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VIVIANE PRISCILA BARROS DE MEDEIROS

EFEITOS DA BIOMASSA DE MICROALGAS DULCÍCOLAS SOBRE A MICROBIOTA COLÔNICA DE ADULTOS SAUDÁVEIS EM SISTEMAS DE FERMENTAÇÃO *IN VITRO*

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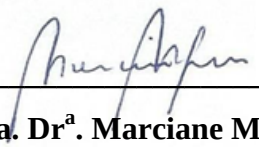
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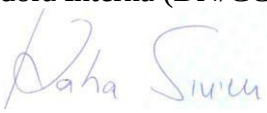
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À minha amada e querida família, dedico.

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RESUMO

Microalgas são microrganismos fotossintetizantes capazes de acumular diversos compostos bioativos com potencial prebiótico, no entanto, essa propriedade funcional é pouco explorada. O presente estudo buscou avaliar os efeitos prebióticos de biomassas de microalgas dulcícolas, utilizando sistemas de fermentação *in vitro.* Inicialmente, quatro espécies de microalgas dulcícolas (*Chlorella vulgaris*, *Desmodesmus maximus*, *Chlorococcum* sp cf *hypnosporum* e *Spirulina platensis*) foram cultivadas para obtenção da biomassa integral. As biomassas foram caracterizadas quanto à composição química e submetidas ao processo de digestão simulada. Posteriormente, a fase intestinal foi liofilizada e utilizada para os ensaios de fermentação com inóculo fecal humano em comparação com frutooligossacarídeos (FOS) como controle positivo. Após 24 e 48 h de fermentação fecal, foram avaliadas as mudanças na abundância relativa de grupos específicos da microbiota fecal por meio de fluorescência de hibridização *in situ* acoplada a citometria de fluxo. Ainda, foram mensurados os indicadores da atividade metabólica bacteriana (pH, açúcares e ácidos orgânicos) e determinado o escore prebiótico de cada microalga.Os resultados revelaram que a biomassa de *S. platensis* apresentou concentrações superioresde nistose (3,71 g/100g) e rafinose (6,14 g/100g), enquanto *D. maximus* obteve maiores concentrações de fibras insolúveis (42,60 g/100g) e kestose (110,62 g/100g). As maiores concentrações de monossacarídeos (glicose e frutose) e ramnose foram identificadas em *C. vulgaris*(16-52g/100g),enquanto a *C.* sp cf *hypnosporum*mostrou concentrações elevadas de procianidina A2, hesperidina e kaempferol-3-glicosídeo. As biomassas digeridas e o FOS aumentaram as abundâncias relativas de *Lactobacillus*, *Enterococcus* e *Bifidobacterium*, enquanto reduziram *Bacteroides spp./Prevotella spp*, *Clostridium histolyticum* e *Eubacterium rectale-Clostridium coccoides* presentes na microbiota fecal.A atividade metabólica microbiana diferiu entre as biomassas, FOS e o tempo avaliado. Após 48 h de fermentação, todas as biomassas microalgais apresentaram índices prebióticos variáveis (7,16-11,29) e superioresao FOS (4,67). Na segunda etapa desta pesquisa, a biomassa digerida de *S. platensis*(0,5 mg/mL) foi avaliada quanto à capacidade de estimular a proliferação e metabolismo de *L. acidophilus* (La-5) durante cultivo em meio laboratorial por 72 h. Na sequência, 1,5g de biomassa digerida de *S. platensis* foi administrada em combinação com La5 (10^6 UFC/g) no Simulador de Ecossistema Microbiano Intestinal (SEMH®), durante cinco dias para avaliação dos efeitos na composição da microbiota fecal de indivíduos saudáveis de meia idade. O *L. acidophilus* na mesma concentração também foi avaliado isoladamente. Ao longo da fermentação, a biomassa digerida de *S. platensis* promoveu impacto superior (9-8.28 log/UFC) ao FOS (8,74-8,05log/UFC) na taxa de proliferação celular e síntese de metabólitos do La-5 em meio laboratorial, reduzindo o pH do meio e aumentando a produção de ácido acético, lático e propiônico. A biomassa de *S. platensis* em combinação com La-5 (Spi-La5) aumentou a abundância de *Erysipelatoclostridium*, *Roseburia*, *Enterococcus*, *Bifidobacterium*, *Coriobacteriaceae* UCG-003, *Enterobacte*r e *Paraclostridium*.Por outro lado, a administração isolada do *L. acidophilus* (La5) aumentou *Bacteroides*, *Megasphaera*, *Lactobacillus*, *Parabacteroide* e reduziu os íons amônia em comparação com Spi-La5. Esses resultados mostram o potencial prebiótico da biomassa integral de microalgas dulcícolas, evidenciado pela mudança da composição, diversidade e atividade metabólica da microbiota fecal humana e sugerem o uso da biomassa de *S. platensis* em combinação com La-5 como uma estratégia para modular a microbiota em adultos saudáveis.

Palavras-chave: propriedades prebióticas; metabólitos; microbioma;modulação; *L. acidophilus*La5; prebiótico.

ABSTRACT

Microalgae are photosynthetic microorganisms capable of accumulating several bioactive compounds with prebiotic potential, however, this functional property is little explored. The present study sought to evaluate the prebiotic effects of freshwater microalgae biomass, using in vitro fermentation systems.The present study sought to evaluate the prebiotic effects of freshwater microalgae biomass, using in vitro fermentation systems.Initially, four freshwater microalgae species (*Chlorella vulgaris*, *Desmodesmus maximus*, *Chlorococcum* sp cf *hypnosporum* and *Spirulina platensis*)were cultivated to obtain the integral biomass. The biomasses were characterized in terms of chemical composition and submitted to the simulated digestion process. Posteriorly, the intestinal phase was lyophilized and used for fermentation assays with human fecal inoculum compared to fructooligosaccharides (FOS) as a positive control. After 24 and 48 h of fecal fermentation, changes in the relative abundance of specific groups of the fecal microbiota were evaluated using in situ hybridization fluorescence coupled to flow cytometry. Furthermore, the indicators of bacterial metabolic activity (pH, sugars and organic acids) were measured and the prebiotic score of each microalgae was determined. The results revealed that the biomass of *S. platensis* showed higher concentrations of nystose (3.71 g/100g) and raffinose (6.14 g/100g), while *D. maximus* had higher concentrations of insoluble fiber (42.60 g/100g) and kestose (110.62g/100g). The highest concentrations of monosaccharides (glucose and fructose) and rhamnose were identified in *C. vulgaris* (16-52 g/100g), while *C.* sp cf *hypnosporum* showed high concentrations of procyanidin A2, hesperidin and kaempferol-3-glycoside. Digested biomass and FOS increased the relative abundances of *Lactobacillus*, *Enterococcus* and *Bifidobacterium*, while reducing *Bacteroides* spp./*Prevotella* spp, *Clostridium histolyticum* and *Eubacterium rectale-Clostridium coccoides* present in the fecal microbiota. The microbial metabolic activity differed between the biomasses, FOS and the time evaluated. After 48 h of fermentation, all microalgal biomasses showed variable prebiotic indices (7.16-11.29) and higher than FOS (4.67).In the second stage of this research, the digested biomass of S. platensis (0.5 mg/mL) was evaluated for its ability to stimulate the proliferation and metabolism of *L. acidophilus* (La-5) during cultivation in laboratory medium for 72 h. Subsequently, 1.5g of digested biomass of *S. platensis* was administered in combination with La5 (10^6 CFU/g) in the Intestinal Microbial Ecosystem Simulator (SEMH®), for five days to evaluate the effects on the composition of the fecal microbiota of healthy medium-aged individuals. *L. acidophilus* at the same concentration was also evaluated separately. During fermentation, the digested biomass of *S. platensis* had a higher impact (9-8.28 log/CFU) than FOS (8.74-8.05 log/CFU) on the rate of cell proliferation and synthesis of La-5 metabolites in laboratory medium, reducing the pH of the medium and increasing the production of acetic, lactic and propionic acid.Biomass of *S. platensis* in combination with La-5 (Spi-La5) increased the abundanceof *Erysipelatoclostridium*, *Roseburia*, *Enterococcus*, *Bifidobacterium*, *Coriobacteriaceae* UCG-003, *Enterobacte*r and*Paraclostridium*. On the other hand, isolated administration of *L. acidophilus* (La5) increased*Bacteroides*, *Megasphaera*, *Lactobacillus*, *Parabacteroide*and reduced ammonium ions compared to Spi-La5.These results show the prebiotic potential of whole freshwater microalgae biomass as evidenced by changing the composition, diversity and metabolic activity of the human gut microbiota and suggest the use of *S. platensis* biomass in combination with La-5 as a strategy to modulate the microbiota in healthy adults.

Keywords: prebiotic properties; metabolites; microbiome; modulation; *L. acidophilus* La5; prebiotic.

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1 INTRODUÇÃO

Microalgas constituem um grupo diverso de microrganismos fotossintetizantes unicelulares que possuem rápido crescimento, são facilmente cultiváveis e podem serencontradas em água doce (lagos e rios), marinhas (mares, oceanos e salobra), solos e águas residuais (ABU-GHOSH *et al*., 2021). Esses microrganismos convertem a luz solar, CO_2, água e nutrientes inorgânicos em oxigênio e biomassa (BARSANTI; GUALTIER, 2018; SILVA *et al*., 2019). Devido a sua composição nutricionale ao avanço crescente da biotecnologia, a biomassa de microalgas tem se tornado ferramentas biotecnológicas para a produção de ingredientes para indústria de alimentos (KUSMAYADI *et al*., 2021). A estimativa de crescimento do mercado mundial de microalgas é de aproximadamente US $ 6,5 bilhões, dos quais cerca de US $ 2,5 bilhões são representados pelo segmento de alimentos saudáveis (SATHASIVAM *et al*., 2019).

A composição da biomassa microalgal inclui carboidratos (polissacarídeos e oligossacarídeos), em proporções que variam de 10 a 52% entre as espécies (BAUDELET *et al*., 2017; CAMACHO *et al*., 2019), ácidos graxos poliinsaturados, pigmentos (clorofilas e carotenóides), proteínas (peptídeos e aminoácidos), vitaminas, fitoesteróis e compostosfenólicos (SILVA *et al*., 2019; LEVASSEUR; PERRÉ; POZZOBON, 2020).A maior parte dos carboidratos presentes na biomassa de microalgas encontra-se na forma de heteropolissacarídeos, os quais são agrupados em três classes distintas de acordo com a sua fucionalidade. Os polissacarídeos estruturais relacionados à parede celular são geralmente constituídos por celulose, hemicelulose, substâncias pécticas e glicoproteínas, os quais estão envolvidos com a rigidez da parede celular (BERNAERTS *et al*., 2018; LEVASSEUR; PERRÉ; POZZOBON, 2020).

A complexidade da parede celular das microalgas confere resitência à digestão por enzimas presentes no trato gastrointestinal superior e permite que esses substratos tornem-se disponíveis para fermentação microbiana no colón humano, o que as torna candidatas para uso como prebióticos (RAPOSO *et al*., 2016). Prebióticos são definidos como substratos utilizados seletivamente por microrganismos do hospedeiroque conferembenefícios à saúde (GIBSON *et al*., 2017; CUNNINGHAM *et al*., 2021). Seu consumo promove impacto positivo à saúde do hospedeiroatravés de mecanismos que envolvem a fermentação do substrato no cólon, alterações na composição e atividade metabólica da microbiota intestinal (SENÉS-GUERRERO *et al*., 2020).

Os principais metabólitos resultantes da fermentação de prebióticossão os ácidos graxos de cadeira curta (acetato, propionatoe butirato), considerados importantes mediadores de funções intestinais e extra-intestinais que contribuem para saúde do hospedeiro (SENÉS-GUERRERO *et al*., 2020). Devido à expansão da demanda mercadológica e a expectativa de crescimento da comercialização de prebióticos para os próximos anos, pesquisas têm se concentrado na busca, produção e extração de ingredientes prebióticos de fontes alimentaresnão convencionais (MANO *et al*., 2018; CUNNINGHAM *et al*., 2021), a exemplo das microalgas (PATEL *et al*., 2021).

As microalgas dulcícolas *Chlorella vulgaris* e *Spirulina platensis* são consideradas seguras para consumo humano [*Generally Recognized as Safe* (GRAS, *United States Food and Drug Administration* FDA, 2011)], razão pela qual têm sido amplamente utilizadas na indústria de alimentos (MEDEIROS *et al*., 2021). Estas microalgas são fontes de fibras insolúveis, oligossacarídeos, proteínas ediversos compostos bioativos (LEAL *et al*., 2017; LAFARGA, 2020). Por outro lado, as espécies *Desmodesmus maximus* e *Chlorococcum* sp cf *hypnosporum*embora sejamsubexploradas para fins alimentares e de saúde,são relatadas como não toxigênicas (EUZING *et al*., 2014) e fonte de diversos compostos bioativoscom potencial para influenciar positivamente a microbiota intestinal humana.

A microbiota intestinal é reconhecida como um dos principais fatores que contribuem para a saúde humana, estabelecendo associação direta com a longevidade do hospedeiro (LYNN *et al*., 2021). Nos últimos anos, a administração de ingredientes prebióticos em combinação com probióticos tem sido sugerida como uma estratégia promissora à saúde humana, associada à modulação e restauração da microbiota intestinal (MOHANTY*et al*., 2018),atividade antioxidante (HESHMATI *et al*., 2018), melhora dosistema imunológico (KAZEMI *et al*., 2020) e ao controle dedoenças(KHALIL *et al*., 2021; ARABI *et al*., 2022).

Em particular, estudos têm demonstrado que *Lactobacillus acidophilus*, uma das cepas probióticas mais utilizadas na indústria de alimentos (ZHANG *et al*., 2018),desempenha atividades de regulação imunológica no hospedeiro (ZHAO *et al*., 2020) através de alterações benéficasna composição da microbiota intestinal,promovida pelo aumento de e grupo de microrganismos benéficos presentes na microbiota intestina, síntese de ácidos orgânicos (VEMURI *et al*., 2018) e inibição da colonização de patógenos (NAJARIAN; SHARIF;GRIFFITHS, 2019).

Pesquisas *in vitro* envolvendo a incorporação da biomassa de *S.platensis* em alimentos fermentados (iogurte, ayran, kefir) mostraram um aumento significativo no crescimento,

viabilidade e metabolismo de *L. acidophilus*(ÇELEKLI *et al*., 2019; SYLWIA; ELŻBIETA, 2020; ATIK *et al*., 2021). No entanto, são escassas na literatura as informações sobre o potencial prebiótico da biomassa integral de microalgas dulcícolas e os possíveis benefícios da associação de biomassa microalgal com*Lactobacillus acidophilus* La-5 na microbiota intestinal de humanos.

Desta forma,o presente estudo teve por objetivo i) determinar a composição (fibra alimentar total, solúvel, insolúvel, oligossacarídeos, monossacarídeos e compostos fenólicos) da biomassa das microalgas dulcícolas de*C. vulgaris*, *D. maximus*, *C.* sp. cf *hypnosporum* e *S. platensis*; ii) avaliar os efeitos da biomassa digerida das microalgas estudadas na abundância relativa de grupos bacterianos específicos presentes na microbiota fecal e na síntese de metabólitos (açúcares, ácidos orgânicos e compostos fenólicos) durante fermentação fecal *in vitro*; iii) verificar o impacto da biomassadigerida de*S. platensis* na taxa de proliferação e parâmetros indicadores do metabolismo de *Lactobacillus acidophilus* (La5) em meio de cultura laboratorial; e iv) avaliar os efeitos da administraçãoda biomassa de*S. platensis*de forma isolada eem combinação com *L. acidophilus*(La5) na microbiotafecalde indivíduos saudáveis e na síntese de metabólitos (ácidos graxos de cadeia curta e íons amônia).

2 REFERENCIAL TEÓRICO

2.1 REVISÃO DE LITERATURA

A abordagem inicial da Tese está descrita no artigo de revisão de literatura intituladocomo “Microalgae as source of functional ingredients in new-generation foods: challenges, technological effects, biological activity, and regulatory issues”, publicado no periódico *Critical Reviews in Food Science and Nutrition*. O artigo refere-se à aplicação de biomassas de microalgas e seus compostos bioativos como fonte de ingredientes com propriedades tecno-funcionais para formulação de diversos produtos alimentícios e fornece informações sobre os potenciaisefeitos tecnológicos de sua incorporação e uso como fonte de compostos bioativos promotores da saúde. Ainda, aborda os aspectos regulatórios da comercialização e consumo de microalgas no mundo, as principais tendências de mercado e suas perspectivas futuras. A publicação do artigo de revisão nesta tese está em consonância coma política de permissões para uso acadêmico descrito no*Taylor & Francis Journal* e encontra-se disponível para acesso através do DOI: 10.1080/10408398.2021.1879729.

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REVIEW

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Microalgae as source of functional ingredients in new-generation foods: challenges, technological effects, biological activity, and regulatory issues

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ABSTRACT

Microalgae feasibility as food ingredients or source of nutrients and/or bioactive compounds and their health effects have been widely studied. This review aims to provide an overview of the use of microalgae biomass in food products, the technological effects of its incorporation, and their use as a source of health-promoting bioactive compounds. In addition, it presents the regulatory aspects of commercialization and consumption, and the main trends and market challenges Microalgae have stood out as sources of nutritional compounds (polysaccharides, proteins, lipids, vitamins, minerals, and dietary fiber) and biologically active compounds (asthaxanthin, β-carotene, omega-3 fatty acids). The consumption of microalgae biomass proved to have several health effects, such as hypoglycemic activity, gastroprotective and anti-steatotic properties, improvements in neurobehavioral and cognitive dysfunction, and hypolipidemic properties. Its addition to food products can improve the nutritional value, aroma profile, and technological properties, with important alterations on the syneresis of yogurts, meltability in cheeses, overrun values and melting point in ice creams, physical properties and mechanical characteristics in crisps, and texture, cooking and color characteristics in pastas. However, more studies are needed to prove the health effects in humans, expand the market size, reduce the cost of production, and tighter constraints related to regulations.

