-	NI	NI	NI	1.472	NI	NI	NI	NI	Moldy and fermented coffee
	Pyrazine, trimethyl-	NI	NI	NI	1.089	NI	NI	NI	NI	Boiled potato
	Pyrazine, 3-ethyl-2,5-dimethyl-	1.372	NI	1.781	1.444	NI	1.734	NI	NI	Earthy, peanut, roasted, cocoa
Several	Methylamine, N,N-dimethyl-	NI	NI	NI	NI	NI	0.152	NI	NI	Fish
	Cyclopentasiloxane, decamethyl-	NI	NI	NI	NI	NI	NI	NI	2.766	
Total compounds		50	42	44	49	37	41	55	35	

NI: not identified.

3.3.5. Volatile organic compounds of Spirulina powders

Microalgae produce a myriad of volatile organic compounds (VOCs) such as aldehydes, ketones, alcohols, sulfur- and nitrogen-containing compounds that are found in many seafood products (Matos et al., 2022). Previous studies have demonstrated the production of VOCs in *Arthrospira* species can be influenced by different culture conditions (Nader et al., 2022), cell disruption techniques like high pressure homogenization (Magpusao et al., 2022), and storage, freezing or drying processes (Coleman et al., 2023a). VOCs are also significantly different between microalgae and within the same species. Consequently, VOCs of the *Spirulina* powders were determined by gas chromatography coupled with a mass detector after solid-phase microextraction. About 190 distinct VOCs in the *Spirulina* powders were detected. For accuracy, only compounds with a retention area greater than 1,000 and a similarity index (SI) greater than 80% were selected. A total of 21 VOCs were detected, resulted from 8 major chemical classes namely hydrocarbons, ketones, aldehyde, alcohols, terpenes, furans, pyrazines, and ester (Table 12). Only the hydrocarbon hexadecane with an average value of 3.49% was reported in all samples. In particular, the S-US2 sample showed the highest number of VOCs, with notable concentrations of hydrocarbons, ketones, aldehyde, and furans compounds. VOCs found for that sample is in consonance with literature data reported for *A. platensis* biomass (Magpusao et al., 2022).

Seven different types of hydrocarbons (tetradecane, decane, dodecane, hexadecane, 8-heptadecene, cyclodecene, and 3-heptadecene) were also found in *Spirulina* powders, illustrating as the major chemical class occurring in this microalga. Hydrocarbons contribute little to the odor of *Spirulina* due to their high odor thresholds (Bao et al., 2018). The branched hydrocarbons, alkanes and alkenes with C15 to C17 carbon chains are detected in various microalgae and are made up from fatty acid metabolism (Milovanović et al., 2015; Nader et al., 2022).

Ketone is an ample organic class of compounds with great importance in biology and in industry (Wohlgemuth, 2010). Cyclohexanone and isophorone are ketones derived from carotenoids compounds, which provide camphor, mint, pepper, tobacco, candy, and honey aromas (Coleman et al., 2023a). These two ketones were detected in five *Spirulina* powders (Table 12), majorly in S-US2 and S-BR2 samples. Production of ketones in *Spirulina* biomass may be related to the high temperature during the drying process, which can destroy heat-sensitive carotenoids.

Regarding aldehyde chemical class, a single compound was detected in six samples of *Spirulina*, and this compound has tropical, sweet, green, and truffled scent. Two alcohols (1-octen-3-ol and 1-hexanol) were detected in moderate quantities (0.55-1.53%) in *Spirulina* powders. In the literature, Moran et al. (2022) described the presence of 1-hexanol, 2-hexen-1-ol, (Z)-, and cyclohexanol,2,4-dimethyl-, as the main alcohols in *A. platensis* biomass. 1-Hexanol is believed to be a component of the odor of freshly mown grass, while 1-octen-3-ol is known as mushroom alcohol (Aisala et al., 2019). These alcohols may be originated from the oxidation of PUFAs such as linoleic and linolenic acids in *Spirulina* species (Coleman et al., 2023a). VOCs from aldehydes, ketones, and alcohols originated from fatty acid lypoxygenase activity or autoxidation of PUFAs have been discussed previously (Coleman et al., 2022; Matos et al., 2022).

Furan is a heterocyclic organic compound, consisting of a five-membered aromatic ring with four carbon atoms and one oxygen atom. Furan is found in heat-treated commercial foods like roasted coffee, instant coffee, and processed baby foods and is produced through thermal degradation of natural food constituents (Seok et al., 2015). The furan 2(4H)-Benzofuranone, 5,6,7,7a-tetra-hydro-4,4,7a-trimethyl- compound was detected in *Spirulina* powders with considerable quantity (2.82-4.30%), and this compound possesses cocoa, bready, coffee, nutty, and malty-like odor features (Coleman et al., 2023a).

Terpenes are a class of natural products consisting of compounds with the formula $(C5H8)n$ for $n \geq 2$. Terpenes are also components of some traditional medicines, such as aromatherapy due to their citrus and floral aroma (Cox-Georgian et al., 2019; Pawase et al., 2024). D-limonene and trans-β-ionone are two terpenes identified in some *Spirulina* samples (S-BR3, S-CH1, and S-CH2). The inherent presence of terpenes in *Spirulina* is due to the abundant carotenoids that are synthesized via carotenoid biosynthetic pathways with appropriate terpene synthases (Magpusao et al., 2022).