KEYWORDS

Freshwater microalgae; marine microalgae; biomass; pigments; Spirulina

Introduction

Microalgae constitute a heterogeneous group of unicellular photosynthetic microorganisms present in both marine and freshwater environments (Bhattacharya and Goswami 2020). The term microalgae has no phylogenetic connotations once it encompasses different groups of eukaryotic organisms such as chlorophylls (green algae), bacillaryiophytes (diatoms), dinophytes (dinoflagellates), euglenophytes, prymnesiophytes (cocolitophorides) and prokaryotes, such as caryophytes (Enzing et al. 2014). Microalgae are commonly used in several biotechnological uses, such as production of biodiesel, bioremediation of wastewater, and in food products (Barkia, Saari, and Manning 2019).

The food pattern established in recent years is focused on the consumption of foods that have functionality for health (Hashempour-Baltork et al. 2020a), which highlighted the microalgae biomass as a potential ingredient for the food industry. Microalgae biomass has been incorporated in a variety of food products, such as mayonnaise (Abd El-Razik and Mohamed 2013), ice cream and soft cheeses (Agustini et al. 2016), rice soy crisp (Bashir et al. 2017), cookies (Batista et al. 2017), smoothies (Castillejo et al. 2018), olive oil (Alavi and Golmakani 2017), sausages (Marti-Quijal, Zamuz, Tomašević, Gómez, et al. 2019), among others. The incorporation of microalgae biomass resulted in suitable sensory acceptance, health benefits to consumer, and improved color (Abd El-Razik and Mohamed 2013). However, the concentration and type of microalgae should be studied, as the incorporation is associated to changes in the color, flavor, and texture of the products (Agustini et al. 2016). Therefore, microalgae biomass can be an innovative resource for the development of functional foods due to its natural origin, unique structuring properties proportionated to the developed products (Bernaerts et al. 2019), and interesting chemical composition (high quantities of vitamins, proteins, polysaccharides, minerals, enzymes, and fibers). Furthermore, they can be a source of bioactive compounds, such as bioactive peptides, polyphenols, essential amino acids, monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids, carotenoids, and phycocyanin. In this way, the consumption of microalgae biomass and its derivatives can result in positive effects on consumer health (Matos et al. 2017; Barba 2017; Niccolai et al. 2019).

China has been considered the largest producer of microalgae biomass, with an estimated production of 35.000 tons/year, representing about two thirds of the global production. *Spirulina platensis* and *Chlorella vulgaris* have been the most widely cultivated species (Muys et al. 2019), being sold in health food stores around the world in various forms, such

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as tablets, capsules, liquids, nutritional supplements or as a natural source for food addition (Batista et al. 2012; Matos 2017). They are classified as safe substances for human consumption (Generally recognized as safe (GRAS) according to the Food and Drug Administration) (FDA, 2011) and validated by the World Health Organization as "superfoods" (Jung et al. 2019). Other species have also been studied, but their exploitation depends on the food safety regulations, market demand, commercial factors, and specific preparation (Abd El-Razik and Mohamed 2013).

Microalgae biomass and its derivatives (extracts and isolated compounds) can have multiple functions, such as pigments and preservatives, improve the health and nutritional profile of foods, and improve the technological properties of the products (Barba 2017). Studies that summarize the utilization of microalgae in food production are scarce (Vaz et al. 2016; Barba 2017, Matos et al. 2017, Barkia, Saari, and Manning 2019), and did not comply the health, regulatory, and technological aspects, being mainly concentrated in the health effects. Therefore, this review aims to provide an overview of the use of microalgae biomass in food products, the technological effects of its incorporation, and their use as a source of health-promoting bioactive compounds. In addition, it presents the regulatory aspects, and the main trends, challenges, and perspectives of this new and growing market niche.

Methodology

A narrative bibliographic review was carried out using the PubMed, Scopus and Lilacs platforms as scientific databases and the following keywords/descriptors as search strategy: "microalgae", "cultivation of microalgae", "bioprospecting", "microalgal biomass", "freshwater microalgae", "*Arthrospira*", "*Spirulina*", "nutritional content of the microalgae", "microalgae for foods production", "technological properties", "bioactive compounds", and "health effects". A total of 165 articles published during the period of 2010–2020 were selected, written exclusively in English language and subsequently screened. Papers that did not coincide with the theme as well as repeated texts published on different platforms were excluded.

A total of 101 articles were researched and categorically subdivided into: I – articles in which the technological properties of the chemical compounds (protein, lipid and carbohydrates) and bioactive compounds from microalgae are exploited (36 articles); II – articles in which whole microalgae (biomasses) or extracts are used in the processing of food matrices (50 articles); and III – articles in which whole microalgae (biomasses) are used isolated or as an additive with a focus on the health benefit to the consumer (15 articles).

Microalgae general characteristics

Microalgae present some interesting features to be exploited, such as fast growth rate, basic cultivation requirements (i.e. solar radiation, water, CO_2, and inorganic nutrients), and ability to survive in harsh conditions (Tang et al. 2020). Furthermore, microalgae biomass cultivation and production have advantages when compared to plant cultivation, namely: can be cultivated in small spaces; presents continuous production during the year (can be grown in cases of seasonality); requires relatively small amount of nutrients for growth; does not compete with agriculture for the land, does not require the use of pesticides for pest control; has superior yield, and can be produced in regions where the weather condition precludes plant production (Koyande et al. 2019; Mata, Martins, and Caetano 2010; Brasil, Silva, and Siqueira 2017).

The main difficulties that limit the expansion of microalgae biotechnology are the high cost and the low scale of cultivation and production of the systems currently employed (Garrido-Cardenas et al. 2018; Gifuni et al. 2019). In this sense, utilization of alternative culture media has been widespread, seeking efficiency in replacing synthetic culture media. Agro-industrial residues can be used as cheap substrates for biotechnological purposes, decreasing the product's total cost (Hashempour-Baltork et al. 2020b). In addition to suitable cell growth, microalgae cultivation in alternative medium with agro-industrial may also improve the nutritional composition and antioxidant activity of the obtained biomass compared to the synthetic medium (Medeiros et al. 2020).

Currently, phototrophic microalgae cultivation systems can be classified as closed systems (vertical or horizontal tubular, flat panel or other photobioreactor) or open systems (open lagoons, paddle wheel tracks and cascading tracks). Closed systems are preferable due to the safety aspects (lower chances of contamination), better control of the conditions of cultivation, lower water use, and higher productivity and biomass yields (due to the higher penetration of light and surface-to-volume ratio) (Di Visconte et al. 2019). On the other hand, the open systems have lower capital operational and energetic costs due to the less complexity (Barkia, Saari, and Manning 2019). It is advisable to use open systems with species that withstand harsh conditions (extreme pH values and high salinity) and have high growth rates (inhibiting the competing microorganisms) (Barreira et al. 2015). The selection of the type of system to be used is related to the nature of the product and the market price assigned. A higher value product will allow the use of a more expensive system, while a cheaper product should be grown in low-cost open lagoons (Torres-Tiji, Fields, and Mayfield 2020).

A conventional microalgae biomass production system consists of the following steps: monitoring the cell growth, collecting and dehydrating the biomass, and disrupt the cell for extraction and/or conversion of biomass into the compound of interest (Matos et al. 2017). The nutritional composition of microalgae varies according to the cultivated species. Other important factors that affect the composition include the physiological responses to biotic and abiotic factors (light intensity, photoperiod, temperature, nutrients, and growth phase). Therefore, these factors can be manipulated by altering the culture conditions and environmental stress to induce the production of higher concentrations of the compound of interest (Vaz et al. 2016; Wild, Steingaß, and Rodehutscord 2018). Consequently, the composition of microalgae is variable, with ranges of 9–77% proteins, 6–54% carbohydrates, and 4–74% lipids (Bernaerts et al. 2019).

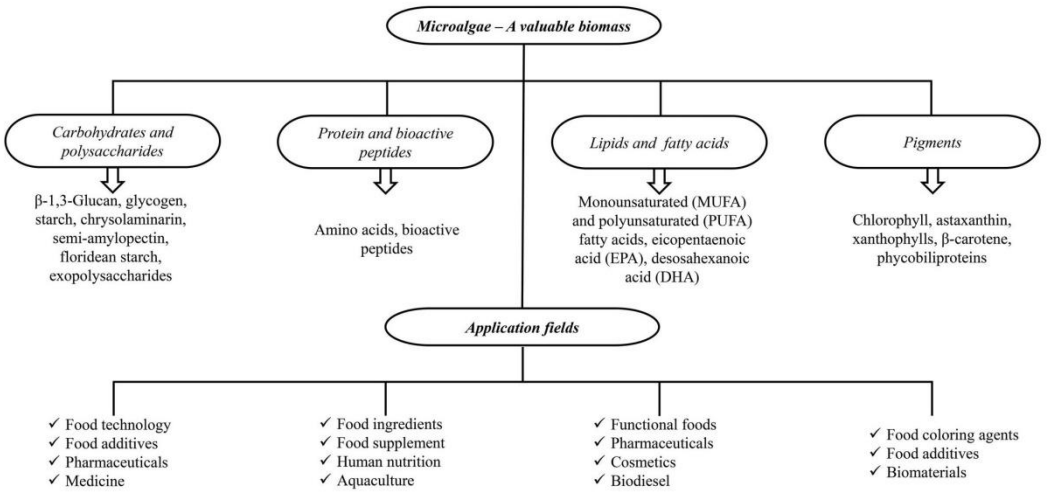


Figure 1. Flowchart scheme showing the main compounds of microalgae and current applications.

Currently, only few species of microalgae are grown, produced and used on a commercial scale: Cyanobacterias, *Arthrospira platensis*, *Chlorella vulgaris*, *Dunaliella salina*, *Isochrysis galbana*, *Bannochloropsis salina*, *Phaeodactylum tricornutum*, *Porphyridium cruentum* and Haematococ which are applied in animal feed, organic fertilizers, biofuel production, bioremediation processes for waste water, soils and combustion gases and in the production of high value compounds, such as PUFA, natural antioxidant and nutraceutical pigments (Delattre et al. 2016).

Despite the longstanding tradition of microalgae consumption in North America and Asia, the multi-commercialization of biomass is still beginning and the increase in the consumption has been due to the disclosure of the effects of its incorporation into food products and improvements in the health of the consumers (Camacho, Macedo, and Malcata 2019). Initial investigations into microalgae applications have limited the use of full biomass in food for human consumption and as feed for aquaculture. Over the years, there has been an evolution in the field of microalgae biotechnology, based on the following different research axes: (i) search for new (or already known) species that present fast and easy growth and contain new bioactive compounds (or induce them); (ii) seeking to understand the biological machinery of the strains in order to evaluate the regulatory mechanisms related to cell performance; (iii) improvement of biomass production systems in terms of efficiency and capacity; and (iv) development of new products to expand consumer markets and in the tracking of bioactive microalgae metabolites (Garrido-Cardenas et al. 2018).

Microalgae chemical composition and bioactive compounds and health-related aspects

Figure 1 presents the main compounds of microalgae and current applications. Microalgae have stood out as natural and promising sources of nutritional compounds (polysaccharides, proteins, lipids, vitamins, minerals, and dietary fiber) or biologically active compounds that can be obtained from the biomass or be released extracellularly into the culture medium (asthaxanthin, β-carotene, omega-3 fatty acids). They can be widely utilized for commercial use in or as pharmaceuticals, cosmetics, food additives and ingredients due to their potential therapeutic activities (De Jesus Raposo, De Morais, and De Morais 2013; Fu et al. 2017). The estimated financial value for products added with microalgae should consider the system of cultivation, harvest and pretreatment of biomass, which includes the stages of washing, purification, dewatering and drying (Rahman 2020). Astaxanthin is commercialized at US$2500–7000/kg, β-carotene at US$300-1500/kg, and microalgae biomass at US$42–44/kg. Therefore, from an industrial perspective, the bioactive compounds present higher economic value than the dried microalgae biomass (Barkia, Saari, and Manning 2019).

Microalgae carbohydrates and polysaccharides

Carbohydrates are considered as important fractions from microalgae biomass, and the content vary with the genera and species (Ho et al. 2013). They are subdivided into storage and structural carbohydrates (Bernaerts et al. 2019), including alginic acid, alginates, carrageenan agar, laminarans, fucoidans, and ulvans and its derivatives. Polysaccharides production is associated to the type of microalgae, as cyanophytes accumulates glycogen and semi-amylopectin, chlorophyta synthesizes starch, rhodophyta produces floridean starch, and diatoms produces chrysolaminarin and β-glucan (Barkia, Saari, and Manning 2019). The extraction of polysaccharides from microalgae biomass is performed using hot water, which is a simple and easy method, although it needs long times and has low efficiency of extraction (Barkia, Saari, and Manning 2019). Therefore, the efficiency can be increased by applying ultrasonic, microwave, and enzymes to assist in the extraction. After

the extraction, a purification step is needed to remove interfering substances, which can be done with solvents or physical methods (separation with membranes or chromatographic methods) (Barkia, Saari, and Manning 2019).

The biological activities of polysaccharides include immunomodulatory (Parages et al. 2012; Qi and Kim 2017), antioxidant (Yu et al. 2019), and anti-cancer activities (Kurd and Samavati 2015). Furthermore, polysaccharides from *Chlorella pyrenoidosa* are effective in dampening the inflammation on motor activity, dopamine expressions, microglial activation, and peripheral immunomodulatory responses of induced Parkinson's disease in mouse model (Chen et al. 2014). Finally, they can have antioxidant activity, which may be related to monosaccharide design, and molecular size, structure, and conformation (Yingying et al. 2014).

Microalgae also produce EPS, which are secreted along their growth or under specific conditions, depending on species and cultivation conditions (nutrient depletion, high salinity, high light fluxes) (Xiao and Zheng 2016; Delattre et al. 2016). The EPS are constituted mainly by glucose, galactose, fucose, xylose, arabinose, rhamnose, mannose, fructose and/or sugar derivatives (Xiao and Zheng 2016). In the face of environmental conditions, EPS may protect against various biotic and abiotic agents, physiological stresses or facilitate the attachment to a solid substrate (Bafana 2013). EPS has received interest due to a large chemical structure's heterogeneity, as a consequence, they have a great variety of physicochemical and biological properties (Delattre et al. 2016; Paniagua-Michel, Olmos-Soto, and Morales-Guerrero 2014). For instance, EPS from microalgae *Chlorella pyrenoidosa*, *Scenedesmus* sp. and *Chlorococcum* sp. showed antitumor effects on the HCT116 and HCT8 human colon cancer cell lines considering the inhibitory effects on cell viability and the decrease in the colony amounts (Zhang et al. 2019). Furthermore, EPS from *Chlamydomonas reinhardtii* presented high antioxidant capacity (Bafana 2013).

Microalgae protein and bioactive peptides

The protein content of several microalgae species has been one of the main reasons for considering these organisms as alternative protein source (Pereira, Lisboa, and Costa 2018; Khemiri et al. 2020; Vieira et al. 2020). Microalgae are considered a non-animal protein source that presents higher protein content than conventional sources (for instance, 55–70% for *S. platensis* and 42–55% for *C. vulgaris* per dry matter) and amino acid quality superior to conventional source such as meat, poultry and dairy products (Koyande et al. 2019). In this sense, several microalgae protein products have been formulated according to cell protein content and purity and are classified into protein concentrates, hydrolysates, and isolates and bioactive peptides (Soto-Sierra, Stoykov, and Nikolov 2018).

The main obstacle to the use of microalgae as protein source is the presence of a cellulosic cell wall, a component that is not digested by human enzymes (Fasolin et al. 2019). In this sense, to promote the release of biofunctional proteins present in microalgae biomass it is necessary to employ effective cell disruption methods (Grossmann, Hinrichs, and Weiss 2019), including enzymatic method (Callejo-López et al. 2020), microwave treatment (Chew et al. 2019), pulsed electric energy and ultrasonication (Zhang et al. 2020), pulsed electric field treatment (Buchmann et al. 2019), high pressure homogenization (Yang et al. 2020), bead milling and enzymatic hydrolysis (Garcia et al. 2018; Alavijeh et al. 2020), chemical and mechanical methods (Phong et al. 2018) and emerging technologies as osmotic shock (Koyande et al. 2020). The processing technique choice largely depends on the peptide structural feature of interest (Ejike et al. 2017).

Wild, Steingaß, and Rodehutscord (2018) evaluated the in vitro protein digestibility of four microalgae genera with and without previous cell disruption. The results show that protein digestibility was lower in *C. vulgaris*, *Phaeodactylum tricornutum*, *S. platensis*, and *Nannochloropsis* not subjected to cell disruption (79%, 77%, 74% and 54%, respectively) compared to damaged cells (84%, 83%, 79%, and 78%, respectively). However, more research is needed to investigate the digestibility and bioavailability of microalgae proteins in vivo (Bleakley and Hayes 2017).

Bioactive peptides can be obtained from microalgae protein using different methods, such as microbial fermentation, chemical hydrolysis, and enzymatic hydrolysis, being the latter considered the most suitable option, as the process is easy to be controlled, the structure of the amino acids is not altered due to the mild conditions, the nutritional value and functionality are preserved, and it does not need high volumes of solvents and toxic chemicals (Barkia, Saari, and Manning 2019). The bioactive peptides are purified using salting out or extraction with solvent, and separated using chromatographic methodologies, membrane separation or electro membrane filtration, and lyophilized (Barkia, Saari, and Manning 2019). The peptides of microalgae can be used in functional foods for promoting cardiovascular health (Ejike et al. 2017). Furthermore, their consumption was associated to antioxidant, anti-hypertensive, anticancer, anti-atherosclerosis, immunosuppressive, and hepatoprotective activities (Bleakley and Hayes 2017).

Microalgae lipids and fatty acids

Microalgae have great potential in biosynthesis of polar lipids (i.e., triacylglycerols molecules) or structural lipids (i.e., MUFA and PUFA), glycolipids or phytosterols and waxes (Santos-Sánchez et al. 2016). The accumulation of lipids in microalgae usually varies in the range of 20–50% of total biomass (dry weight), and depends on the metabolic status of the cells and growth, and the conditions of cultivation such as medium composition, environmental solar radiation and temperature (Katiyar and Arora 2020). The fatty acid profile may vary considerably among microalgae species, but the most abundant are those with an even number of carbons with lengths chain of 14, 16 or 18 carbons (Barreira et al. 2015).

Microalgae production of PUFA is of great commercial interest because they are sources of natural edible oils with lower levels of contaminants (dioxins, polychlorinated

biphenyls, methyl mercury) than fish oils (Charles et al. 2019; Barkia, Saari, and Manning 2019). Furthermore, they are considered natural, of high purity and quality, from "vegetarian" origin and sustainable food matrices, and with improved sensory qualities when compared to animal-based oils. Species with high levels of n-3 PUFA (higher than 50% of total fatty acid) can be found in Chlorophyta (Dunaliella sp., *Tetraselmis viridis*, *Nephroselmis* sp.), Cryptophyta (*Chroomonas salina*, *Cryptomonas* sp.), and *Rhodomonas* sp (Martins et al. 2013).

Microalgae can produce eicopentaenoic acid (EPA) and dososahexanoic acid (DHA), which are generally used in the supplementation of infant formula (Barkia, Saari and & Manningg 2019). *Isochrysis galbana* and *Phaeodactylum tricornutum* species produce closely $\geq$2 folds higher EPA and *Crytthecodinium cohnii* produce nearly $\geq$6 folds higher DHA than cod liver oil (Katiyar and Arora 2020). Oil derived from *Schizochytrium* spp. has been found to contain at least 14% DHA (Kaewsutas et al. 2017).

The PUFA extraction has some steps, such as disruption of the cells, extraction of lipids with organic solvents, separation of PUFA from the other lipids (usually using winterization or fractional distillation), and purification (bleaching, filtration, polishing, deodorization, and addition of antioxidants) (Jesus et al. 2019; Barkia, Saari, and Manning 2019). The extraction step can be assisted with ultrasound (Sivaramakrishnan and Incharoensakdi 2019), supercritical fluids (Elst et al. 2018), electroporation or enzyme (Santos-Sánchez et al. 2016).

Some criteria are established to promote an increase in the content of intracellular PUFA in microalgae, namely: (i) control of culture conditions and deviation of cellular metabolism to maximize the synthesis of lipids, EPA and DHA; (ii) species selection to increase the production of biomass and lipids; and (iii) genetic engineering application for natural production of EPA and DHA (Chauton et al. 2015). For instance, culture medium nutritional stress by deprivation of nitrogen (N) or phosphorous (P) promotes overproduced radicals and the antioxidant levels are insufficient to counteract the radicals, resulting in the elevation of oxidative stress and consequently the accumulation of intracellular lipid (Hu et al. 2019).

Several researches demonstrate PUFA effects in health: for an example, at heart diseases prevention (Mozaffarian and Wu 2011), inhibition of cancerous tumors growth (Qian and Xu 2014), modulation of lipid metabolism disorders and gut microbiota (Li et al. 2019), children central nervous system development (Guesnet and Alessandri 2011) and neurodegenerative diseases prevention (Kaewsutas et al. 2017). Nevertheless, most regions of the world present low levels of intake of PUFA omega-3, EPA and DHA emphasizing the economically and environmentally need for sustainable sources to raise the food nutritional quality (Pierre et al. 2019). To supply this market demand, several companies have produced concentrates of these compounds from microalgae, with emphasis on omega-3 i.e. alpha-linolenic acid, EPA, DHA and omega-6 (Katiyar and Arora 2020).

More recently, several studies have evaluated the supplementation of essential fatty acid extracted from microalgae in animal feed and the possible effects on the nutritional quality of the products. Milk obtained from cows fed with *Aurantiochytrium* sp. DHA showed changes in the fatty acid profile with decreases in the saturated fatty acid (SFA) and increases in PUFA (Marques et al. 2019). Feng et al. (2020) evaluated the effects on table eggs (fatty acid composition, sensory profile, and general acceptability) of the supplementation of laying hens with oil extracted from microalgae. The results indicate that there was a DHA and total PUFA n-3 levels increase in the yolk as well as satisfactory sensory acceptability, when compared to fish oil. Similarly, the addition of 2% DHA extracted from biomass microalgae (*Schizochytrium* sp.) through feeding of laying hens resulted in an increase in the essential fatty acid content in eggs, and their consumption by young and healthy subjects resulted in improvements in the cognitive function (cortical activity) (Kaewsutas et al. 2017). Finally, Tibbetts, Scaife, and Armenta (2020) evaluated the effect of using oil derived from the microalgae *Schizochytrium* sp. as a DHA source in the juvenile Atlantic salmon as a replacer of fish meal feeding. The results suggest that the PUFA digestibility was not affected and there was an improvement in the digestibility of MUFA.

Microalgae pigments

With the increasing of the consumer interest for natural products and consolidation of functional food and nutraceuticals' market, the demand for natural pigments to replace synthetic colorants used in food is on the rise (Fernandes et al. 2020). In this context, microalgae are a group of promising microorganisms. The pigments responsible for color are part of the photosynthetic system and include chlorophyll a, b and c, β-carotene, astaxanthin, xanthophylls and phycobiliproteins (Christaki, Bonos, and Florou-Paneri 2015). The synthesis of pigments depends on the species of microalgae and cultivation conditions such as temperature, salinity, wavelength, light intensity, and photoperiods (Thawechai et al. 2016), pH and nutrient availability (Mc Gee et al. 2020).

The β-carotene is an orange pigment usually extracted from *Dunaliella salina,* a green alga which grows in ambient with high light and salinity, and can used in butter/margarines, cheeses, and soft drinks. Astaxanthin is a reddish-salmon pigment produced by *Haematococcus pluvialis*, a green alga from freshwater (Barkia, Saari, and Manning 2019). Phycobuliproteins are usually extracted from *Porphyridium cruentum*, *Synechococcus* spp., and *Arthrospira* spp., and can be used as natural colorants in dairy products, candies, chewing gums, and ice creams (Buono et al. 2014). Phycocyanin is a blue color pigment that can be used in food formulations to increase the appearance attractiveness (Camacho, Macedo, and Malcata 2019). Other pigments include chlorophylls (green color, all phototrophic alga), bixin (yellow to peach color, *Dunaliella salina),* canthaxanthin (golden-orange, *Haematococcus pluvialis* and *Chlorella zofngiensis),* fucoxanthin (brown, *Phaeodactylum tricornutum*), lutein (orange-yellow, *Dunaliella salina, Chlorella*

Table 1. Studies assessing the functional and technological effects of pigments isolated from different microalgae specie.

Functional properties				
Microalgae specie	Pigments	Assay	Major outcomes	References
Scenedesmus obliquus	Carotenoids	*In vivo*	Improvement the activity of antioxidant enzymes and reduce lipid peroxidation.	Nascimento et al. (2020)
Spirulina platensis	Phycocyanin	*In vitro*	Antidiabetic activity through inhibition of α-amylase and α-glucosidase; anti-cancer, anti-diabetic and anti-inflammatory action.	Prabakaran et al. (2020)
Spirulina maxima	Phycocyanin	*In vitro*	Protect renal cells against oxidative stress and cellular damage in the kidney.	Rodríguez-Sánchez et al. (2012)
Spirulina platensis	Phycocyanin	*In vivo*	Regulation of lipid metabolism and reduction of fat accumulation in the liver caused by nonalcoholic fatty liver disease	Ma et al. (2020)
Technological properties				
Spirulina platensis	Phycocyanin	Yogurt	Improvement in physical-chemical parameters (syneresis, color, texture) without negatively affecting the growth of initial cultures.	Mohammadi-Gouraji and Soleimanian-Zad (2019)
Spirulina platensis	Phycocyanin	Ice creams	Minimum color change of the ice creams (during 182 d), increased antioxidant capacity after in vitro digestion (ORAC e ABTS).	Amarante et al. (2020)

salina, and *C. zofngiensis*), violaxanthin (orange, *Dunaliella tetrio-lecta* and *Botryococcus braunii*), zeaxanthin (yellow-orange, *Phaeodactylum tricornutum),* phycoerythrin (red, *Porphyridium),* and tocopherol (brown, *Euglena gracilis*, *Nannochlropsis oculata*, and *Chlorella* sp.) (Matos 2017).

Pigment extraction involve some steps, such as pretreatment of the biomass (enzyme lysis, treatment with acid and bases, mechanical disruption), extraction with solvents or alternatives (supercritical fluid, microwave, ultrasound, enzyme), and purification (to concentrate, purify, and remove contaminants) (Barkia, Saari, and Manning 2019). Besides the functionality as pigments, several studies demonstrate the functional and health-related potential of these compounds, as shown in the Table 1.

Health effects of microalgae biomass and derivatives

Figure 2 presents the main health effects associated to the consumption of microalgae biomass and derivatives. Table 2 shows the main studies assessing the in vivo health effects associated to microalgae biomass consumption.

Microalgae could be used as an alternative gastroprotective agent. Anantharajappa, Dharmesh and Ravi (2019) evaluated the effect of the consumption of Spirulina biomass (SB) and Spirulina extract (SE) by rats (male, adult, and albino) on the swim induced gastric ulcers. The microalgae biomass inhibited 45% of ulcers formation, while the microalgae extract inhibited 60%. The authors mentioned that the effects were associated to the bioavailability of vitamin B_{12} and reported some mechanisms of action related to the inhibition of lipid peroxidation, alteration of the levels of antioxidant enzymes, and upregulation of the activity of H^+, K^+-ATPase. Fields et al. (2020) reported that human volunteers with varied gastrointestinal symptoms had less diarrhea, bowel discomfort, bloating, and gas, as well as, higher regularity in the bowel movements, and better consistency of the stool after consumption of *Chlamydomonas reinhardtii* biomass (1 or 3 g, 30 days).

The consumption of microalgae biomass can have an impact on the oxidative stress. Ghanbari et al. (2019) evaluated the effect of *Spirulina fusiformis* biomass consumption by *Wistar* rats (200 mg/kg) on the deficit of memory induced by scopolamine. The authors reported that the microalgae biomass could improve the deficit of memory induced by scopolamine by reducing the malondialdehyde levels, being related to the inhibition of oxidative stress. Peter et al. (2017) reported that *Spirulina platensis* biomass consumption by rats (400 mg/kg) with hepato-renal injury induced by diclofenac resulted in decreases in the damages caused by oxidative stress in kidney and liver and prevented the ulcer formation.

Microalgae biomass consumption can improve the lipid profile and modulate the antioxidant status. Nascimento et al. (2019) evaluated the effect of *Scenedesmus obliquus* biomass consumption by mice (400 or 800 mg/kg/day for 4 weeks) and observed that the highest concentration increased the high-density lipoprotein (HDL) cholesterol and triglycerides levels, the activity of glutathione reductase in liver, the activity of glutathione peroxidase in kidneys, and the heart antioxidant potential. The lower concentration increased the activity of glutathione peroxidase in kidneys, and the antioxidant potential. Furthermore, the lipid peroxidation was increased in all tissues, regardless of the concentration. Decreases of triglycerides levels, atherogenic index, and serum glucose concentration in rats was also observed by Silva et al. (2020) for consumption of *Scenedesmus obliquus* biomass (20 g/day). Sengupta et al. (2018) reported that the consumption of soy yogurt fortified with *Spirulina platensis* biomass (0.5%, 1.0% and 1.5%) by hypercholesterolemic mice (totaling 8.5% of the diet) resulted in improved lipid profile (reduction in the total cholesterol and increase in the HDL cholesterol), anti-atherogenic properties, and improved liver function. Bigagli et al. (2017) evaluated the effects of a diet enriched with *A. platensis* F&M-C256 in rats (12 g/Kg body weight/day, 1 month) and reported that the diet was well tolerated, without adverse effects, or changes in body weight, food consumption, growth, or behavior. Furthermore, an increase in the HDL-cholesterol levels and

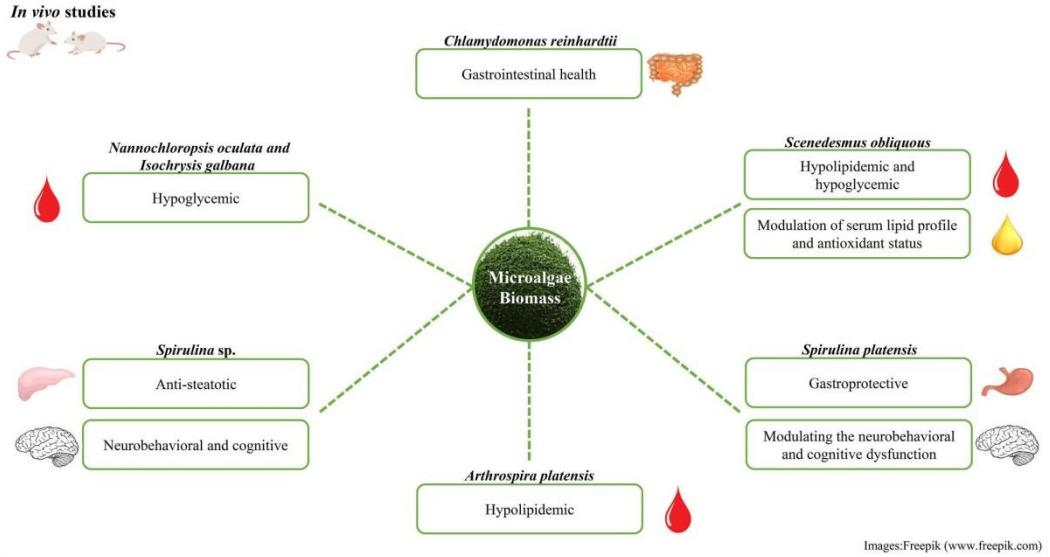


Figure 2. Main health benefits of microalgae biomass and derived products (extracts or isolated compounds) in vivo studies.

a decrease in the triglycerides were observed, suggesting the application of the biomass in dyslipidemia.

Microalgae consumption can have anti-steatotic activity and promote therapeutic properties in nonalcoholic fatty liver (NAFL). Oriquat (2018) studied the effect of consumption of Spirulina (100, 200, and 500 mg/kg for 30 days) by rats with NAFL induced by a high-fat diet. The microalgae consumption improved the liver functions, body weight, insulin, fasting blood glucose, and homeostasis model assessment index for insulin resistance. Furthermore, it modulated the hepatic expression (miR-122, miR-34a, and miR-21) and the genes (SREBP-1c, SIRT1, and HPB1). The effects were dose dependent.

The consumption of microalgae can decrease malnutrition that is related to cognitive and behavioral deficits. Sinha, Patro, and Patro (2020) evaluated the impact of the consumption of Spirulina biomass by female rats (malnourished protein, 400 mg/kg, pregnancy and lactation) on the reflex of offspring, and cognitive and behavioral development. The supplementation resulted in higher reproductive performance, neurological reflexes, neuromuscular strength, spatial learning, memory, and partly normalized protein malnutrition induced anxiolytic, anhedonic and hyperactivity behavior in offspring. Furthermore, it improved the physical appearance of the rats.

Microalgae consumption can delay chronic kidney disease and the cardiovascular complications associated to it. Memije-Lazaro et al. (2018) reported that the consumption of *Arthrospira maxima* (Spirulina) by rats (1 g/kg/day) decreased the causes of hypertension, renal dysfunction, and left ventricular hypertrophy associated to chronic kidney disease, as well as, oxidative stress in heart and kidney.

The health effect of the microalgae biomass consumption may be associated to the type of microalgae used. Nuño et al. (2013) evaluated the effects of the microalgae *Isochrysis galbana* and *Nannochloropsis oculata* (50 mg/day, 8 weeks) on glucose, body weight, lipids, lipoproteins, nitrogen compounds and intestine histopathology in a diabetic rat model. The consumption of *Isochrysis galbana* resulted in decreased glucose, triacylglycerol and cholesterol levels, and higher lactic acid bacteria counts. On the other hand, the consumption of *Nannochloropsis oculate* resulted in absence of alterations in the clinical values, lower lactic acid bacteria count, and some intestinal damage.

Clinical studies on the health effects of microalgae biomass consumptions are needed to prove the health effects observed in animals and to stablish the recommended dosage, and the form of consumption of the food products (Buono et al. 2014).

Technological aspects of microalgae biomass and its derivatives

Figure 3 presents the main technological effects associated to the incorporation of microalgae biomass and extracts into food products. In recent years, research has described a variety of innovative products formulated through enriched food matrices with microalgae biomass from different microalgae species or their bioactive compounds. Table 3 presents the main studies assessing the incorporation of microalgae biomass and extracts in new food products.

The addition of microalgae biomass can improve the nutritional value of the products, being observed increases in the vitamin B_{12} and fucose contents in smoothies (Castillejo et al. 2017), higher concentrations of protein, minerals, and β-carotene in ice cream, soft cheese, and extrudates (Agustini et al. 2016; Joshi, Bera, and Panesar 2014), higher protein content and improved balance of the

Table 2. Studies assessing the in vivo (murine model) physiology effects from microalgae biomass and bioactive compounds.