Pyrazine is a heterocyclic aromatic organic compound generally found in baked and roasted goods (Fayek et al., 2023). Pyrazine compounds are formed during the final stage of the Maillard reaction, where water activity, moisture and temperature are believed to be the important factors in controlling the Maillard reaction (Mottram, 2007). As an example, high quantity of trimethylpyrazine was detected in treated samples of Arthrospira sp. by high-pressure homogenization (HPH), proving that high temperature during HPH can cause pyrazine production (Magpusao et al., 2022). In our study, three pyrazines were detected in

moderate quantity in S-BR4 sample. Curiously, this sample had the highest levels of moisture (15.44%) and water activity (0.66) among all samples studied, evidencing the influence of moisture and aw on pyrazines production (Wong et al., 2015). Esters compounds, i.e., acetic acid and hexyl ester, which are associated with sweet aroma, were the remaining chemical class only found in S-BR4 sample with trace level (1.17%).

3.4. Final considerations of the chapter

Food quality parameters in terms of biochemical composition, physical characteristics, technofunctional properties, and volatile organic compounds of commercial powders of *Spirulina* biomass were investigated. Coming from different producers, the biochemical composition of *Spirulina* powders showed large variations, which may be related to the cultivation practices such as the amount of nutrients, culture systems employed, growth factors, and drying method used. Except for S-BR2, all *Spirulina* powders had elevated protein content (60-70 g/100g), moderate quantities of palmitic (42-69%) and linoleic (8-19%) acids, and small amount of phycocyanin (1-4 g/100g). Ample differences on physical characteristics and technofunctional properties of *Spirulina* powders were noted and can be related to the microalgal processing like harvesting techniques and drying process. In particular, the powder of the S-BR4 sample had elevated moisture (15.44 g/100g) and water activity (0.66), rendering the production of pirazyne volatile compounds due to the Maillard reaction. The S-BR1 sample showed remarkable physical properties in terms of color (L* = 20.08), particle size (67.79 μm), real density (1.40 g/cm3), porosity (0.63 g /cm3), and dispersion time (6.75 min), which is desired for developing instant foods with high solubility. The S-US2 sample had great water/oil holding (3.28 and 1.25 g/g, respectively), and foaming (55.5%) capacities, good protein content (60 g/100g), and notable ketones and furans volatile compounds, denoting premium quality biomass for developing *Spirulina* supplements bars carrying on woody, cocoa, malty-like aroma. In conclusion we have tested the potential of *Spirulina* biomass for food applications, bringing closer reliable food analyses and attributes necessary for developing *Spirulina*-based foods.

CHAPTER IV

4 ULTRASOUND APPLICATION ON SPIRULINA POWDER TO DEVELOP A HIGH TECHNOLOGICAL QUALITY ALGAE-BASED INGREDIENT

Based on the promising results presented in Chapter 3, *Spirulina* biomass exhibits good nutritional, functional, and technological properties, making it suitable for using as an ingredient in food formulations. Therefore, this chapter evaluates the impact of a two-step processing method (ultrasound and drying) on *Spirulina* biomass. This study investigated how these methods influence the sample's biochemical, physical, and techno-functional properties. Evaluations of the drying process (kinetics), phycocyanin content, color, *in vitro* protein digestibility, and volatile organic compound profile were performed.

The chapter is based on the ongoing paper "***Effects of ultrasound pretreatment on chemical and functional properties and drying of Spirulina***" which will be submitted as an original article.

Abstract

Spirulina, commonly used as a dehydrated powder in food, faces challenges in ingredient stability and sensory properties. Advances in technology, particularly ultrasound, are explored to enhance microalgae's nutritional quality and sensory appeal in food products, addressing issues associated with the conventional methods that are linked with high energy consumption and compound degradation. The study investigates the impact of a two-step process on commercial *Spirulina* involving ultrasound pre-treatment for cell disruption and subsequent vacuum drying process to evaluate the sensory characteristics of *Spirulina* biomass, aiming to comprehensively understand the combined effects on volatile organic compounds, color, phycocyanin content, and *in vitro* digestibility. Ultrasound application significantly reduced the drying duration. No significant differences between samples subjected to ultrasound pretreatment and those that were not treated were observed. Ultrasound pre-treatment did not show a correlation with increased *in vitro* digestibility of *Spirulina* samples. While not statistically significant, a slight rise in phycocyanin content was noted from the control sample to the ultrasound-treated samples. Ultrasound demonstrated the ability to eliminate specific compounds from the samples, including dean, dodecane,

2,6,10-trimethyl-, and hexadecane, 2,6,11,15-tetramethyl-. Despite ultrasound pretreatment, D-Limonene, acetic acid, and pentadecane remained the three major compounds found in all samples.

Keywords: *Spirulina*; Ultrasound; Pre-treatment; Vacuum-drying; Volatile compounds.

4.1. Introduction

Spirulina is commonly used in the form of dehydrated powder for food purposes, either consumed as a dietary supplement or used as an ingredient in food products. However, the use of dehydrated *Spirulina* as an ingredient has some limitations, including the stability of certain components during the downstream production and storage processes as well as its sensory properties (Lafarga et al., 2020). Algae and cyanobacteria naturally produce volatile substances and flavors, and microalgae biomasses can be immediately rejected by humans due to strong palatability (Colonia et al., 2023). In addition to their numerous beneficial properties, microalgae also release a variety of volatile organic compounds that can result in unpleasant odors reminiscent of mustiness, fishiness, and earthiness. Blooms and accumulations of microalgae can emerge in diverse freshwater and brackish water ecosystems, leading to musty odors and the generation of harmful toxins (Smith; Boyer; Zimba, 2008).

Food processing techniques can be employed to enhance the nutritional quality of alternative food sources, such as microalgae biomass, by improving digestibility for humans. Efforts in technological advancements have been made to enhance the sensory characteristics of microalgae biomass, making it an appealing by-product to be incorporated into food products, as demonstrated in a study conducted by Nunes, Ferreira & Raymundo (2023). Conventional thermal methods are widely used for this purpose but have disadvantages, such as high time demand and energy consumption, large amounts of water usage, and degradation of desirable compounds like phycocyanin. Emerging technologies have been investigated as strategies to improve the quality of protein products subjected to subsequent stabilization processes. These technologies include ultrasound, microwave, pulsed electric field, high pressure, ohmic heating, cold plasma, and enzymatic processes. In some cases, these technologies offer advantages when used as pretreatment of algae biomass before dehydration processes (Wang et al., 2019; Pojić, Misan, & Tiwari, 2018; Al-Ruwaih et al., 2019).

Ultrasound technology stands out as a modern non-thermal processing method capable of reducing processing time, conserving energy, and elevating food quality when compared to conventional thermal techniques (Chen, Zhang, Yang, 2020). The use of pretreatments such as ultrasound with the aim of improving the extraction process also involves the exploration of environmentally friendly alternative techniques, with the aim of minimizing the use of solvents and maximizing the extraction of valuable biocompounds. This approach not only reduces environmental impact, but also establishes a pathway for greater appreciation of extracted components as value-added ingredients (Nunes, Ferreira & Raymundo, 2023). Throughout the ultrasound treatment, a sequence of events takes place within the liquid medium, encompassing the formation of bubbles, and eventual implosive collapse due to a phenomenon cavitation, characterized by the periodic stretching and compression of molecules within a liquid medium as ultrasound waves travel through it (Awad, et al., 2012; Singla, Sit, 2021). The collapse of these bubbles generates high local pressures and temperatures, along with micro-jets of liquid that disrupt cell membranes. This process increases the permeability of tissues, facilitating the penetration of solvents into the internal plant tissue and the release of its contents (Suslick et al., 2011; Zhao, Yan, Cheng, 2021; Shirsath, Sonawane, Gogate, 2012). Furthermore, ultrasound triggers the production of highly reactive radicals from water molecules ($H2O \rightarrow OH\bullet + H\bullet$), which can induce oxidation reactions with other molecules (Tiwari, 2015). Consequently, downstream operations in microalgal biomass production, encompassing activities such as cell wall disruption and drying, may play a crucial role in influencing their flavor. Cell disruption, induced by drying or other pretreatments such as ultrasound, facilitates the release of intracellular compounds, thereby altering the functionality and bioactivity of the algae (Nunes, Ferreira & Raymundo, 2023).

Additionally, the dehydration of microalgae biomass for human consumption involves either heating methods such as spray drying or thin-layer oven drying, or the application of a vacuum, as occurring in freeze-drying. The utilization of heat in the drying process has the potential to degrade heat-sensitive compounds, depending on the temperature and duration of exposure (Coleman et al., 2023). In a study by Demarco et al. (2021), the impact of temperature and drying time using different methods on the degradation of phycocyanin in *Spirulina* biomass was observed. Additionally, the drying process may influence the flavor characteristics of microalgae, with the potential loss of certain volatiles during vacuum-based drying methods (Coleman et al., 2023).

This study focuses on exploring the effects of a two-step process involving ultrasound as a pre-treatment for cell disruption, followed by vacuum drying, on the sensory characteristics of *Spirulina* biomass. Ultrasound is particularly associated with cell disruption, potentially making certain compounds more accessible. Simultaneously, vacuum drying is known to influence deodorization. The investigation includes an examination of volatile organic compounds, color, phycocyanin content, and *in vitro* digestibility in commercial *Spirulina,* aiming to provide a comprehensive understanding of the combined effects of ultrasound and vacuum drying on the overall sensory quality of the biomass.