Microalgae specie	Bioactive compound	Administered amount	Objectives	Major outcomes	References
Scenedesmus obliquous	Biomass	20g/day	Hypolipidemic and hypoglycemic	Decrease in TG, atherogenic index and serum glucose; No histological changes were identified of the liver, spleen, and kidney tissues.	Silva et al. (2020).
	Biomass	400 or 800 mg/kg/day	Modulation of serum lipid profile and antioxidant status	Increase in HDL-c, antioxidant enzymes and antioxidant potential of all tissues as well as reduction of lipid peroxidation.	Nascimento et al. (2019).
Isochrysis galbana and *Nannochloropsis oculata*	Biomass	5 μg, 5 mg, or 50 mg	Hypoglycemic	Body weight loss, reduced glucose and cholesterol levels and increased the LAB count. However, the consumption of the microalgae N. oculata produced negative effects on the entire gastrointestinal tract	Nuño et al. (2013).
S. platensis	Biomass and extract	Equivalent to the amount as defined by "The Nutritional requirements of rats"	Gastroprotective	Biomass and extract inhibited ulcers induced by swim stress formation; Promoted the inhibition of upregulated H+, K+, ATPase activity increased in mucin level and changed the antioxidant enzyme levels; Rats pretreated with Spirulina extract and biomass showed normal histology or only very superficial lesions.	Anantharajappa, Dharmesh and Ravi (2019).
	Extract	500 mg/kg /day	Cardioprotective	Reduction biomarkers in the inflammatory, apoptotic protein markers, boosted the enzymatic/non-enzymatic antioxidants and alleviated the induced-lipid peroxidation damage caused by nicotine. Moreover, it attenuated the nicotine-induced histopathological alterations of the kidney	Zahran and Emam, (2018).
	Biomass	400 mg/kg	Modulating the neurobehavioral and cognitive dysfunction	Accelerated acquisition of neurological reflexes, better physical appearance, enhanced neuromuscular strength, improved spatial learning and memory and partly normalized protein malnutrition in protein malnourished offspring rats.	Sinha, Patro and Patro (2020).
S. maxima	Biomass and C-phycocyanin	1 g/kg/day	Cardiovascular and renal protection	Partially prevented alterations in urinary protein, creatinine clearance and seric uric acid concentration. The C-phycocyanin offers a better cardiorenal axis protection.	Memije-Lazaro et al. (2018).
	Phycobiliproteins	100 mg/kg/day	Antihypertensive, antiglycemic and endothelial protective	Decreased systolic blood pressure and reestablished glucose, weight gain and vascular contractility as well as enzyme activities of rats considered pre-eclamptic.	Castro-García et al. (2018).
Spirulina sp	Biomass	100, 200 and 500 mg/kg	Anti-steatotic	Reduction body weight; modulation glucose/lipid profile, insulin sensitivity; anti-steatotic properties (lower of serum activities of transaminases; AST and ALT, level of total bilirubin and decreased inflammatory hepatocytes rats) activities associated with hepatic microRNA expression.	Oriquat (2018).
Spirulina fusiformis	Biomass	400 mg/kg	Hepato-renal and gastroprotective	Protection to oxidative stress-induced damages to the liver and kidney and, reduction in AST, ALT and ALP activities; levels of total and direct bilirubin. Promoted the restoration normal antioxidant	Peter S et al. (2017).

(*continued*)

Table 2. Continued.

Microalgae specie	Bioactive compound	Administered amount	Objectives	Major outcomes	References
				status as well as pathological alterations and improvement liver function by reducing the extent of hepatic cellular damage or injury.	
Chlamydomonas reinhardtii	Biomass	1 or 3 g	Gastrointestinal health	Murine weight loss; Human reduction of intestinal discomfort, diarrhea, gas and bloating; increase in bowel regularity and quality of stools improvement without modification of the intestinal microbiota.	Fields et al. (2020).
Arthrospira platensis	Biomass	12 g/kg/day	Hypolipidemic	Increase in HDL-c and reduction in plasmatic TG due to increased excretion of fecal lipids and expression of the PPAR-α gene.	Bigagli et al. (2017).
	Lipid extract	4 g/100 g diet	Anti-obesity	Body weight and total adipose tissue loss; Reduction of liver lipid and suppression of hepatic steatosis.	Yang et al. (2020).
C. pyrenoidosa and S. platensis	Lipid extract	55 g/ 100 g diet	Hypoglycemic	Control of hypoglycemic function and regulation of the gut microbiota.	Wan et al. (2019).

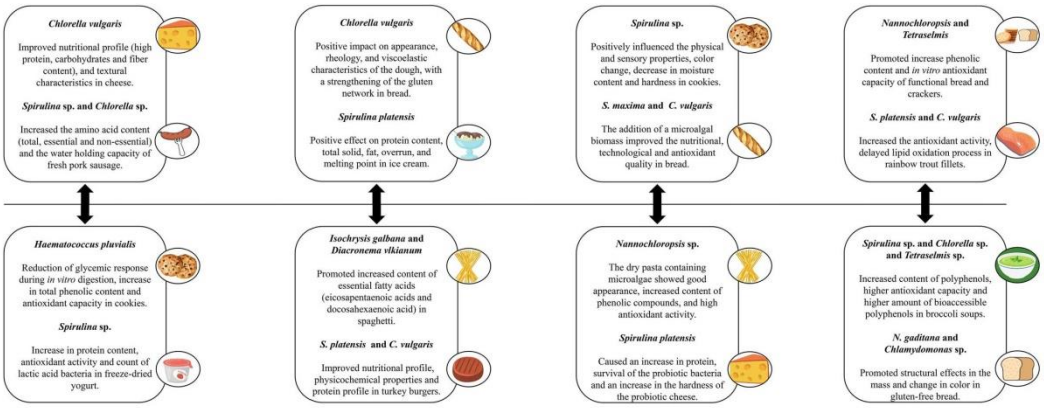


Figure 3. Main technological effects after incorporation of microalgae biomass and derived products (extracts or isolated compounds) in food products.

amino acids in rice soy crisps (Bashir et al. 2017), higher total amino acid content and the ratio of essential/non-essential amino acids in fresh pork sausages and turkey burgers (Marti-Quijal, Zamuz, Tomašević, Gómez, et al. 2019; Marti-Quijal, Zamuz, Tomašević, Rocchetti, et al. 2019), improved total phenolic content and antioxidant capacity in cookies, crostini, and shortbread biscuits (Batista et al. 2017; Marcinkowska-Lesiak et al. 2018; Niccolai et al. 2019), increased carotenoids and chlorophyll in olive oils (Alavi and Golmakani 2017), and increased protein, fat, and minerals in fresh spaghetti and snacks (Fradique et al. 2010; Lucas et al. 2018).

The addition of microalgae biomass can also improve the technological properties of food products, being the effect dependent on the food matrix. For yogurts, Barkallah et al. (2017) observed that the addition of 0.25% of *Spirulina* biomass accelerated the fermentation process and improved the syneresis and antioxidant activity. In cheeses, the addition of *Spirulina platensis* (0.3%, 0.5%, and 0.8%) increased the hardness and the probiotic viability (*Lactobacillus acidophilus*) (Mazinani, Fadaei, and Khosravi-Darani 2016), and the addition of *Chlorella vulgaris* (1%, 2%, and 3%) increased the firmness, and decreased the oil separation and meltability. In rice soy crisps, improved physical properties and mechanical characteristics were obtained by adding 6% of *Spirulina* biomass (Bashir et al. 2017). In fresh spaghetti, the addition of *Chlorella vulgaris* and *Spirulina maxima* biomass (0.5%, 1.0%, and 2.0%) resulted in attractive color (orange and green), and higher firmness, which is interesting for this type of product (Fradique et al. 2010). In pasta, higher swelling index and firmness, and lower cooking loss were observed after addition of *Arthrospira platensis* (1%, 2% or 3%) (Zouari et al. 2011). In wheat breads, the addition of *Isochrysis galbana, Tetraselmis suecica, Scenedesmus almeriensis,* and *Nannochloropsis gaditana* biomass (1.5%) promoted a yellow color (higher b* values and lower a* values)

Table 3. Studies assessing the microalgae biomass and extracts incorporation in new food products.

Microalgae species	Food products	Incorporated amounts	Effect of incorporation	References
Spirulina platensis	Supplement and shake	750 mg/100 g	Carbohydrate content reduction, increased lipid content and sensorially accepted.	Freitas et al. (2019).
	Donuts	2.59 to 5.41% w/w	Improvement in nutritional quality (content of the protein, mineral, fiber and lipid composition) and sensorially accepted.	Rabelo et al. (2013).
	Probiotic cheese	0.3, 0.5 and 0.8% w/v	Increased protein and iron content during refrigerated storage; color change and increasing hardness of samples; stimulation the growth and survival of probiotic bacteria (*L. acidophilus*).	Mazinani, Fadaei and Khosravi-Darani (2016).
	Soft cheese	0.5 %, 1 %, 1.5 % w/w	Positive effect at protein content, water, fat, β-carotene, and texture.	Agustini et al. (2016).
	Ice cream	0.6% and 1.2% w/w	Positive effect at protein content, total solid, fat, total sugar, overrun, melting point and attributes sensory.	Agustini et al. (2016).
Spirulina platensis	Energy gel	0.35% and 0.50% w/w	Sensorially accepted.	Moreira et al. (2018).
	Pasta	1, 2 and 3 g/100 g	Increased the protein content and antioxidant activity. Furthermore, improved the color parameters, cooking properties, firmness, and sensory characteristics.	Zouari et al. (2011).
	Croissants	0.5, 1.0 and 1.5% w/w	Increased the protein content, moisture content, water-holding capacity and improvement the textural and sensory properties. Furthermore, inhibited the growth of both yeasts and molds in croissants throughout the entire storage period.	Massoud et al. (2016).
	Gluten-free pasta	1–15% w/w	Positively affected the nutritional composition, phenolic content, chlorophylls and carotenoids, resulting increase in the antioxidant capacity.	Fradinho et al. (2020).
	Sourdough crostini	2, 6 and 10% w/w	Color change, increase in protein content, ash, phycocyanin, phenolic content and antioxidant capacity.	Niccolai et al. (2019)
Spirulina sp.	Corn extrudates	2, 4, 6 and 8% w/w	Increase of protein, fat, fiber, and ash content of carotenoids and chlorophylls. Sensorially accepted.	Tańska et al. (2017).
Spirulina sp.	Crisps	2, 4, 6 and 8% w/w	Increase of content protein, crude fat, ash, dietary fiber contents and moisture. The samples were sensorially accepted.	Bashir et al. (2017).
	Maize extrudates	5, 7.5, 10, 12.5 and 15%.w/w	Increase content of protein, carotenoids and zinc.	Joshi et al. (2012).
	Yogurts	0.25, 0.5, 0.75 and 1% w/v	Improvement of nutritional attributes, technological and antioxidant activity.	Barkallah et al. (2017).
	Cookies	1, 2 and 3% w/w	Positively influenced the physical and sensory properties of cookies. Promoted color change, decrease in moisture content and hardness.	Onacik-Gür, Zbikowska and Majewska (2018).
	Edible film	0, 2.5, 5, 10, 15 and 20% w/v	Improvement in mechanical and barrier properties, increase the antioxidant activity and properties and antibacterial.	Balti et al. (2017).
	Snacks	2.6% w/w	The addition of *Spirulina* sp resulted in a protein increase, of carotenoids and minerals, without significantly affecting physical properties. High nutritional content and sensory acceptance.	Lucas et al. (2018).
	Olive oil	0.5, 1.0, and 1.5% w/w	Improvement stability of olive oil by reducing the production of lipid oxidation products	Alavi and Golmakani (2017).
	Olive oil	0.25, 0.5, and 1% w/v	*Spirulina* nanoparticles were effective in delaying lipid oxidation, positively impacting color and prolonging oil life	Morsy et al. (2019).

(*continued*)

Table 3. Continued.

Microalgae species	Food products	Incorporated amounts	Effect of incorporation	References
	Yogurts	1.6 g/100 g	Increase in protein content, antioxidant activity and count of lactic bacteria. Sensory acceptance.	Yamaguchi et al. (2019).
	Shakes	750 mg/100 g	Increased protein content. The incorporation of microalgae biomass did not influence the acceptance of the product by the target consumer.	Santos et al. (2016).
	Baby food formulas	2.5, 5 and 7.5% w/w	The chemical composition indicated that these formulas were suitable as a food supplement for children aged 1-3 years (good proportions of essential amino acids), presented low cost, can produced on the scale of domestic and industrial scale, and were sensorially accepted.	Sharoba, (2014).
	Meat analogues	15, 30 and 50% w/w	Positively affected the physical parameters (texture, cooking yield and humidity), increase of total phenolic concentration, total flavonoid concentration and antioxidant activity.	Palanisamy et al. (2019).
	Shortbread biscuits	1, 3, and 6% w/w	Color change, hardness increased, reduction water activity, increase of phenolic compounds and antioxidant activity, inhibition the production of MDA.	Marcinkowska-Lesiak et al. (2017).
Spirulina sp. and *Chlorella sp.*	Meat products	1 and 5% w/w	The enrichment of meat products with Chlorella and Spirulina proteins provided products with high amino acid concentration that may be related to bitter taste, generating low acceptability. However, Chlorella and Spirulina protein pork liver pate had good acceptance.	Zamuz et al. (2019).
	Smoothies	2.2% w/v	*Chlorella* and *spirulina* smoothies showed the highest vitamin B12 content during a shelf – life of 17 days at 5°C, established based on sensory and microbiological quality. Promoted color changes, nevertheless, such color changes did not negatively affect to the consumer acceptance.	Castillejo et al. (2017).
	Beef patties	1% w/w	Beef patties elaborated with biomass microalgae showed the highest values for the total amino acids content (aspartic, glutamic acids and lysine were the predominant amino acids found).	Žugčić et al. (2018).
	Sausage	1% w/w	The inclusion of biomass microalgae promoted increased the amino acid content total, essential and non-essential and of the water holding capacity of fresh pork sausage. The color parameters changed.	Marti-Quijal, Zamuz, Tomašević, Gómez, et al. (2019).
	Sausages fermented "chorizo"	3% w/w	Color change; increased protein content; few changes in hardness, adhesiveness and gumminess. The profile amino acid of was similar to that obtained for the control samples	Thirumdas et al. (2018).
S. platensis and *Chlorella vulgaris*	*Oncorhynchus mykiss* fillets	0.05 and 0.1% v/w	The concentration of extract for both microalgae led to an increase at antioxidant activity, delayed lipid oxidation process, maintenance or increase the sensory attributes and increase the shelf life of fish refrigerated.	Takyar et al. (2019).
	Burgers	1% w/w	Improvement nutritional profile, physicochemical properties and protein profile and amount of total amino acids.	Marti-Quijal, Zamuz, Tomašević, Rocchetti, et al. (2019)

(*continued*)

Table 3. Continued.

Microalgae species	Food products	Incorporated amounts	Effect of incorporation	References
S. maxima and *Chlorella vulgaris*	Pasta	0.5, 1.0 and 2.0% w/w	Dough color stability after baking, increased firmness and sensory acceptance by tasters.	Fradique et al. (2010).
Chlorella vulgaris	Bread	1% w/w	Improved nutritional, technological and antioxidant properties.	Nunes et al. (2020).
Chlorella vulgaris	Bread	1.0, 2.0, 3.0, 4.0 and 5.0 g/ 100 g	Positive impact on appearance, rheology and viscoelastic characteristics the dough, with strengthening of the gluten network. No impact on the kinetics of yeast fermentation, neither on the time required for fermentation was induced by the biomass addition. The firmness and aging kinetics were similar to reference wheat bread.	Graça et al. (2018).
	Cheese	1, 2 and 3% w/w	; Improvement nutritional profile (high protein, carbohydrates and fiber content), the textural characteristics (namely firmness). The addition of 2% *C.vulgaris* did not have any effect on the overall acceptability sensory.	Mohamed et al. (2013).
	Mayonnaise	10, 15 and 20% w/w	Improvement of properties nutritional, rheological (dynamic viscosity and emulsion stability) and effect positive on the sensory characteristics (appearance, color, aroma, taste and overall acceptability).	Abd El-Razik and Mohamed., (2013).
Nannochloropsis oculata, Diacronema vlkianum and *Porphyridium cruentumd*	Ice cream	0.10, 0.20 and 0.30g/ 100 g	Changes of color, increase the total phenolic content, decrease in apparent viscosity and physical stability. The samples were accepted sensorially, however with some suggestions to improve the taste and odor (deodorization process and the use of aromatic components).	Durmaz et al. (2020).
Nannochloropsis and *Tetraselmis*	Breads and crackers	1 to 3% w/w for bread and 1.25 to 3.75% w/w for crackers	Increased phenolic content and in vitro antioxidant capacity and bioaccessible polyphenols in both matrices. Sensory evaluation showed that microalgae containing products were competitive with the controls. Moreover, microalgae-containing products showed an increased emission of some volatile compounds.	Lafarga, Mayre, et al. (2019).
Spirulina sp., Chlorella sp. and *Tetraselmis* sp.	Broccoli soups	0,5, 1,0, 1,5 and 2,0% (w/v)	Increased content of polyphenols and to a higher antioxidant capacity. Microalgae-containing soups showed a higher amount of bioaccessible polyphenols. The acceptability index of soups formulated using lower microalgae concentrations was over 70% suggesting that the soups would be well accepted.	Lafarga, Acién-Fernández, et al. (2019).
Arthrospira platensis, C. vulgaris, Tetraselmis suecica and *Phaeodactylum tricornutum*	Cookies	2 and 6% w/w	It is possible to improve the antioxidant properties of biodegradable films from cassava starch by adding microalgae biomass or extract.	Batista et al. (2017).
Heterochlorella luteoviridis and *Dunaliella tertiolecta*	Biodegradable starch film	0.5, 1.0, and 2.0% w/w	Improved the antioxidant properties. The extract *H. luteoviridis* resented the lowest water vapor permeability and good mechanical characteristics, was applied in salmon packaging. Of the films for salmon packaging was efficient to decrease lipid oxidation rate but did not avoid moisture loss of the fish	Carissimi, Flôres and Rech (2018).
Tetraselmis suecica, Scenedesmus almeriensis and *Nannochloropsis gaditana*	Bread wheat	1.5% w/w	There were no significant changes in the properties physicochemical or microbiological counts of the fermented mass. There were color changes that were related pigment content in microalgae.	Garcıa-Segovia et al. (2017).

(*continued*)

Table 3. Continued.

Microalgae species	Food products	Incorporated amounts	Effect of incorporation	References
Nannochloropsis gaditana	Hen eggs	5.0% and 10.0% w/w	Accumulated eicosapentaenoic acid (EPA) in the egg was converted to docosahexaenoic acid (DHA) and deposited in the yolk. Change in color egg yolk.	Bruneel et al. (2013).
Isochrysis sp., *Nannochloropsis* sp. *Phaeodactylum* sp. and *Schizochytrium* sp.	Tomato puree	80 mg n-3 LC-PUFA/ 100 g	Higher oxidative stability attributed to the presence of endogenous carotenoids in microalgae biomass.	Gheysen et al. (2019).
Isochrysis galbana and *Diacronema vlkianum*	Spaghetti	0.5, 1.0 and 2.0 g/100 g	Increased content of essential fatty acids (eicosapentaenoic acids and docosahexaenoic acid) and sensorially accepted	Fradique et al. (2013).
Nannochloropsis sp.	Dry pasta	10, 20, 30 and 40 g/100 g	Good appearance, pleasant color, light fish flavor, phenolic compounds attributed to high antioxidant activity. Sensory acceptance.	De Marco et al. (2017).
Nannochloropsis oculata and *Isochrysis galbana*	Chewing gum	0.5 and 1g/100g	Biomass of *N. oculata* and *I. galbana* species acted as coloring agent natural in chewing gum matrix. The spray dryer inlet temperature and microalgae usage level did not cause significant difference in hardness, springiness, gumminess, and chewiness properties in the gum samples. Sensory acceptance.	Palabiyik et al. (2018).
Auxenochlorella protothecoides	Meat analogues	5, 10, 20, 30, 40 and 50% w/w	Effects on the fibrous texture of the extrudate, attractive coloring and increase in fat content and levels of vitamins B and E.	Caporgno et al. (2020).
Haematococcus pluvialis	Cookies	5, 10 and 15% w/w	Color change, reduction of glycemic response during in vitro digestion, increase in total phenolic content and antioxidant capacity of foods.	Hossain et al. (2017).
Nannochloropsis gaditana and *Chlamydomonas sp.*	Gluten-free bread	1.0 and 3.0 g/100 g	Increase in the content of proteins, lipids (improves in the proportion of essential fatty acids), ash, iron and calcium contents. Promoted structural effects in the mass (firmness and adhesiveness) and change in color. The breads were accepted sensorially.	Khemiri et al., (2020).

(García-Segovia et al. 2017). In croissants, the addition of Spirulina biomass (0.5–1.5%) resulted in croissants with higher water holding capacity, and lower crumb firmness, being recommended the concentration of 1% due to the higher sensory scores (Massoud et al. 2017). In ice cream and soft cheese, the addition of *Spirulina platensis* biomass (1.2% and 1%, respectively) resulted in products with lower water content (associated to higher shelf life), and enhanced quality properties (higher overrun values and melting point in ice creams) (Agustini et al. 2016). Finally, in meats and meat analogues, softer textures (lower values of hardness, adhesiveness, elasticity and/or chewiness) were observed after addition of *Chlorella* or *Spirulina* (3%) to fermented Spanish "chorizo" sausage (Thirumdas et al. 2018), *S. platensis* and *Chlorella vulgaris* (1%) to turkey burgers (Marti-Quijal, Zamuz, Tomašević, Rocchetti, et al. 2019), and *Chlorella* or *Spirulina* (1%) to sausages (Marti-Quijal, Zamuz, Tomašević, Gómez, et al. 2019).

The impact of microalgae addition on the sensory acceptance of food products is dependent on the food matrix. Improvements in the sensory acceptance after microalgae biomass were reported for rice soy crisps (Bashir et al. 2017) and pasta (Zouari et al. 2011). No impact compared to the products without biomass was reported for spaghetti (Fradique et al. 2010), cassava donuts (Rabelo et al. 2013), rice-soy crisps (Bashir et al. 2017), chocolate shake powder (Santos et al. 2016), shake (Freitas et al. 2019), freeze-dried yogurts (Yamaguchi et al. 2019), cheese analogue (Mohamed, Abo-El-Khair and Shalaby, 2013), and yogurts (Barkallah et al. 2017). However, the addition of microalgae biomass reduced the acceptance of ice creams (Agustini et al. 2016), croissants (Massoud et al. 2017), high-calorie supplement (Freitas et al. 2019), cookies (Onacik-Gür, Zbikowska, and Majewska 2018, Tanska, Konopka and Ruszkowska, 2017), and meat products (Zamuz et al. 2019). The negative effect was associated, in some cases, to the fish odor, dark green color, and altered texture and taste (Agustini et al. 2016). In these cases, alternatives may be used, such as using fruits to mask the microalgae impact. Adiba et al. (2011) obtained food tablets with improved flavor characteristics by mixing the powders from date fruit and spirulina. The authors mentioned that the food tablets could be consumed by all types of consumers, and by people that have difficulties to chew or swallow food products.

The addition of microalgae biomass can improve the aroma profile of the food products. Lafarga, Mayre, et al.

(2019) observed that the addition of *Tetraselmis* and *Nannochloropsis* biomass (2.5% in baked crackers and 1–2% in breads) resulted in an increase in the number of volatile compounds, with the presence of p-cymene (associated to the citrus, fresh, woody, and terpenic aromas) and (Z)-2-heptenal (oily, fatty, and fruit aromas).

The concentration of the microalgae biomass has a significant effect on the quality parameters of food products. The addition of higher than 10% of *Arthrospira platensis* (F&M-C256 and Ox Nature) biomass increased the adhesiveness of gluten-free pasta and provided unsuitable manufacturing abilities, precluding the lamination of the product, while the addition of 4–5% of biomass promoted a very intense fish aroma and increased the cooking loss. On the other hand, the addition of 1–3% increased the nutritional profile (chlorophylls, phenolic compounds, and carotenoids), with no impact on the texture properties. The product added with 2% of *Arthrospira platensis* biomass was preferred by consumers (Fradinho et al. 2020). At the same time, the addition of up to 3% of *Chlorella vulgaris* biomass to a wheat flour dough resulted in improved viscoelastic and rheological properties and higher strength in the gluten network. Higher concentrations negatively impacted the characteristics of the products, resulting in breads with altered texture and flavor, and with accelerated aging process (Graça et al. 2018). Finally, the addition of 0.5–1% of *Spirulina* sp., *Tetraselmis* sp. or *Chlorella* sp. to broccoli soup resulted in increased nutritional profile (polyphenols and antioxidant activity) and suitable sensory acceptance. However, when higher concentrations were used the flavor and overall acceptance decreased, especially in the products supplemented with *Chlorella* sp (Lafarga, Acién-Fernández, et al. 2019). The results indicate that it is of paramount importance to study the concentration of microalgae biomass to be added to the food products in order to not compromise the technological and sensory properties.

The type of microalgae can impact on the quality parameters of food products. Batista et al. (2017) evaluated the effect of the addition of 2% or 5% microalgae biomass (*Arthrospira platensis* F & M-C256, *Chlorella vulgaris* Allma, *Tetraselmis suecica* F & M-M33 and *Phaeodactylum tricornutum* F & M-M40) on the quality parameters of cookies. Cookies with *Arthrospira platensis* would be the most recommended, as they had increased protein and total phenolic contents, more structured texture, and were preferred by consumers. Durmaz et al. (2020) evaluated the effect of the addition of 0.1%, 0.2% or 0.3% microalgae biomass (*Nannochloropsis oculata*, *Porphyridium cruentum* and *Diacronema vlkianum*) on the technological parameters of ice cream. Ice creams with *Porphyridium cruentum* would be the most recommended, as they had higher consistency index, more suitable color (pink color), and were preferred by consumers. Gheysen et al. (2019) evaluated the effect of the addition of photoautotrophic (*Nannochloropsis, Isochrysis,* and *Phaeodactylum*) or heterotrophic *(Schizochytrium)* microalgae biomass (0.24–6.60% depending on the microalgae) on the oxidative stability of omega-3 PUFA supplemented tomato puree. The utilization of photoautotrophic microalgae biomass would be advisable, resulting in tomato puree with high omega-3 PUFA concentration and without impact on the oxidative stability.

The form of addition of the microalgae can have impact on the characteristics of food products. Carissimi, Flôres, and Rech (2018) evaluated the application of biomass or biomass extract from *Heterochlorella luteoviridis* and *Dunaliella tertiolecta* microalgae in biodegradable films and posterior application in salmon. The addition of microalgae biomass resulted in films with high solubility, precluding its application in moist foods, such as salmon. The utilization of 2% of *Heterochlorella luteoviridis* extract was suggested, as films with low vapor permeability and suitable mechanical properties were obtained, which resulted in salmons with decreased moisture content and reduced lipid oxidation. The addition of Spirulina nanoparticles (0.5%) as antioxidant in olive oil was more efficient than the whole cell (0.25%, 0.5%, or 1%), resulting in lower primary and secondary oxidation products (thiobarbituric acid and peroxide contents, among others), improved green color, and higher sensory attributes (Morsy et al. 2019). The addition of disrupted cells (high-pressure homogenizer) of *Chlorella vulgaris* biomass resulted in breads with higher firmness and increased antioxidant activity compared to the product with raw biomass (Nunes et al. 2020). Finally, the addition of encapsulated *Spirulina platensis* (spray-dried in maltodextrin or maltodextrin + citric acid) to yogurts resulted in more homogeneous appearance. The utilization of maltodextrin + citric acid as encapsulating agent was advisable, as products with higher nutritional value, antioxidant activity and attractive color were obtained (Silva et al. 2019). The results suggest that in some cases it is advisable to use the microalgae in alternative forms, such as extracts, disrupted cells, encapsulated, or nanoparticles.

The parameters of the process used to obtain the microalgae biomass has impact on the quality properties of the supplemented products. Palabiyik et al. (2018) observed that the inlet temperature of the spray drying technique used to dry the microalgae biomass impacted on the color stability and the sensory acceptance of chewing gums added with *Isochrysis galbana* and *Nannochloropsis oculata* biomass (0.5% and 1%). The products processed at 150 °C were the least accepted, while temperatures of 170–180 °C provided higher concentrations of pigment. Therefore, microalgae biomass could be used as a natural colorant in chewing guns, but the parameters of spray drying process should be optimized.

Microalgae biomass can be used to substitute traditional ingredients in food products. Acid casein curd associated to microalgae biomass (*Chlorella vulgaris,* 10%, 15%, and 20%) was used to replace 25% and 50% of total egg in the processing of mayonnaise, resulting in products with good consistency, low cholesterol levels, high stability, and high acceptability (Abd El-Razik and Mohamed 2013). Microalgae biomass (*Nannochloropsis* sp) could be used to replace up to 30% of the wheat flour in dry pasta without compromising the technological properties and with the

Table 4. Studies assessing the technological properties from proteinaceous, lipidic and glicidic components of the microalgae.

Sources	Assay	Technological properties evaluated	Microalgae evaluated	References
Proteinaceus		Gelling capacity	*Spirulina maxima* and *Haematococcus pluvialis*	Batista et al. (2012)
			Chlorella sorokiniana	Grossmann et al. (2019).
		Foaming properties	*Arthrospira platensis*	Buchmann et al. (2019).
		Emulsifying capacity	*Chlorella vulgaris*	Ursu et al. (2014).
			Tetraselmis sp.	Schwenzfeier et al. (2013).
			Haematococcus pluvialis	Ba et al. (2016).
	Hydrolysates	Solubility	*Chlorella protothecoides*	Grossmann et al. (2018);
		Emulsifying properties	*Chlorella protothecoides*	Dai et al. (2020).
		Solubility; oil absorption, foaming capacity and emulsifying activity; Foam stability	Chlorella pyrenoidosa, *Arthrospira* platensis and Nannochloropsis oceanica	Chen et al. (2019).
		Foaming and stability capacity	*Spirulina platensis*	Pereira et al. (2018).
	Isolate	Emulsifying ability	*N. gaditana, T. impellucida and A. maxima*	Teuling et al. (2019).
		Water and oil absorption capacity/;es; Foaming, foam stability and viscosity; Film forming properties.	*Spirulina platensis*	Benelhad et al. (2016).
	Concentrate	Water absorption capacity and emulsifying and foaming properties	*Spirulina platensis*	Menegotto et al. (2019).
Glicidic		Viscosity	*Dixoniella grisea* and *Porphyridium aerugineum*	Liberman et al. (2020).
Lipidic		Emulsifying properties and biosurfactants	*Nannochloropsis salina*	Law et al. (2018).
		Emulsions	*Chlorella protothecoides*	Caporgno et al. (2019).

advantage of increasing the EPA concentration (De Marco et al. 2018).

Microalgae biomass or extract can be used as antioxidant in food products and edible packages. Alavi and Golmakani (2017) evaluated the utilization of *Spirulina* biomass (0.5%, 1%, and 1.5%) and/or citric acid to improve the oxidative stability of olive oil. The authors observed that the addition of *Spirulina* enhanced the oxidative stability of the olive oil and improved the shelf life of the products. Balti et al. (2017) observed that the addition of Spirulina extract (0%, 2.5%, 5%, 10%, 15%, and 20%) to crab chitosan films improved the mechanical and barrier properties and increased the antioxidant and antibacterial activities. The results were associated to the presence of polyphenols in the extracts.

Components extracted from the microalgae biomass or produced during its growth can also have technological properties. Table 4 presents the technological properties of proteinaceous, lipidic and glicidic components of the microalgae. EPS produced by microalgae have technological applications as gelling agents, stabilizing, emulsifying, or thickening in new food products (Paniagua-Michel, Olmos-Soto, and Morales-Guerrero 2014; Raposo, Morais, and Morais 2015; Bernaerts et al. 2019). Protein isolate from microalgae have relevant technological properties such as emulsifying (Ursu et al. 2014), foaming (Benelhadj et al. 2016) and gelling (Batista et al. 2012) properties and could be used as ingredients in the formulation of new food products or for improvement of the existing ones (Gifuni et al. 2019). He et al. (2019) evaluated the microalgae triglycerides obtained from twelve microalgae species aiming the application in infant formulas. Among the tested species, the *Isochrysis* species (*Isochrysis* sp. and *I. galbana*) presented fatty acid composition, fatty acid positional distribution of triacylglycerol, and melting and crystallization properties suitable for application in infant formula as human milk fat substitutes. The use of whole microalgae *Chlorella protothecoides* promoted improvement in the quality of the emulsion (oil/water) through the presence of lipids and carotenoids (Caporgno et al. 2019)

Despite the significant number of studies using microalgae biomass as a source of bioactive compounds, it is also necessary to evaluate the effect of food processing techniques on the bioactive content, stability and microalgae effectiveness in order to increase the incorporation and type of products (Raja et al. 2018).

Regulatory aspects for commercialization and consumption

The use of microalgae biomass may raise some concerns about toxicity, mainly associated to the presence of toxins, heavy metals, or pathogenic microorganisms arising on culturing and manufacturing conditions (Buono et al. 2014; Rzymski et al. 2015). When selecting a microalgae for utilization in food products, it is important to observe the possible production of allergens, toxins, or any other harmful compound (Enzing et al. 2014). Therefore, prior to registration, the microalgae biomass or microalgae-based products must be assessed regarding safety aspects to ensure that marketed products comply with legal and regulatory requirements (Rzymski et al. 2015).

Concerning the food safety, the utilization and development of products incorporated with microalgae have been regulated by different international entities. Despite the great existing microalgae diversity, only the species *Chlorella vulgaris* and *Spirulina/Arthrospira sp, Euglena gracilis, Dunaliella bardawil, Dunaliella salina, Chlamydomonas reinhardtii* are classified as Generally Recognized as Safe (GRAS), according to FDA and the European Food Safety

Authority (EFSA). These species have been widely cultivated for large-scale production and are available in the health food sector in the forms as tablets, capsules, liquids, nutritious supplements or as a natural source for food addition. Besides, astaxanthin extracts of *Haematococcus pluvialis* and oils derived from *Chlorella protothecoides, Crypthecodinium cohnii*, Ulkenia sp. Schizochytrium sp are also considered safe (Batista et al. 2012; Vigani et al. 2015).

The GRAS status is officially applied to the jurisdiction of the United States and different rules may be followed in other countries (Torres-Tiji, Fields, and Mayfield 2020). For instance, the microalgae *Chlorophyta, Tetraselmis* sp, *Dunaliella* sp., *Chlorococcum* sp., *Scenedesmus* sp., *Desmodesmus* sp, *Chlorella* sp., *Chlamydomonas reinhard, Synechococcus* sp., *Chlamydomonas reinhardtii, Haematococcus pluvialis* and *Parietochloris incisa* are classified as safe based on classification 'no toxins known' established by European Comission (Enzing et al. 2014).

In the European Union, the main regulations about the production and marketing of microalgae food products are the Food Safety Regulation (EC 178/2002) and the Novel Food Regulation (EC 258/97), re-designated in 2015 by a new regulation (EC 2015/2283) effectively applied in 2018. This new regulation seeks to improve efficiency during the process of authorization of new foods, as well as to promote greater agility to present safe foods suitable for market insertion. More specifically, the marketing of whole microalgae (biomass) or food products formulated with microalgae (biomass, extracts or isolated compounds) is subjected to the Food Safety Regulation that covers all food products (EC 178/2002). In turn, the regulation of novel foods is applied to foods and ingredients that were not on the European market before 15 May 1997. EPA and DHA oils as well as colorants obtained from microalgae are subjected to this Regulation. Overall, United States and European Union regulations approach different requirements for the authorization of microalgae products. In the United States the main focus is related to product safety assessment, while the European regulation prioritizes the technology used to obtain the final product (Enzing et al. 2014).

In Brazil, the Brazilian Health Regulatory Agency (ANVISA) also consider *Spirulina/Arthrospira* sp safe. These genera are inserted in the list of "new ingredients" approved in 2018 with a recommended daily dose should not exceed 1.6 g. The specifications of the species, system of cultivation and content of inorganic contaminants must be presented in the label. In addition, oils obtained from microalgae *Crypthecodinium cohnii*, Ulkenia sp and *Schizochytrium* sp can be used in food supplements source DHA and EPA except for administration to infants and children in early childhood (Brasil 2018a). Resolution N° 1.965 of 2018 authorizes the sale of *Chlorella* sp. in powder, capsules and tablets (Brasil 2018b).

The Food Standards Australia New Zealand (FSANZ) regulates the safety of novel foods in the country. More specifically, the Food Standards Code includes the use of *Schizochytrium* sp. microalgae dried or DHA-rich oil derived from *Schizochytrium* sp. in food products, as alternative source of novel food ingredients such as omega-3 fatty acids (ANZFA, 2002). The Health Canada Agency establishes the safety use of lyophilized *Tetraselmis chui* as novel foods and emphasizes that the manufacturing, distribution, and marketing steps must be carried out in accordance with official regulations. Similarly, it was established that whole flour (biomass) of *Chlorella protothecoides* is safe for human consumption based on the lack of microbiological, toxicological, or allergenic risk and can be used as an alternative protein source. In addition, Health Canada Agency also concluded that the oil obtained from the genetically modified microalgae *Prototheca moriformis* S8695B (high oleic acid content) poses no risk to human health and may be an equivalent alternative to commercially available conventional oils.

Overall, factors such as time and costs to perform safety tests have limited the designation of the safe status to microalgae. Considering the increasing number of studies showing the feasibility of microalgae cultivation to obtain biomass or derivatives with high-added-value it is expected that this scenario change in a soon future.