4.2. Material and methods

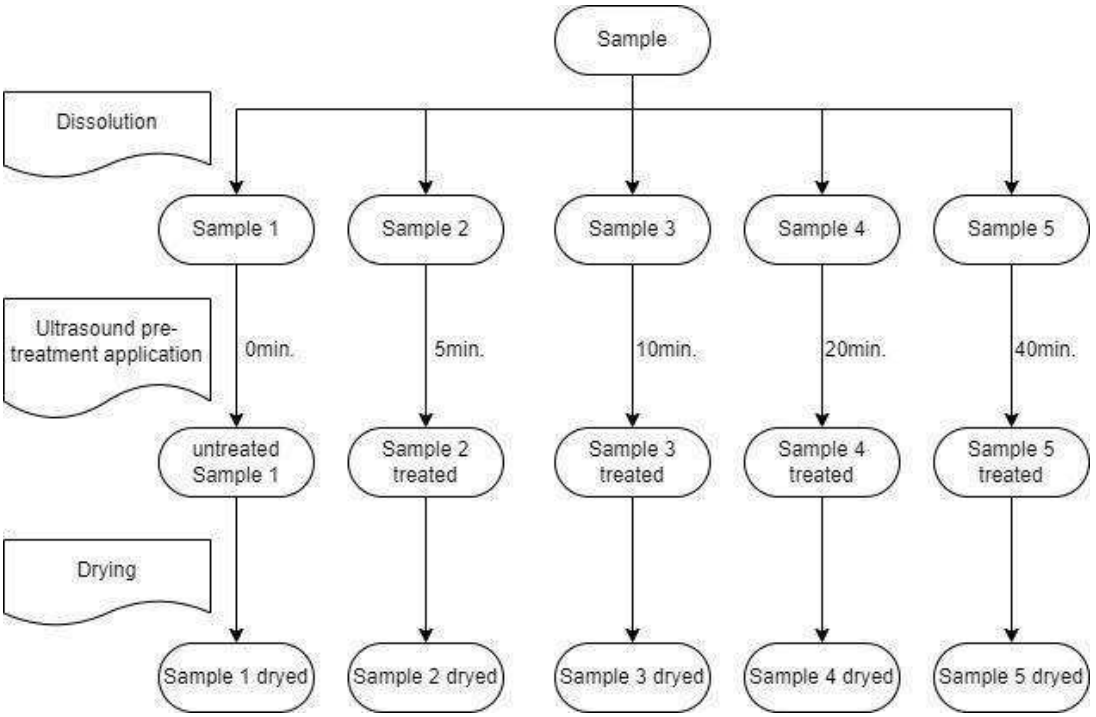
4.2.1. Spirulina powder

The commercial *Spirulina* sample was purchased from a local market (Brazil). The sample was kept in a hermetically sealed metallic packaging and refrigerated during the analysis. The sample was reconstituted with distilled water at a ratio of 1:5 (*Spirulina*/water) to maintain appropriate viscosity for ultrasound application and sample spreading during the drying process. All samples, including the control, underwent rehydration and drying to ensure standardization among them.

4.2.2. Ultrasound application

The samples were pre-treated with an ultrasonic probe. Tests on diluting *Spirulina* powder in distilled water were carried out reaching a 1:5 ratio so that it reaches a consistency suitable for the application of ultrasound. For this purpose, 80 mL of diluted *Spirulina* samples were processed using ultrasound equipment applying a power of 500 W, with the processing time varying 5, 10, 20 and 40 minutes. The temperature was maintained throughout the pretreatment at 25°C to avoid sample degradation by heat. Afterwards, the samples were packed in containers protected from the light (Figure 5).

Figure 5: Flowchart of sample preparation and ultrasound application.



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4.2.3. Drying kinetics

The control and pre-treated *Spirulina* samples were dried in a vacuum oven called conductive multi-flash drying (KMFD), described by Link (2016).

The experimental apparatus consists of a 100 L drying chamber (440-DE, Ethik Technology, Brazil) connected to a vacuum pump with a pumping capacity of 350 $m^3\ h^{-1}$ (LC305-DVP Vacuum Technology, Italy). Inside the drying chamber, the plates were kept warm (40 °C) with electrical resistances, with temperatures controlled by a PID controller (proportional-integral-derivative). The system pressure was monitored during the drying process using a digital manometer (IT-MN-DG, Velki, Itu, Brazil).

The *Spirulina* samples with (0, 5, 10, 20, and 40 minutes of ultrasound) and without pretreatment were spread on Teflon® with a thickness of 3 mm and inserted inside the drying chamber on the heated plates. Plate temperature was maintained at 40 °C, as well as chamber temperature. The internal chamber pressure was maintained at 80 mbar. Drying kinetics were obtained through the difference in Teflon ® + sample mass and data collected at different drying times, as was carried out in a study by Demarco et al. (2022) using the vacuum cast-tape drying method.

4.2.3.1. Moisture

Moisture analysis was performed according to section 3.2.2.1 in Chapter III.

4.2.3.2. Water Activity (a_w)

Water activity was performed according to section 3.2.3.6 in Chapter III.

4.2.3.3. Mathematical modeling of drying kinetics

A linear model was able to fit the initial drying period, indicating the presence of a constant drying rate period, as described by De Moraes, Scheibe, Carciofi, and Laurindo (2015). The constant drying rate period fitting was performed only for the coefficient of determination (R^2) values higher than 0.91. Besides, the end of this period can be estimated when, suddenly, a_W starts to decrease.

4.2.4. Color

Color measurement was performed using a conventional colorimeter DELTA VISTA®, model 450 G, Software i7. The values of parameters L*, a* and b* given by the equipment automatically were obtained.

4.2.5. In vitro protein digestibility (IVPD)

The *in vitro* digestibility of *Spirulina* proteins was determined according to section 3.2.5.4 in Chapter III.

4.2.6. Profile of volatile organic compounds

The volatile component profiles was determined according to section 3.2.6 in Chapter III.

4.2.7. Phycocyanin determination

Phycocyanin content was determined according to section 3.2.2.3 in Chapter III.

4.2.8. Statistical analysis

The software Statistica® (v.13.5, Statsoft Inc.) was used to perform the experimental data statistical analysis. One-way ANOVA was applied to compare samples and Tukey test

for pairwise comparisons was used for significant values reported, adopting a confidence level of 95%.

4.3. Results and discussions

The drying parameters are presented in Table 13. The corresponding Table details the average drying rates after 0, 5, 10, 20, and 40 minutes of ultrasound pre-treatment during the constant drying rate period, along with the coefficients of determination (R^2) from the linear model.

Table 13: Moisture and water activity of *Spirulina* biomass with and without ultrasound pretreatment and drying rates (d*X* /dt) and determination coefficients (R^2) for linear adjustments to the constant rate period.