Market challenges and future perspectives

The microalgae biomass producing market for food purposes is basically dominated by four specific genera, which include: *Arthrospira* sp with annual production estimated at over 12.000 tons, *Chlorella* sp with approximately 5.000 tons per year, *Dunaliella salina*, widely marketed as a source of β-carotene, with annual production of 3.000 tons and *Scenedesmus* (Mu et al. 2019; Levasseur, Perré, and Pozzobon 2020).

Currently, the market for microalgae-based products is led by the United States, Asia and Oceania, however, it is expected that soon Europe will achieve a prominent position in the world ranking. In 2017, the global market for products made with microalgae was around US$32.60 billion and it is currently expected to reach an average of US$53.43 billion in 2026. About 75% of the production of products derived from microalgae is for applications in food, animal feed or nutraceuticals and this production is expected to increase due to the growing demand for healthy food products (Rahman 2020).

Despite the market expansion observed, the cost involved in the production/processing stages of microalgae and derived products has limited their use as food substitutes. The development of advanced technology, automation and artificial intelligence can overcome these obstacles. In general, application, safety evaluation and cost are the main concerns. Improvements for microalgae production have been suggested, such as: strict contamination control with the use of photobioreactors; low operating cost and rational use of energy; optimization of microalgal harvesting and drying and development of sustainable technologies for extraction of bioactive compounds (Wang et al. 2020).

Microalgae biomass and its derivatives (extracts and bioactive compounds) can have multiple functions, such as natural pigments and preservatives, health-related and nutritional compounds to be added to food products, and ingredients with improved technological properties. Several

in vivo studies attest to the functional potential of microalgae biomass and derived bioactive compounds, however, it is worth noting that clinical trials in humans are necessary to attest to the efficacy, bioavailability, safety, side effects and beneficial effects of microalgae based products. Despite the advances over the years, some challenges need to be overcome in order to achieve broad manufacturing and commercialization of microalgae biomass and derived bioactive compounds. The main ones include high cost of operation, infrastructure and maintenance of facilities necessary for cultivation and obtaining biomass, the selection of protein source strains, as well as the harvesting and dehydration stages on a commercial scale. In addition, the financial market, and the reliability of statistical data on microalgae market opportunities are pointed out as limiting factors for the actual evaluation of the potential of these microorganisms.

Therefore, future studies should be conducted in order to evaluate the stability of the species, identify new species aiming higher growth and cell densities, optimize the conditions of the medium to increase the biomass and bioactive compounds production, replace the synthetic media for alternative media with lower costs, among others. Furthermore, some drawbacks need to be overcome, such as increase the market size, reduce the cost of production, and tighter constraints related to regulations, such as specifications related to quality, safety, and reduction of the environmental impact.

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References

Abd El-Razik, M. M., and A. G. Mohamed. 2013. Utilization of acid casein curd enriched with *Chlorella vulgaris* biomass as substitute of egg in mayonnaise production. *World Applied Sciences Journal* 26: 917–25. doi: 10.5829/idosi.wasj.2013.26.07.13523.

Adiba, B. D., B. Salem, S. Nabil, and M. Abdelhakim. 2011. Preliminary characterization of food tablets from date (*Phoenix dactylifera* L.) and Spirulina (*Spirulina* sp.) powders. *Powder Technology* 208 (3):725–30. doi: 10.1016/j.powtec.2011.01.016.

Agustini, T. W., W. F. Ma'ruf, W. Widayat, M. Suzery, H. Hadiyanto, and S. Benjakul. 2016. Application of *Spirulina platensis* on ice cream and soft cheese with respect to their nutritional and sensory perspectives. *Journal Teknologi* 78:245–51. doi: 10.11113/jt.v78.8216.

Alavi, N., and M. T. Golmakani. 2017. Improving oxidative stability of olive oil: Incorporation of Spirulina and evaluation of its synergism with citric acid. *Grasas y Aceites* 68 (1):178. doi: 10.3989/gya.0940162.

Alavijeh, R. S., K. Karimi, R. H. Wijffels, C. Van den Berg, and M. Eppink. 2020. Combined bead milling and enzymatic hydrolysis for efficient fractionation of lipids, proteins, and carbohydrates of *Chlorella vulgaris* microalgae. *Bioresource Technology* 309:123321. doi: 10.1016/j.biortech.2020.123321.

Australian New Zealand Food Authority (ANZFA). 2002. *DHA-rich dried marine micro algae (Schizochytrium sp.) and DHA-rich oil derived from Schizochytrium sp. as novel food ingredients*. Canberra, Austrália: Australian Government Publishing Service.

Amarante, M. C. A., A. R. C. Braga, L. Sala, and J. S. S. Kalil. 2020. Colour stability and antioxidant activity of C-phycocyanin-added ice creams after *in vitro* digestion. *Food Research International (Ottawa, Ont.)* 137:109602. doi: 10.1016/j.foodres.2020.109602.

Anantharajappa, K., S. M. Dharmesh, and S. Ravi. 2020. Gastro-protective potentials of *Spirulina*: Role of vitamin B 12. *Journal of Food Science and Technology* 57 (2):745–53. doi: 10.1007/s13197-019-04107-8.

Ba, F., A. Violeta, C. Laroche, and G. Djelveh. 2016. *Haematococcus pluvialis* soluble proteins: Extraction, characterization, concentration/fractionation and emulsifying properties. *Bioresource Technology* 200:147–52. doi: 10.1016/j.biortech.2015.10.012.

Bafana, A. 2013. Characterization and optimization of production of exopolysaccharide from *Chlamydomonas reinhardtii*. *Carbohydrate Polymers* 95 (2):746–52. doi: 10.1016/j.carbpol.2013.02.016.

Balti, R., B. M. Mansour, N. Sayari, L. Yacoubi, L. Rabaoui, N. Brodu, and A. Massé. 2017. Development and characterization of bioactive edible films from spider crab (*Maja crispata*) chitosan incorporated with *Spirulina* extract. *International Journal of Biological Macromolecules* 105:1464–72. doi: 10.1016/j.ijbiomac.2017.07.046.

Barba, F. J. 2017. Microalgae and seaweeds for food applications: Challenges and perspectives. *Food Research International (Ottawa, Ont.)* 99 (Pt 3):969–70. doi: 10.1016/j.foodres.2016.12.022.

Barkallah, M., M. Dammak, I. Louati, F. Hentati, B. Hadrich, T. Mechichi, M. A. Ayadi, I. Fendri, H. Attia, and S. Abdelka. 2017. Effect of *Spirulina platensis* fortification on physicochemical, textural, antioxidant and sensory properties of yogurt during fermentation and storage. *LWT - Food Science and Technology* 84:323–30. doi: 10.1016/j.lwt.2017.05.071.

Barkia, I., N. Saari, and S. R. Manning. 2019. Microalgae for high-value products towards human health and nutrition. *Marine Drugs* 17 (5): 304. doi: 10.3390/md17050304.

Barreira, L., H. Pereira, K. N. Gangadhar, L. Custódio, and J. Varela. 2015. Medicinal effects of microalgae-derived fatty acids. In S. K. Kim (Ed.), *Handbook of marine microalgae: Biotechnology advances*, 209–31. United States: Academic Press Elsevier Inc.

Bashir, S., M. K. Sharif, M. S. Butt, S. S. H. Rizvi, I. Paraman, and R. Ejaz. 2017. Preparation of micronutrients fortified *Spirulina* supplemented rice-soy crisps processed through novel supercritical fluid extrusion. *Journal of Food Processing and Preservation* 41 (3):e12986. doi: 10.1111/jfpp.12986.

Batista, A. P., M. C. Nunes, P. Fradinho, L. Gouveia, I. Sousa, A. Raymundo, and J. M. Franco. 2012. Novel foods with microalgal ingredients – Effect of gel setting conditions on the linear viscoelasticity of *Spirulina* and *Haematococcus* gels. *Journal of Food Engineering* 110 (2):182–9. doi: 10.1016/j.jfoodeng.2011.05.044.

Batista, A. P., A. Niccolai, P. Fradinho, S. Fragoso, I. Bursic, L. Rodolfi, N. Biondi, M. R. Tredici, I. Sousa, and A. Raymundo. 2017. Microalgae biomass as an alternative ingredient in cookies: Sensory, physical and chemical properties, antioxidant activity and *in vitro* digestibility. *Algal Research* 26:161–71. doi: 10.1016/j.algal.2017.07.017.

Benelhadj, S., A. Gharsallaoui, P. Degraeve, H. Attia, and D. Ghorbel. 2016. Effect of pH on the functional properties of *Arthrospira* (*Spirulina*) *platensis* protein isolate. *Food Chemistry* 194:1056–63. doi: 10.1016/j.foodchem.2015.08.133.

Bernaerts, T. M. M., L. Gheysen, I. Foubert, M. E. Hendrickx, and A. M. Van Loey. 2019. The potential of microalgae and their biopolymers as structuring ingredients in food: A review. *Biotechnology Advances* 37 (8):107419. doi: 10.1016/j.biotechadv.2019.107419.

Bhattacharya, M., and S. Goswami. 2020. Microalgae – A green multi-product biorefinery for future industrial prospects. *Biocatalysis and Agricultural Biotechnology* 25:101580. doi: 10.1016/j.bcab.2020.101580.

Bigagli, E., L. Cinci, A. Niccolai, M. R. Tredici, N. Biondi, L. Rodolfi, M. Lodovici, M. D'Ambrosio, G. Mori, and C. Luceri. 2017. Safety evaluations and lipid-lowering activity of an *Arthrospira platensis*

enriched diet: A 1-month study in rats. *Food Research International (Ottawa, Ont.)* 102:380–6. doi: 10.1016/j.foodres.2017.09.011.

Bleakley, S., and M. Hayes. 2017. Algal proteins: Extraction, application, and challenges concerning production. *Foods* 6 (5):33. doi: 10.3390/foods6050033.

Boiago, M. M., J. D. Dilkin, M. A. Kolm, M. Barreta, C. F. Souza, M. D. Baldissera, I. D. Santos, R. Wagner, F. C. Tavernari, M. L. B. Silva, et al. 2019. *Spirulina platensis* in Japanese quail feeding alters fatty acid profiles and improves egg quality: Benefits to consumers. *Journal of Food Biochemistry* 43 (7):1–9. doi: 10.1111/jfbc.12860.

Brasil, B. S. A. F., F. C. P. Silva, and F. G. Siqueira. 2017. Microalgae biorefineries: The Brazilian scenario in perspective. *New Biotechnology* 39 (Pt A):90–8. doi: 10.1016/j.nbt.2016.04.007.

Brasil. 2018a. Instrução Normativa n° 28 de 26 de julho de 2018. *Estabelece as listas de constituintes, de limites de uso, de alegações e de rotulagem complementar dos suplementos alimentares. Agência Nacional de Vigilância Sanitária*. Brasília: Diário Oficial da União.

Brasil. 2018b. Resolução RE-n° 1.665 de 28 de junho de 2018. *Relatório de conferência de alimentos. Agência Nacional de Vigilância Sanitária*. Brasília: Diário Oficial da União.

Bruneel, C., C. Lemahieu, I. Fraeye, E. Ryckebosch, K. Muylaert, J. Buyse, and I. Foubert. 2013. Impact of microalgal feed supplementation on omega-3 fatty acid enrichment of hen eggs. *Journal of Functional Foods* 5 (2):897–904. doi: 10.1016/j.jff.2013.01.039.

Buchmann, L., P. Bertsch, L. Böcker, U. Krähenmann, P. Fischer, and A. Mathys. 2019. Adsorption kinetics and foaming properties of soluble microalgae fractions at the air/water interface. *Food Hydrocolloids* 97:105182. doi: 10.1016/j.foodhyd.2019.105182.

Buono, S., A. L. Langellotti, A. Martello, F. Rinna, and V. Fogliano. 2014. Functional ingredients from microalgae. *Food & Function* 5 (8):1669–85. doi: 10.1039/c4fo00125g.

Callejo-López, J. A., M. Ramírez, D. Cantero, and J. Bolívar. 2020. Versatile method to obtain protein- and/or amino acid-enriched extracts from fresh biomass of recalcitrant microalgae without mechanical pretreatment. *Algal Research* 50:102010. doi: 10.1016/j.algal.2020.102010.

Camacho, F., A. Macedo, and F. Malcata. 2019. Potential industrial applications and commercialization of microalgae in the functional food and feed industries: A short review. *Marine Drugs* 17 (6):312. doi: 10.3390/md17060312.

Carissimi, M., S. H. Flôres, and R. Rech. 2018. Effect of microalgae addition on active biodegradable starch film. *Algal Research* 32:201–9. doi: 10.1016/j.algal.2018.04.001.

Castillejo, N., G. B. Martínez-Hernández, V. Goffi, P. A. Gómez, E. Aguayo, F. Artés, and F. Artés-Hernández. 2018. Natural vitamin B12 and fucose supplementation of green smoothies with edible algae and related quality changes during their shelf life. *Journal of the Science of Food and Agriculture* 98 (6):2411–21. doi: 10.1002/jsfa.8733.

Castro-García, S. Z., G. Chamorro-Cevallos, L. Quevedo-Corona, M. F. McCarty, and R. A. Bobadilla-Lugo. 2018. Beneficial effects of phycobiliproteins from *Spirulina maxima* in a preeclampsia model. *Life Sciences* 211:17–24. doi: 10.1016/j.lfs.2018.09.011.

Caporgno, M. P., I. Haberkorn, L. Böcker, and A. Mathys. 2019. Cultivation of *Chlorella protothecoides* under different growth modes and its utilisation in oil/water emulsions. *Bioresource Technology* 288:121476. doi: 10.1016/j.biortech.2019.121476.

Caporgno, M. P., L. Böcker, C. Müssner, E. Stirnemann, I. Haberkorn, H. Adelmann, S. Handschin, E. J. Windhab, and A. Mathys. 2020. Extruded meat analogues based on yellow, heterotrophically cultivated *Auxenochlorella protothecoides* microalgae. *Innovative Food Science & Emerging Technologies* 59:102275. doi: 10.1016/j.ifset.2019.102275.

Charles, C. N., T. Msagati, H. Swai, and M. Chacha. 2019. Microalgae: An alternative natural source of bioavailable omega-3 DHA for promotion of mental health in East Africa. *Scientific African* 6:e00187. doi: 10.1016/j.sciaf.2019.

Chauton, M. S., K. I. Reitan, N. H. Norsker, R. Tveterås, and H. T. Kleivdal. 2015. A techno-economic analysis of industrial production of marine microalgae as a source of EPA and DHA-rich raw material for aquafeed: Research challenges and possibilities. *Aquaculture* 436:95–103. doi: 10.1016/j.aquaculture.2014.10.038.

Chen, P. B., H. C. Wang, Y. W. Liu, S. H. Lin, H. N. Chou, and L. Y. Sheen. 2014. Immunomodulatory activities of polysaccharides from *Chlorella pyrenoidosa* in a mouse model of Parkinson's disease. *Journal of Functional Foods* 11:103–13. doi: 10.1016/j.jff.2014.08.019.

Chew, K. W., S. R. Chia, S. Y. Lee, L. Zhu, and P. L. Show. 2019. Enhanced microalgal protein extraction and purification using sustainable microwave-assisted multiphase partitioning technique. *Chemical Engineering Journal* 367:1–8. doi: 10.1016/j.cej.2019.02.131.

Christaki, E., E. Bonos, and P. Florou-Paneri. 2015. Innovative microalgae pigments as functional ingredients in nutrition. In *Handbook of marine microalgae: Biotechnology advances*, ed. S. K. Kim, 233–43. United States: Academic Press Elsevier Inc.

Dai, L., J. Hinrichs, and J. Weiss. 2020. Emulsifying properties of acid-hydrolyzed insoluble protein fraction from *Chlorella protothecoides*: Formation and storage stability of emulsions. *Food Hydrocolloids* 108:105954. doi: 10.1016/j.foodhyd.2020.105954.

Delattre, C., G. Pierre, C. Laroche, and P. Michaud. 2016. Production, extraction and characterization of microalgal and cyanobacterial exopolysaccharides. *Biotechnology Advances* 34 (7):1159–79. doi: 10.1016/j.biotechadv.2016.08.001.

De Jesus Raposo, M. F., R. M. S. C. De Morais, and A. M. M. B. De Morais. 2013. Health applications of bioactive compounds from marine microalgae. *Life Sciences* 93 (15):479–86. doi: 10.1016/j.lfs.2013.08.002.

De Marco, R. E., M. E. Steffolani, M. Martínez, and A. E. León. 2018. The use of *Nannochloropsis* sp. as a source of omega-3 fatty acids in dry pasta: Chemical, technological and sensory evaluation. *International Journal of Food Science & Technology* 53 (2):499–507. doi: 10.1111/ijfs.13609.

Di Visconte, G. S., A. Spicer, C. J. Chuck, and M. J. Allen. 2019. The microalgae biorefinery: A perspective on the current status and future opportunities using genetic modification. *Applied Sciences* 9 (22):4793. doi: 10.3390/app9224793.

Durmaz, Y., M. Kilicli, O. S. Toker, N. Konar, I. Palabiyik, and F. Tamtürk. 2020. Using spray-dried microalgae in ice cream formulation as a natural colorant: Effect on physicochemical and functional properties. *Algal Research* 47:101811. doi: 10.1016/j.algal.2020.101811.

Ejike, C. E. C. C., S. A. Collins, N. Balasuriya, A. K. Swanson, B. Mason, and C. C. Udenigwe. 2017. Prospects of microalgae proteins in producing peptide-based functional foods for promoting cardiovascular health. *Trends in Food Science & Technology* 59:30–6. doi: 10.1016/j.tifs.2016.10.026.

Enzing, C., M. Ploeg, M. Barbosa, and L. Sijtsma. 2014. Microalgae-based products for the food and feed sector: An outlook for Europe. In *JRC scientific and policy reports*. Luxembourg: European Comission. doi: 10.2791/3339.

Elst, K., M. Maesen, G. Jacobs, L. Bastiaens, S. Voorspoels, and K. Servaes. 2018. Supercritical CO_2 Extraction of *Nannochloropsis* sp: A lipidomic studyon the influence of pretreatment on yield and composition. *Molecules* 23 (8):1854. doi: 10.3390/molecules23081854.