Analysis	Control	5 min.	10 min.	20 min.	40 min.
***X* db (g.g^{-1})**	0.0557 ± 0.003^{b}	0.0707 ± 0.004^{a}	0.0723 ± 0.003^{a}	0.0643 ± 0.002^{ab}	0.0680 ± 0.004^{a}
a_w	0.2342 ± 0.019^{b}	0.2860 ± 0.000^{ab}	0.3148 ± 0.027^{a}	0.2968 ± 0.029^{a}	0.2887 ± 0.014^{ab}
d*X*/dt (g.g^{-1}.min^{-1})	0.0059	0.0063	0.0056	0.0057	0.0059
R^2	0.9939	0.9914	0.9965	0.9964	0.9939
Constant rate (min.)	0-90	0-90	0-120	0-120	0-120

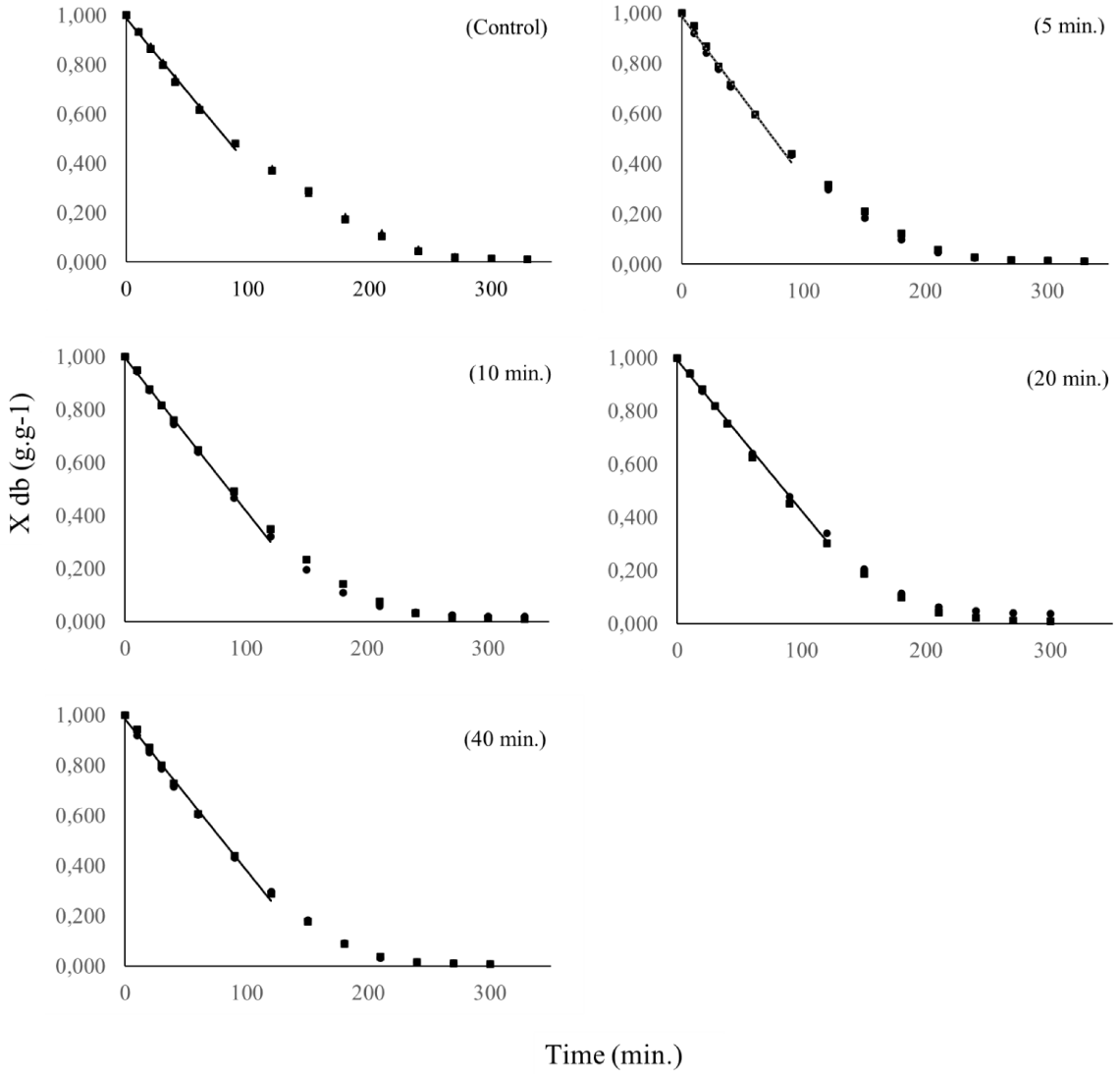
Values in the same line with different superscript letters are significantly different ($p < 0.05$). Data represent the mean ± standard deviation (SD).

Ultrasound pretreatment did not influence the drying rate but extended the period of drying rate in samples treated with ultrasound at 10-, 20- and 40-min. Ultrasound treatment alters the structure of food materials through the "holes effect" and "degassing effect," consequently enhancing the efficiency of the drying process (Duan, Zhang, Li, & Mujumdar, 2008; Mothibe, Zhang, Nsoratindana, & Wang, 2011). Subjecting the material to ultrasound treatment involves the repetitive application of compression and stretching, causing the material to continually contract and expand, ultimately forming a sponge-like structure (Ojha,

Mason, O'Donnell, Kerry, & Tiwari, 2017). When the force resulting from this structural effect exceeds the surface adhesion of the moisture within the material, the moisture easily traverses through the ducts during the drying process (Fan, Gallão, & Rodrigues, 2008).

In Figure 6 the drying kinetics of all samples are illustrated, including the untreated control and those subjected to ultrasound treatment at various intervals. All curves are normalized in dimensionless form, originating from a shared starting point. The drying process unfolds in two distinct phases: firstly, the constant rate phase (indicated by the straight line) where moisture is consistently extracted from the water-saturated surface, and secondly, the decreasing rate phase where the drying speed diminishes as the surface water decreases. Building upon insights from previous curves, the values of drying rates are visually apparent. The application of ultrasound resulted in a noteworthy decrease in the drying duration as visualized in 20- and 40-min. samples. The same was observed by Kadam, Tiwari & O'Donnell (2015) in drying kinetics of brown seaweed *Ascophyllum nodosum* submitted to ultrasound pre-treatment. Fernandes et al. (2008) employed ultrasound treatment prior to Melon dehydration, leading to a reduction in the overall drying time from 814 minutes to 734 minutes. This decrease in drying time has the potential to lower energy consumption, as highlighted in previous studies by Azoubel et al. (2010) and Nowacka et al. (2012).