Fasolin, L. H., R. N. Pereira, A. C. Pinheiro, J. T. Martins, C. C. P. Andrade, O. L. Ramos, and A. A. Vicente. 2019. Emergent food proteins - Towards sustainability, health and innovation. *Food Research International (Ottawa, Ont.)* 125:108586. doi: 10.1016/j.foodres.2019.108586.

Feng, J., S. Long, H. Zhang, S. Wu, G. Qi, and W. Jing. 2020. Comparative effects of dietary microalgae oil and fish oil on fatty acid composition and sensory quality of table eggs. *Poultry Science* 99 (3):1734–43. doi: 10.1016/j.psj.2019.11.005.

Fernandes, A. S., F. C. Petry, A. Z. Mercadante, E. Jacob-Lopes, and L. Q. Zepka. 2020. HPLC-PDA-MS/MS as a strategy to characterize and quantify natural pigments from microalgae. *Current Research in Food Science* 3:100–12. doi: 10.1016/j.crfs.2020.03.009.

Fields, F. J., F. Lejzerowicz, D. Schroeder, S. M. Ngoi, M. Tran, D. McDonald, L. Jiang, J. T. Chang, R. Knight, and S. Mayfield. 2020. Effects of the microalgae *Chlamydomonas* on gastrointestinal health. *Journal of Functional Foods* 65:103738. doi: 10.1016/j.jff.2019.103738.

Fradique, M., A. P. Batista, M. C. Nunes, L. Gouveia, N. M. Bandarra, and A. Raymundo. 2010. Incorporation of *Chlorella vulgaris* and *Spirulina maxima* biomass in pasta products. Part 1: Preparation and evaluation. *Journal of the Science of Food and Agriculture* 90 (10):1656–64. doi: 10.1002/jsfa.3999.

Fradique, M., A. Paula, M. C. Nunes, L. Gouveia, N. M. Bandarra, and A. Raymundo. 2013. *Isochrysis galbana* and *Diacronema vlkianum* biomass incorporation in pasta products as PUFA' s source. *LWT - Food Science and Technology* 50 (1):312–9. doi: 10.1016/j.lwt.2012.05.006.

Fradinho, P., A. Niccolai, R. Soares, L. Rodol, N. Biondi, M. R. Tredici, I. Sousa, and A. Raymundo. 2020. Effect of *Arthrospira platensis (Spirulina)* incorporation on the rheological and bioactive properties of gluten-free fresh pasta. *Algal Research* 45:101743. doi: 10.1016/j.algal.2019.101743.

Freitas, B. C. B., T. D. Santos, J. B. Moreira, K. Zanfonato, M. G. Morais, and J. A. V. Costa. 2019. Novel foods: A meal replacement shake and a high-calorie food supplemented with *Spirulina* biomass. *International Food Research Journal* 26:59–65.

Food and Drug Administration (FDA). 2011. *Spirulina platensis* GRAS self affirmation 7-1-2011. *Original Submission* 477:1–126.

Fu, W., D. R. Nelson, Z. Yi, M. Xu, B. Khraiwesh, and K. Jijakli. 2017. Bioactive compounds from microalgae: Current development and prospects. In *Studies in Natural Products Chemistry*, ed. A. Rahman, 54 , 199–225. Amsterdam: Elsevier.

García-Segovia, P., M. J. Pagán-Moreno, I. F. Lara, and J. Martínez-Monzó. 2017. Effect of microalgae incorporation on physicochemical and textural properties in wheat bread formulation. *Food Science and Technology International = Ciencia y tecnologia de los alimentos internacional* 23 (5):437–47. doi: 10.1177/1082013217700259.

Garcia, E. S., J. Van Leeuwen, C. Safi, L. Sijtsm, M. H. M. Eppink, R. H. Wijffels, and C. Van den Berg. 2018. Selective and energy efficient extraction of functional proteins from microalgae for food applications. *Bioresource Technology* 268:197–203. doi: 10.1016/j.biortech.2018.07.131.

Garrido-Cardenas, J. A., F. Manzano-Agugliaro, F. G. Acien-Fernandez, and E. Molina-Grima. 2018. Microalgae research worldwide. *Algal Research* 35:50–60. doi: 10.1016/j.algal.2018.08.005.

Ghanbari, A., A. A. Vafaei, F. S. Naghibi Nasab, M. Attarmoghaddam, A. R. Bandegi, and N. Moradi- Kor. 2019. *Spirulina* microalgae improves memory deficit induced by scopolamine in male pup rats: Role of oxidative stress. *South African Journal of Botany* 127:220–5. doi: 10.1016/j.sajb.2019.08.045.

Gheysen, L., R. Demets, J. Devaere, T. Bernaerts, P. Goos, A. Van Loey, L. De Cooman, and I. Foubert. 2019. Impact of microalgal species on the oxidative stability of n-3 LC-PUFA enriched tomato puree. *Algal Research* 40:101502. doi: 10.1016/j.algal.2019.101502.

Gifuni, I., A. Pollio, C. Safi, A. Marzocchell, and G. Olivieri. 2019. Current bottlenecks and challenges of the microalgal biorefinery. *Trends in Biotechnology* 37 (3):242–52. doi: 10.1016/j.tibtech.2018.09.006.

Graça, C., P. Fradinho, I. Sousa, and A. Raymundo. 2018. Impact of *Chlorella vulgaris* on the rheology of wheat flour dough and bread texture. *LWT - Food Science and Technology* 89:466–74. doi: 10.1016/j.lwt.2017.11.024.

Grossmann, L., S. Ebert, J. Hinrichs, and J. Weiss. 2018. Production of protein-rich extracts from disrupted microalgae cells: Impact of solvent treatment and lyophilization. *Algal Research* 36:67–76. doi: 10.1016/j.algal.2018.09.011.

Grossmann, L., J. Hinrichs, and J. Weiss. 2019. Solubility and aggregation behavior of protein fractions from the heterotrophically cultivated microalga *Chlorella protothecoides*. *Food Research International (Ottawa, Ont.)* 116:283–90. doi: 10.1016/j.foodres.2018.08.037.

Guesnet, P., and J. M. Alessandri. 2011. Docosahexaenoic acid (DHA) and the developing central nervous system (CNS) - Implications for dietary recommendations. *Biochimie* 93 (1):7–12. doi: 10.1016/j.biochi.2010.05.005.

Hashempour-Baltork, F., K. Khosravi-Darani, H. Hosseini, P. Farshi, and S. F. S. Reihani. 2020a. Mycoproteins as safe meat substitutes. *Journal of Cleaner Production* 253:119958. doi.org/10.1016/j.jclepro.2020.119958. doi: 10.1016/j.jclepro.2020.119958.

Hashempour-Baltork, F., S. M. Hosseini, M. A. Assarehzadegan, K. Khosravi-Darani, and H. Hosseini. 2020b. Safety assays and nutritional values of mycoprotein produced by *Fusarium venenatum* IR372C from date waste as substrate. *Journal of the Science of Food and Agriculture* 100 (12):4433–41. doi: 10.1002/jsfa.10483.

He, Y., T. Wu, H. Sun, P. Sun, B. Liu, M. Luo, and F. Chen. 2019. Comparison of fatty acid composition and positional distribution of microalgae triacylglycerols for human milk fat substitutes. *Algal Research* 37:40–50. doi: 10.1016/j.algal.2018.11.004.

Ho, S. H., S. W. Huang, C. Y. Chen, T. Hasunuma, A. Kondo, and J. S. Chang. 2013. Bioethanol production using carbohydrate-rich microalgae biomass as feedstock. *Bioresource Technology* 135:191–8. doi: 10.1016/j.biortech.2012.10.015.

Hossain, A. K. M. M., M. A. Brennan, S. L. Mason, X. Guo, X. A. Zeng, and C. S. Brennan. 2017. The effect of astaxanthin-rich microalgae "*Haematococcus pluvialis*" and wholemeal flours incorporation in improving the physical and functional properties of cookies. *Foods* 6 (8):57. doi: 10.3390/foods6080057.

Hu, H.,. J. Y. Li, X. R. Pan, F. Zhang, L. L. Ma, H. J. Wang, and R. J. Zeng. 2019. Different DHA or EPA production responses to nutrient stress in the marine microalga *Tisochrysis lutea* and the freshwater microalga *Monodus subterraneus*. *The Science of the Total Environment* 656:140–9. doi: 10.1016/j.scitotenv.2018.11.346.

Jesus, S. S., G. F. Ferreira, L. S. Moreira, M. M. R. Wolf, and F. R. Maciel. 2019. Comparison of several methods for effective lipid extraction from wet microalgae using green solvents. *Renewable Energy* 143:130–41. doi: 10.1016/j.renene.2019.04.168.

Joshi, S. M., M. B. Bera, and P. S. Panesar. 2014. Extrusion cooking of maize/Spirulina mixture : factors affecting expanded product characteristics and sensory quality. *Journal of Food Processing and Preservation* 38 (2):655–64. doi: 10.1111/jfpp.12015.

Jung, F., A. Krüger-Genge, P. Waldeck, and J.-H. Küpper. 2019. *Spirulina platensis*, a super food. *Journal of Cellular Biotechnology* 5 (1):43–54. ? doi: 10.3233/JCB-189012.

Kaewsutas, M., A. Sarikaphuti, T. Nararatwanchai, P. Sittiprapaporn, and P. Patchanee. 2017. Electroencephalographic study of microalgae DHA omega-3 egg consumption on cognitive function. *Journal of Functional Foods* 29:46–52. doi: 10.1016/j.jff.2016.12.004.

Katiyar, R., and A. Arora. 2020. Health promoting functional lipids from microalgae pool: A review. *Algal Research* 46:101800. doi: 10.1016/j.algal.2020.101800.

Khemiri, S., N. Khelifi, M. C. Nunes, A. Ferreira, L. Gouveia, I. Smaali, and A. Raymundo. 2020. Microalgae biomass as an additional ingredient of gluten-free bread: Dough rheology, texture quality and nutritional properties. *Algal Research* 50:101998. doi: 10.1016/j.algal.2020.101998.

Koyande, A. K., K. W. Chew, K. Rambabu, Y. Tao, D.-T. Chu, and P.-L. Show. 2019. Microalgae: A potential alternative to health supplementation for humans. *Food Science and Human Wellness* 8 (1):16–24. doi: 10.1016/j.fshw.2019.03.001.

Koyande, A. K., V. Tanzil, H. Murraly Dharan, M. Subramaniam, R. N. Robert, P. L. Lau, I. Khoiroh, and P. L. Show. 2020. Integration of osmotic shock assisted liquid biphasic system for protein extraction from microalgae *Chlorella vulgaris*. *Biochemical Engineering Journal* 157:107532. doi: 10.1016/j.bej.2020.107532.

Kurd, F., and V. Samavati. 2015. Water soluble polysaccharides from *Spirulina platensis*: Extraction and in vitro anti-cancer activity. *International Journal of Biological Macromolecules* 74:498–506. doi: 10.1016/j.ijbiomac.2015.01.005.

Lafarga, T., E. Mayre, G. Echeverria, I. Viñas, S. Villaró, F. G. Acién-Fernández, M. Castellari, and I. Aguiló-Aguayo. 2019. Potential of the microalgae *Nannochloropsis* and *Tetraselmis* for being used as innovative ingredients in baked goods. *LWT - Food Science and Technology* 115:108439. doi: 10.1016/j.lwt.2019.108439.

Lafarga, T., F. G. Acién-Fernández, M. Castellari, S. Villaró, G. Bobo, and I. Aguiló-Aguayo. 2019. Effect of microalgae incorporation on the physicochemical, nutritional, and sensorial properties of an

innovative broccoli soup. *LWT - Food Science and Technology* 111: 167–74. doi: 1016/j.lwt.2019.05.037.

Law, S. Q. K., S. Mettu, M. Ashokkumar, P. J. Scales, and G. J. O. Martin. 2018. Emulsifying properties of ruptured microalgae cells: Barriers to lipid extraction or promising biosurfactants? *Colloids and Surfaces. B, Biointerfaces* 170:438–46. doi: 10.1016/j.colsurfb.2018.06.047.

Levasseur, W., P. Perré, and V. Pozzobon. 2020. A review of high value-added molecules production by microalgae in light of the classification. *Biotechnology Advances* 41:107545. 1075. doi: 10.1016/j.biotechadv.2020.

Li, T. T., A. J. Tong, Y. Y. Liu, Z. R. Huang, X. Z. Wan, Y. Y. Pan, R. B. Jia, B. Liu, X. H. Chen, and C. Zhao. 2019. Polyunsaturated fatty acids from microalgae *Spirulina platensis* modulates lipid metabolism disorders and gut microbiota in high-fat diet rats. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association* 131:110558 doi: 10.1016/j.fct.2019.06.005.

Liberman, G., G. Ochbaum, M. Mejubovsky-Mikhelis, R. Bitton, and S. M. Arad. 2020. Physico-chemical characteristics of the sulfated polysaccharides of the red microalgae *Dixoniella grisea* and *Porphyridium aerugineum*. *International Journal of Biological Macromolecules* 145:1171–9. doi: 10.1016/j.ijbiomac.2019.09.205.

Lucas, B. F., M. G. de Morais, T. D. Santos, and J. A. V. Costa. 2018. *Spirulina* for snack enrichment: Nutritional, physical and sensory evaluations. *LWT - Food Science and Technology* 90:270–6. doi: 10.1016/j.lwt.2017.12.032.

Ma, P., R. Huang, J. Jiang, Y. Ding, T. Li, and Y. Ou. 2020. Potential use of C-phycocyanin in non-alcoholic fatty liver disease. *Biochemical and Biophysical Research Communications* 526 (4): 906–12. doi: 10.1016/j.bbrc.2020.04.001.

Marques, J. A., T. A. Del Valle, L. G. Ghizzi, E. M. C. Zilio, L. S. Gheller, A. T. Nunes, T. B. P. Silva, M. S. D. S. Dias, N. T. S. Grigoletto, A. F. Koontz, et al. 2019. Increasing dietary levels of docosahexaenoic acid-rich microalgae: Ruminal fermentation, animal performance, and milk fatty acid profile of mid-lactating dairy cows. *Journal of Dairy Science* 102 (6):5054–65. doi: 10.3168/jds.2018-16017.

Marcinkowska-Lesiak, M., A. Onopiuk, M. Zalewska, A. Ciepłoch, and L. Barotti. 2018. The effect of different level of *Spirulina* powder on the chosen quality parameters of shortbread biscuits. *Journal of Food Processing and Preservation* 42 (3):e13561. doi: 10.1111/jfpp.13561.

Martins, D. A., L. Custódio, L. Barreira, H. Pereira, R. Ben-Hamadou, J. Varela, and K. M. Abu-Salah. 2013. Alternative sources of n-3 long-chain polyunsaturated fatty acids in marine microalgae. *Marine Drugs* 11 (7):2259–81. doi: 10.3390/md11072259.

Marti-Quijal, F. J., S. Zamuz, I. Tomašević, B. Gómez, G. Rocchetti, L. Lucini, F. Remize, F. J. Barba, and J. M. Lorenzo. 2019. Influence of different sources of vegetable, whey and microalgae proteins on the physicochemical properties and amino acid profile of fresh pork sausages. *LWT - Food Science and Technology* 110:316–23. doi: 10.1016/j.lwt.2019.04.097.

Marti-Quijal, F. J., S. Zamuz, I. Tomašević, G. Rocchetti, L. Lucini, K. Marszałek, F. J. Barba, and J. M. Lorenzo. 2019. A chemometric approach to evaluate the impact of pulses, *Chlorella* and *Spirulina* on proximate composition, amino acid, and physicochemical properties of turkey burgers. *Journal of the Science of Food and Agriculture* 99 (7):3672–80. doi: 10.1002/jsfa.9595.

Massoud, R., K. Khosravi-Darani, F. Nakhsaz, and L. Varga. 2017. Evaluation of physicochemical, microbiological and sensory properties of croissants fortified with *Arthrospira platensis* (Spirulina). *Czech Journal of Food Sciences* 34 (No. 4):350–5. doi: 10.17221/289/2015-CJFS.

Mata, T. M., A. A. Martins, and N. S. Caetano. 2010. Microalgae for biodiesel production and other applications: A review. *Renewable and Sustainable Energy Reviews* 14 (1):217–32. doi: 10.1016/j.rser.2009.07.020.

Matos, A. P. 2017. The impact of microalgae in food science and technology. *Journal of the American Oil Chemists' Society* 94 (11): 1333–50. doi: 10.1007/s11746-017-3050-7.

Matos, J., C. Cardoso, N. M. Bandarra, and C. Afonso. 2017. Microalgae as healthy ingredients for functional food: A review. *Food & Function* 8 (8):2672–85. doi: 10.1039/c7fo00409e.

Mazinani, S., V. Fadaei, and K. Khosravi-Darani. 2016. Impact of *Spirulina platensis* on physicochemical properties and viability of *Lactobacillus acidophilus* of probiotic UF feta cheese. *Journal of Food Processing and Preservation* 40 (6):1318–24. doi: 10.1111/jfpp.12717.

Mc Gee, D., L. Archer, G. T. A. Fleming, E. Gillespie, and N. Touzet. 2020. The effect of nutrient and phytohormone supplementation on the growth, pigment yields and biochemical composition of newly isolated microalgae. *Process Biochemistry* 92:61–8. doi: 10.1016/j.procbio.2020.03.001.

Medeiros, V. P. B., T. C. Pimentel, R. C. R. Varandas, S. A. Santos, G. T. Souza Pedrosa, C. F. C. Sassi, M. M. Conceição, and M. Magnani. 2020. Exploiting the use of agro-industrial residues from fruit and vegetables as alternative microalgae culture medium. *Food Research International (Ottawa, Ont.)* 137:109722. doi.org/10.1016/j.foodres.2020.109722. doi: 10.1016/j.foodres.2020.109722.

Memije-Lazaro, I. N., V. Blas-Valdivia, M. Franco-Colín, and E. Cano-Europa. 2018. *Arthrospira maxima* (Spirulina) and C-phycocyanin prevent the progression of chronic kidney disease and its cardiovascular complications. *Journal of Functional Foods* 43:37–43. doi: 10.1016/j.jff.2018.01.013.

Menegotto, A. L. L., L. E. S. Souza, L. M. Colla, J. A. V. Costa, E. Sehn, P. R. S. Bittencourt, É. L. M. Flores, C. Canan, and E. Coll. 2019. Investigation of techno-functional and physicochemical properties of *Spirulina platensis* protein concentrate for food enrichment. *LWT - Food Science and Technology* 114:108267. doi: 10.1016/j.lwt.2019.108267.