Figure 6: Drying kinetics of 0, 5, 10, 20 and 40 minutes of ultrasound pre-treated samples.



The author (2024).

Huang et al. (2020) reported in their review that ultrasound pre-treatment can lead to water loss or gain in food products, with an escalation in ultrasonic parameters such as sonication time, amplitude, and ultrasound power intensifying the effect. The application of ultrasound technology before the drying process consistently resulted in an enhancement of drying kinetics, although varied outcomes were also documented.

In Table 14 are presented the color results for control and treated samples. There were no significant differences between the samples that were submitted to ultrasound pretreatment and the one that was not. Changes in color parameters are expected, since the ultrasound induces degassing effects, physical harm, and membrane destruction in cells,

thereby facilitating the easier elution of pigment substances from biological materials (Kadam, Tiwari & O'Donnell, 2015).

Table 14: Color parameters of *Spirulina* biomass with and without ultrasound pretreatment.

Sample	Color		
	L*	**a***	**b***
Control	19.403 ± 0.344^{b}	-5.530 ± 0.520^{a}	3.867 ± 0.319^{a}
5 min	17.437 ± 0.307^{a}	-4.543 ± 0.673^{a}	3.197 ± 0.482^{a}
10 min	19.763 ± 0.119^{b}	-5.900 ± 0.310^{a}	4.450 ± 0.593^{a}
20 min	18.680 ± 0.892^{ab}	-5.010 ± 0.926^{a}	3.943 ± 0.666^{a}
40 min	17.167 ± 0.774^{a}	-5.383 ± 0.378^{a}	3.970 ± 0.225^{a}

Values in the same column with different superscript letters are significantly different ($p < 0.05$).
Data represent the mean ± standard deviation (SD).

The influence of ultrasound pre-treatment on the *in vitro* protein digestibility and phycocyanin compound was investigated, and the results are presented in Table 15. The structural integrity of algal cells can limit the accessibility and availability of various intracellular compounds (Tavanandi, Devi, & Raghavarao, 2019). Microalgae cells, characterized by their small size and a relatively thick cell wall, typically store products in globules or bind them to cell membranes. This arrangement poses challenges in extracting intracellular metabolites (Wang, Zhang, & Fang, 2019). Ultrasound as a pretreatment method can cause a cell disruption, which may enhance their digestibility and bioavailability (Demarco et al., 2022), playing a significant role in ensuring the digestibility of proteins during gastrointestinal (GI) digestion (Machado, Carvalho & Pereira, 2022).

Table 15: *In vitro* protein digestibility (IVPD), phycocyanin content of *Spirulina* biomass with and without ultrasound pretreatment.

Sample	IVPD (%)	Phycocyanin content (mg. g^{-1})

Control	91.903 ± 0.627^{ac}	308.897 ± 18.636^{a}
5 min	93.173 ± 0.627^{a}	333.839 ± 36.204^{a}
10 min	89.190 ± 0.724^{bc}	345.117 ± 3.272^{a}
20 min	92.150 ± 0.418^{a}	324.286 ± 6.065^{a}
40 min	87.200 ± 1.907^{b}	330.685 ± 18.620^{a}

Values in the same column with different superscript letters are significantly different ($p < 0.05$).
Data represent the mean ± standard deviation (SD).

Arthrospira platensis exemplifies microalgae with a delicate cell wall primarily composed of peptidoglycan (Niccolai et al., 2019; Safi et al., 2013). Unlike some other microalgae, *Arthrospira platensis* lacks a robust cell wall, making it easily breakable (Lupatini et al., 2017) and showcasing a considerable potential for enhanced digestibility and bioaccessibility of its constituents (Machado, Carvalho & Pereira, 2022). Vacuum drying can significantly contribute to breaking down *Spirulina* fragile walls. Similarly, the enzymes employed in *in vitro* tests can also contribute to achieving favorable digestibility values naturally. Consequently, the utilization of ultrasound is overlooked in this context.

It was not possible to observe a correlation between the ultrasound pre-treatment and an increase in the *in vitro* digestibility of *Spirulina* samples. Despite not showing a statistically significant difference, a slight increase in the phycocyanin content was noticeable from the control sample to the ultrasound-treated samples. This can be explained by the rupture of the cell wall, exposing the phycocyanin to extraction more effectively.

The volatile organic compounds (VOCs) found in commercial *Spirulina* are presented in Table 16. The volatile compounds (VOCs) of the samples were determined by gas chromatography coupled with a mass detector (GC/MS) after solid phase microextraction (SPME).

Table 16: Relative contents of the main volatile compounds (SI ≥ 80) in *Spirulina* biomass with and without ultrasound pretreatment.

	Compound	Relative content (%) in each sample					
		Control	**5min**	**10min**	**20min**	**40min**	**Odor**
Ester	Acetic acid	18,756	10,752	14,155	10,499	14,954	Acid, sour
	Oxalic acid, 6-etiloct-3-yl propyl ester	NI	NI	NI	2,144	NI	Fruity
	Oxalic acid, butyl 6-ethyloct-3-yl ester	NI	3,614	NI	NI	NI	Fruity
	Oxalic acid, 2-ethylhexyl isohexyl ester	NI	5,608	NI	5,573	NI	Fruity
Terpenes	D-Limonene	33,817	26,174	35,322	30,353	33,170	Citric
	trans-. beta. -Ionone	6,836	NI	NI	6,201	6,954	Violet, flower, raspberry
Aldehyde	1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-	NI	1,887	2,482	1,953	NI	Tropical, sweet, green, truffled
	Hexanal	NI	2,524	4,192	2,694	3,781	Fresh, green, fruity
Ketones	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclo-hexen-1-yl)-	NI	8,238	10,005	NI	NI	Woody, dry, violet, fruity
Furans	2(4H)-Benzofuranone, 5,6,7,7a-tetra-hydro-4,4,7a-trimethyl-	NI	1,884	2,367	NI	NI	Musk, coumarin, licorice
	Furan, 2-pentyl-	NI	NI	NI	2,672	2,875	Bean, green, butter
Hydrocarbons	Tetradecane	NI	NI	4,732	NI	6,500	Alkane
	Dean	2,136	NI	NI	NI	NI	Alkane
	Decane, 3,4-dimethyl-	NI	NI	NI	2,297	NI	Alkane
	Decane, 3,7-dimethyl-	6,567	NI	NI	NI	5,174	Alkane
	Dodecane, 2,6,10-trimethyl-	5,405	NI	NI	NI	NI	Alkane
	Pentadecane	10,138	11,077	14,901	11,199	12,735	Alkane
	Hexadecane	5,142	5,913	7,775	5,778	NI	Alkane
	Hexadecane, 2,6,11,15-tetramethyl-	4,098	NI	NI.	NI	NI	Alkane
	2,6-dimethyldecane	NI	2,242	NI	NI	NI	Alkane

	Heptadecane, 2,6,10,15-tetramethyl-	NI	NI	NI	6,115	NI	Alkane
	Heptane, 2,2,4,6,6-pentamethyl-	5,017	3,137	4,071	3,720	5,012	Alkane
	Heptane, 2,4-dimethyl-	2,088	2,144	NI	2,174	2,154	Alkane
	Hexane, 2,3,4-trimethyl-	NI	2,104	NI	NI	NI	Alkane
	Undecane	NI	NI	NI	NI	6,693	Alkane
	Undecane, 3,8-dimethyl-	NI	6,084	NI	NI	NI	Alkane
	Octane, 5-ethyl-2-methyl-	NI	6,616	NI	6,628	NI	Alkane

NI: not identified.

It was possible to detect 161 distinct volatile compounds in the commercial *Spirulina* samples. For VOC identification, compounds with a retention area greater than 1,000 and a similarity index (SI) greater than 80% were selected, resulting in 4 esters, 2 terpenes, 2 aldehyde, 1 ketone, 2 furans and 16 hydrocarbons. Of these, acetic acid, D-Limonene, pentadecane, hexadecane, heptane, 2,2,4,6,6-pentamethyl- and heptane, 2,4-dimethyl- were noted in all samples even after the ultrasound pretreatment. It was possible to observe a little decrease in acetic acid content with the ultrasound pretreatment compared to control.

Sixteen distinct hydrocarbon types were identified, and despite *Spirulina* having a profile dominated by hydrocarbons (Moran et al., 2022), their contribution to the odor of *Spirulina* is minimal due to their high odor thresholds (Bao et al., 2018). In contrast, these hydrocarbons were recognized as significant odor compounds in seaweed species (Yamamoto et al., 2014). Even though, it was possible to observe that ultrasound could eliminate dean, dodecane, 2,6,10-trimethyl-, and hexadecane, 2,6,11,15-tetramethyl- of the samples, bringing a positive result since hydrocarbons have been described as responsible for off-flavors in *Spirulina* (Aguero et al., 2003).

The three major compounds found in all samples, even after ultrasound pretreatment, were D-Limonene, acetic acid and pentadecane, respectively. The large presence of D-Limonene is not common in *Spirulina*; however, Streptomyces and Cyanobacteria are examples of rare microbial species that produce monoterpenes (Warmling et al., 2022). Warmling et al., (2022) also found D-Limonene from *Spirulina sp.* (2.82%). Acetic acid identified in large quantities, characterized by a distinctive sour and pungent aroma, exhibiting low detection thresholds and occurring in high concentrations across various microalgae species, was also found in dried *Arthrospira platensis* (Taiti et al., 2024) and in *Arthrospira fusiformis* (Hamad et al., 2023) as the main constituent in both studies. Despite not presenting the highest values in terms of Pentadecane, hydrocarbons contained the greatest number of volatile compounds in the samples. Hydrocarbons exhibit moderate variation in both the number of individual compounds and the percentage of relative abundance among algal strains (Moran et al., 2022). A study carried out by Moran et al. (2022) revealed that the most notable variability of volatile compounds was observed in branched hydrocarbons unique to two freshwater species of *Spirulina*.

As part of the objective of this work, the effects of the drying process could be observed. Subjecting the material to heat treatment has been shown to elevate the levels of aldehydes and ketones (Li et al., 2012). Aldehydes typically result from the auto-oxidation of lipids, while ketones predominantly originate from the thermal oxidation or degradation of

unsaturated fatty acids (Liu et al., 2020). This phenomenon was not observed in the present study, although a slight increase in hexanal was observed. It may be related to the fact that *Spirulina* does not have significant amounts of lipids. In general, vacuum drying decreases alkane aroma (from hydrocarbons) and increases fresh, green, fruity aroma from hexanal, which impacts positively in *Spirulina* final product odors.

4.4. Final considerations of the chapter

In conclusion, our research delved into technological advancements aimed at improving the sensory characteristics of microalgae biomass for integration into food products, emphasizing the use of environmentally friendly pretreatment methods. Ultrasound, a promising technique, was employed to enhance the extraction process by reducing drying duration through cell disruption. While no significant differences were observed between samples subjected to ultrasound pretreatment and those that were not treated, subtle increases in phycocyanin content hinted at improved extraction efficiency. This suggests that ultrasound-induced cell wall rupture may contribute to the enhanced accessibility of valuable biocompounds, notably phycocyanin.

Furthermore, our findings highlight the potential of ultrasound as a pretreatment method to influence the digestibility and bioavailability of microalgae proteins during gastrointestinal (GI) digestion. The 161 distinct volatile compounds detected in powdered commercial *Spirulina* samples revealed that ultrasound pretreatment led to a decrease in acetic acid content, indicating its deodorizing effect. Notably, specific volatile compounds, including dean, dodecane, 2,6,10-trimethyl-, and hexadecane, 2,6,11,15-tetramethyl-, were eliminated by ultrasound. This outcome is particularly remarkable as hydrocarbons, known for imparting off-flavors to *Spirulina*, were successfully mitigated through ultrasound treatment.

Despite these advancements, three major compounds, D-Limonene, acetic acid, and pentadecane, persisted in all samples, even after ultrasound pretreatment. These compounds may play a crucial role in the overall composition and flavor profile of the *Spirulina* samples. In conclusion, our study underscores the multifaceted impact of ultrasound pretreatment on microalgae biomass, providing insights into its potential applications for improved extraction and product quality in the food industry. The study highlighted that vacuum drying, in

general, leads to a decrease in alkane aroma derived from hydrocarbons, while concurrently enhancing fresh, green, and fruity aromas, particularly from hexanal. This dual effect positively influences the overall odor profile of the *Spirulina* final product. These results underscore the potential of vacuum drying as a method to enhance the sensory attributes of *Spirulina* biomass, contributing to its desirability in the market.

CHAPTER V

5 FINAL CONCLUSIONS

Algae, particularly *Spirulina*, offer numerous advantages as a protein source for food formulations, including rapid growth, low water consumption, and no need for arable land. *Spirulina* stands out with its high protein content, essential amino acids, and significant polyunsaturated fatty acids like gamma-linolenic acid, which is linked to health benefits. The biochemical composition of *Spirulina* can vary based on cultivation conditions, nutrient availability, and processing methods.

The composition of algae plays a vital role in its digestibility and absorption by the human body. While carbohydrates and polysaccharides in algae have moderate digestibility due to high fiber content, lipids and fatty acids are not highly bioaccessible due to inefficient breakdown in the digestive tract. However, proteins and amino acids from algae exhibit high bioaccessibility during gastrointestinal digestion. Dietary supplementation with algal biomass has shown health benefits in animals, and *in vitro* studies support the digestibility and bioaccessibility of natural bioactive compounds in functional foods enriched with algal biomass, promoting the potential for algae consumption to enhance human health. It's worth noting that conditions like temperature, sonication, pressure, and enzymatic treatments significantly influence the digestibility and bioaccessibility of algal biomass or compounds, potentially doubling these values, and offering improved methods for obtaining highly absorbable algal products.

Overall, it was possible to evaluate the nutritional, functional, and technological potential of *Spirulina* to be used as a food ingredient when comparing different commercial samples. Moreover, the physical properties of powdered *Spirulina*, including solubility, dispersion, water/oil holding capacity, and color parameters, are pivotal in its application in food products. These properties are influenced by drying methods, particle size, chemical composition, and surface properties.

The suitability of powdered *Spirulina* in food formulations depends on its intended usage, such as for enhancing nutrition or serving a specific technological function. The presence of volatile organic compounds in *Spirulina* contributes to its aroma and flavor, though they have limited impact on overall odor due to their high odor thresholds.

In conclusion, this research explored technological advancements to improve the sensory characteristics of microalgae biomass for food integration, emphasizing

environmentally friendly pretreatment methods. Focusing on ultrasound, a promising technique, was found that it enhances the extraction process by reducing drying duration through cell disruption. While no significant differences were observed between ultrasound-pretreated samples and those without, a subtle increase in phycocyanin content hinted at improved extraction efficiency. This suggests that ultrasound-induced cell wall rupture may enhance the accessibility of valuable biocompounds like phycocyanin. Additionally, ultrasound pretreatment positively influenced the digestibility and bioavailability of microalgae proteins during gastrointestinal digestion.

The findings also highlighted the potential of ultrasound to impact the volatile compound profile of powdered commercial *Spirulina*. Ultrasound pretreatment reduced acetic acid content, demonstrating a deodorizing effect, and eliminated specific hydrocarbons known for off-flavors, thereby improving the odor profile. However, three major compounds—D-Limonene, acetic acid, and pentadecane—persisted across all samples, indicating their crucial role in the overall flavor profile. Moreover, vacuum drying was shown to decrease alkane-derived aromas while enhancing fresh, green, and fruity aromas, particularly from hexanal. This dual effect improves the overall sensory attributes of Spirulina, making it more desirable for the market. This study underscores the multifaceted impact of ultrasound and vacuum drying on microalgae biomass, providing valuable insights for enhanced extraction and product quality in the food industry.

In conclusion, *Spirulina* exhibits a promising sustainable protein source with versatile nutritional and functional attributes. Ongoing research is essential for refining cultivation and processing methods, addressing sensory and stability challenges, and optimizing its potential to contribute to protein needs sustainably, thereby reducing the environmental impact of animal-based proteins.

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