Mohamed, A. G., B. E. Abo-El-Khair, and S. M. Shalaby. 2013. Quality of novel healthy processed cheese analogue enhanced with marine microalgae *Chlorella vulgaris* biomass. *World Applied Sciences Journal* 23:914–25. doi: 10.5829/idosi.wasj.2013.23.07.13122.

Mohammadi-Gouraji, E., S. Soleimanian-Zad, and M. Ghiaci. 2019. Phycocyanin-enriched yogurt and its antibacterial and physicochemical properties during 21 days of storage. *LWT - Food Science and Technology* 102:230–6. doi: 10.1016/j.lwt.2018.09.057.

Moreira, J. B., L. F. Carvalho, T. D. Santos, M. S. Oliveira, and J. A. V. Costa. 2018. Microalgal biotechnology for development of energy gel in sports. *International Food Research Journal* 25:1942–7.

Morsy, M. K., O. M. Morsy, H. A. Elbarbary, and M. A. Saad. 2019. Enhancing of oxidative stability and quality attributes of olive oil using spirulina (*Arthrospira platensis*) nanoparticles. *LWT - Food Science and Technology* 101:444–55. doi: 10.1016/j.lwt.2018.11.056.

Mozaffarian, D., and J. H. Y. Wu. 2011. Omega-3 fatty acids and cardiovascular disease: Effects on risk factors, molecular pathways, and clinical events. *Journal of the American College of Cardiology* 58 (20):2047–67. doi: 10.1016/j.jacc.2011.06.063.

Mu, N., J. G. Mehar, S. N. Mudliar, and A. Y. Shekh. 2019. Recent advances in microalgal bioactives for food, feed, and healthcare products: Commercial potential, market space, and Sustainability. *Comprehensive Reviews in Food Science and Food Safety* 18 (6): 1882–97. doi: 10.1111/1541-4337.12500.

Muys, M., Y. Sui, B. Schwaiger, C. Lesueur, D. Vandenheuvel, P. Vermeir, and S. E. Vlaeminck. 2019. High variability in nutritional value and safety of commercially available *Chlorella* and *Spirulina* biomass indicates the need for smart production strategies. *Bioresource Technology* 275:247–57. doi: 10.1016/j.biortech.2018.12.059.

Nascimento, T. C., C. B. B. Cazarin, Jr. M. R. Maróstica, E. M. Risso, J. Amaya-Farfan, R. Grimaldi, A. Z. Mercadante, E. Jacob-Lopes, and L. Q. Zepka. 2019. Microalgae biomass intake positively modulates serum lipid profile and antioxidant status. *Journal of Functional Foods* 58:11–20. doi: 10.1016/j.jff.2019.04.047.

Niccolai, A., M. Venturi, V. Galli, N. Pini, L. Rodolfi, N. Biondi, M. D'Ottavio, A. P. Batista, A. Raymundo, L. Granchi, et al. 2019. Development of new microalgae-based sourdough "crostini": functional effects of Arthrospira platensis (spirulina) addition"". *Scientific Reports* 9 (1):19433. doi: 10.1038/s41598-019-55840-1.

Nunes, M. C., C. Graça, S. Vlaisavljević, A. Tenreiro, I. Sousa, and A. Raymundo. 2020. Microalgal cell disruption: Effect on the bioactivity and rheology of wheat bread. *Algal Research* 45:101749. doi: 10.1016/j.algal.2019.101749.

Nuño, K., A. Villarruel-López, A. M. Puebla-Pérez, E. Romero-Velarde, A. G. Puebra-Mora, and F. Ascencio. 2013. Effects of the marine microalgae *Isochrysis galbana* and *Nannochloropsis oculata* in diabetic rats. *Journal of Functional Foods* 5 (1):106–15. doi: 10.1016/j.jff.2012.08.011.

Onacik-Gür, S., A. Zbikowska, and B. Majewska. 2018. Effect of Spirulina (*Spirulina platensis*) addition on textural and quality properties of cookies. *Italian Journal of Food Science* 30:1–12. doi: 10.14674/IJFS-702.

Oriquat, G. A. 2018. Therapeutic effects of Spirulina against experimentally-induced non- alcoholic fatty liver in rats may involve miR-21, -34a and -122. *Meta Gene* 18:115–21. doi: 10.1016/j.mgene.2018.08.008.

Palabiyik, I., Y. Durmaz, B. Öner, O. S. Toker, G. Coksari, N. Konar, and F. Tamtür. 2018. Using spray-dried microalgae as a natural coloring agent in chewing gum: Effects on color, sensory, and textural properties. *Journal of Applied Phycology* 30 (2):1031–9. doi: 10.1007/s10811-017-1324-y.

Palanisamy, M., S. Töpfl, R. G. Berger, and C. Hertel. 2019. Physico-chemical and nutritional properties of meat analogues based on Spirulina/lupin protein mixtures. *European Food Research and Technology* 245 (9):1889–98. doi: 10.1007/s00217-019-03298-w.

Paniagua-Michel, J. J., J. Olmos-Soto, and E. R. Morales-Guerrero. 2014. Algal and microbial exopolysaccharides: New insights as biosurfactants and bioemulsifiers. In *Advances in food and nutrition research*, ed. S. K. Kim, *73*, 221–57. United States: Academic Press Elsevier Inc.

Parages, M. L., R. M. Rico, R. T. Abdala-Díaz, M. Chabrillón, T. G. Sotiroudis, and C. Jiménez. 2012. Acidic polysaccharides of *Arthrospira* (Spirulina) *platensis* induce the synthesis of TNF-α in RAW macrophages. *Journal of Applied Phycology* 24 (6):1537–46. doi: 10.1007/s10811-012-9814-4.

Pereira, A. M., C. R. Lisboa, and J. A. V. Costa. 2018. High protein ingredients of microalgal origin: Obtainment and functional properties. *Innovative Food Science & Emerging Technologies* 47:187–94. doi: 10.1016/j.ifset.2018.02.015.

Peter S, J., S. K. Basha, R. Giridharan, B. U. Lavinya, and E. P. Sabina. 2017. Suppressive effect of *Spirulina fusiformis* on diclofenac-induced hepato-renal injury and gastrointestinal ulcer in *Wistar* albino rats: A biochemical and histological approach. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie* 88:11–8. doi: 10.1016/j.biopha.2017.01.032.

Phong, W. N., P. L. Show, T. C. Ling, J. C. Juan, E. P. Ng, and J. S. Chang. 2018. Mild cell disruption methods for bio-functional proteins recovery from microalgae—Recent developments and future perspectives. *Algal Research* 31:506–16. doi: 10.1016/j.algal.2017.04.005.

Pierre, G., C. Delattre, P. Dubessay, S. Jubeau, C. Vialleix, J. P. Cadoret, I. Probert, and P. Michaud. 2019. What is in store for EPS microalgae in the next decade. *Molecules* 24 (23):4296. doi: 10.3390/molecules24234296.

Prabakaran, G., P. Sampathkumar, M. Kavisri, and M. Moovendhan. 2020. Extraction and characterization of phycocyanin from *Spirulina platensis* and evaluation of its anticancer, antidiabetic and antiinflammatory effect. *International Journal of Biological Macromolecules* 153:256–63. doi: 10.1016/j.ijbiomac.2020.03.009.

Qi, J., and S. M. Kim. 2017. Characterization and immunomodulatory activities of polysaccharides extracted from green alga *Chlorella ellipsoidea*. *International Journal of Biological Macromolecules* 95:106–14. doi: 10.1016/j.ijbiomac.2016.11.039.

Qian, S., and Y. Xu. 2014. Anti-cancer activities of ω-6 polyunsaturated fatty acids. *Biomedical Journal* 37 (3):112–9. doi: 10.4103/2319-4170.131378.

Rabelo, S. F., A. C. Lemes, K. P. Takeuchi, M. T. Frata, J. C. M. de Carvalho, and E. D. G. Danesi. 2013. Development of cassava doughnuts enriched with Spirulina platensis biomass. *Brazilian Journal of Food Technology* 16 (1):42–51. doi: 10.1590/S1981-67232013005000001.

Rahman, K. M. 2020. Food and high value products from microalgae: Market opportunities and challenges. In *Microalgae biotechnology for food, health and high value products*, eds. M. A. Alam, J. L. Xu, and Z. Wang, 3–28. Singapore: Springer Nature.

Raja, R.,. A. Coelho, S. Hemaiswary, P. Kumar, I. S. Carvalho, and A. Alagarsamy. 2018. Applications of microalgal paste and powder as food and feed: An update using text mining tool. *Beni-Suef University Journal of Basic and Applied Sciences* 7 (4):740–7. doi: 10.1016/j.bjbas.2018.10.004.

Raposo, M. F. D. J., A. M. D. M. B. Morais, and R. M. S. C. Morais. 2015. Carotenoids from marine microalgae: A valuable natural source for the prevention of chronic diseases. *Marine Drugs* 13 (8):5128–55. doi: 10.3390/md13085128.

Rodríguez-Sánchez, R.,. R. Ortiz-Butrón, V. Blas-Valdivia, A. Hernández-García, and E. Cano-Europa. 2012. Phycobiliproteins or C-phycocyanin of *Arthrospira* (*Spirulina*) *maxima* protect against HgCl(2)-caused oxidative stress and renal damage . *Food Chemistry* 135 (4):2359–65. doi: 10.1016/j.foodchem.2012.07.063.

Rzymski, P., P. Niedzielski, N. Kaczmarek, T. Jurczak, and P. Klimaszyk. 2015. The multidisciplinary approach to safety and toxicity assessment of microalgae-based food supplements following clinical cases of poisoning. *Harmful Algae* 46:34–42. doi: 10.1016/j.hal.2015.05.003.

Santos, T. D., B. C. B. Freitas, J. B. Moreira, K. Zanfonato, and J. A. V. Costa. 2016. Development of powdered food with the addition of *Spirulina* for food supplementation of the elderly population. *Innovative Food Science & Emerging Technologies* 37:216–20. doi: 10.1016/j.ifset.2016.07.016.

Santos-Sánchez, N. F., R. Valadez-Blanco, B. Hernández-Carlos, A. Torres-Ariño, P. C. Guadarrama-Mendoza, and R. Salas-Coronado. 2016. Lipids rich in ω-3 polyunsaturated fatty acids from microalgae. *Applied Microbiology and Biotechnology* 100 (20):8667–84. doi: 10.1007/s00253-016-7818-8.

Schwenzfeier, A., A. Helbig, P. A. Wierenga, and H. Gruppen. 2013. Food hydrocolloids emulsion properties of algae soluble protein isolate from *Tetraselmis* sp. *Food Hydrocolloids.* 30 (1):258–63. doi: 10.1016/j.foodhyd.2012.06.002.

Sengupta, S.,. H. Koley, S. Dutta, and J. Bhowal. 2018. Hypocholesterolemic effect of *Spirulina platensis* (SP) fortified functional soy yogurts on diet-induced hypercholesterolemia. *Journal of Functional Foods* 48:54–64. doi: 10.1016/j.jff.2018.07.007.

Sharoba, A. 2014. Nutritional value of Spirulina and its use in the preparation of some complementary baby food formulas. *Journal of Food and Dairy Sciences* 5 (8):517–38. doi: 10.21608/jfds.2014.53033.

Silva, S. C., I. P. Fernandes, L. Barros, Â. Fernandes, M. J. Alves, R. C. Calhelha, C. Pereira, J. C. M. Barreira, Y. Manrique, E. Colla, et al. 2019. Spray-dried *Spirulina platensis* as an effective ingredient to improve yogurt formulations: Testing different encapsulating solutions. *Journal of Functional Foods* 60:103427. doi: 10.1016/j.jff.2019.103427.

Silva, M. E. T., K. P. Correa, M. A. Martins, S. L. P. Matta, H. S. D. Martino, and J. S. R. Coimbra. 2020. Food safety, hypolipidemic and hypoglycemic activities and *in vivo* protein quality of microalga *Scenedesmus obliquus* in *Wistar* rats. *Journal of Functional Foods* 65:103711. doi: 10.1016/j.jff.2019.103711.

Sinha, S., N. Patro, and I. K. Patro. 2020. Amelioration of neurobehavioral and cognitive abilities of F1 progeny following dietary supplementation with Spirulina to protein malnourished mothers. *Brain, Behavior, and Immunity* 85:69–87. doi: 10.1016/j.bbi.2019.08.181.

Sivaramakrishnan, R., and A. Incharoensakdi. 2019. Low power ultrasound treatment for the enhanced production of microalgae biomass and lipid content. *Biocatalysis and Agricultural Biotechnology* 20:101230. doi: 10.1016/j.bcab.2019.101230.

Soto-Sierra, L., P. Stoykov, and Z. L. Nikolov. 2018. Extraction and fractionation of microalgae-based protein products. *Algal Research* 36:175–92. doi: 10.1016/j.algal.2018.10.023.

Takyar, T. M. B., H. K. S. Khajavi, and R. Safari. 2019. Evaluation of antioxidant properties of *Chlorella vulgaris* and *Spirulina platensis*

and their application in order to extend the shelf life of rainbow trout (*Oncorhynchus mykiss*) fillets during refrigerated storage. *LWT - Food Science and Technology* 100:244–9. doi: 10.1016/j.lwt.2018.10.079.

Tańska, M., I. Konopka, and M. Ruszkowska. 2017. Sensory, physicochemical and water sorption properties of corn extrudates enriched with Spirulina. *Plant Foods for Human Nutrition (Dordrecht, Netherlands)* 72 (3):250–7. doi: 10.1007/s11130-017-0628-z.

Tang, D. Y. Y., K. S. Khoo, K. W. Chew, Y. Tao, S. H. Ho, and P. L. Show. 2020. Potential utilization of bioproducts from microalgae for the quality enhancement of natural products. *Bioresource Technology* 304:122997. doi: 10.1016/j.biortech.2020.122997.

Teuling, E., J. W. Schrama, H. Gruppen, and P. A. Wierenga. 2019. Characterizing emulsion properties of microalgal and cyanobacterial protein isolates. *Algal Research* 39:101471. doi: 10.1016/j.algal.2019.101471.

Thawechai, T.,. B. Cheirsilp, Y. Louhasakul, P. Boonsawang, and P. Prasertsan. 2016. Mitigation of carbon dioxide by oleaginous microalgae for lipids and pigments production: Effect of light illumination and carbon dioxide feeding strategies. *Bioresource Technology* 219:139–49. doi: 10.1016/j.biortech.2016.07.10

Thirumdas, R., M. Brnčić, S. R. Brnčić, F. J. Barba, F. Gálvez, S. Zamuz, R. Lacomba, and J. M. Lorenzo. 2018. Evaluating the impact of vegetal and microalgae protein sources on proximate composition, amino acid profile, and physicochemical properties of fermented Spanish "chorizo" sausages. *Journal of Food Processing and Preservation* 42 (11):e13817. doi: 10.1111/jfpp.13817.

Tibbetts, S. M., M. A. Scaife, and R. E. Armenta. 2020. Apparent digestibility of proximate nutrients, energy and fatty acids in nutritionally-balanced diets with partial or complete replacement of dietary fish oil with microbial oil from a novel *Schizochytrium* sp. (T18) by juvenile Atlantic salmon (Salmo salar L.). *Aquaculture* 520:735003. doi: 10.1016/j.aquaculture.2020.735003.

Torres-Tiji, Y., F. J. Fields, and S. P. Mayfield. 2020. Microalgae as a future food source. *Biotechnology Advances* 41:107536. doi: 10.1016/j.biotechadv.2020.107536.

Ursu, A., A. Marcati, T. Sayd, V. Sante-Lhoutellier, G. Djelveh, and P. Michaud. 2014. Extraction, fractionation and functional properties of proteins from the microalgae *Chlorella vulgaris*. *Bioresource Technology* 157:134–9. doi: 10.1016/j.biortech.2014.01.071.

Vaz, B. S., J. B. Moreira, M. G. Morais, and J. A. V. Costa. 2016. Microalgae as a new source of bioactive compounds in food supplements. *Current Opinion in Food Science* 7:73–7. doi: 10.1016/j.cofs.2015.12.006.

Vieira, M. V., S. M. Oliveira, I. R. Amado, L. H. Fasolin, A. A. Vicente, L. M. Pastrana, and P. Fuciños. 2020. 3D printed functional cookies fortified with *Arthrospira platensis*: Evaluation of its antioxidant potential and physical-chemical characterization. *Food Hydrocolloids* 107:105893. doi: 10.1016/j.foodhyd.2020.105893.

Vigani, M., C. Parisi, E. Rodríguez-Cerezo, M. J. Barbosa, L. Sijtsma, M. Ploeg, and C. Enzing. 2015. Food and feed products from microalgae: Market opportunities and challenges for the EU. *Trends in Food Science & Technology* 42 (1):81–92. doi: 10.1016/j.tifs.2014.12.004.

Wan, X., T. Li, R. Zhong, H. Chen, X. Xia, L. Gao, X. Gao, B. Liu, H. Zhang, and C. Zhao. 2019. Anti-diabetic activity of PUFAs-rich extracts of *Chlorella pyrenoidosa* and *Spirulina platensis* in rats. *Food and Chemical Toxicology : An International Journal Published for the British Industrial Biological Research Association* 128:233–9. doi: 10.1016/j.fct.2019.04.017.

Wang, A., K. Yan, D. Chu, M. Nazer, N. T. Lin, E. Samaranayake, and J. Chang. 2020. Microalgae as a mainstream food ingredient: Demand and supply perspective. In *Microalgae for food, health and high value products*, eds. M. A. Alam, J. L. Xu, and Z. Wang, 29–80. Singapore: Springer Nature.

Wild, K. J., H. Steingaß, and M. Rodehutscord. 2018. Variability in nutrient composition and in vitro crude protein digestibility of 16 microalgae products. *Journal of Animal Physiology and Animal Nutrition* 102 (5):1306–19. doi: 10.1111/jpn.12953.

Xiao, R., and Y. Zheng. 2016. Overview of microalgal extracellular polymeric substances (EPS) and their applications. *Biotechnology Advances* 34 (7):1225–44. doi: 10.1016/j.biotechadv.2016.08.004.

Yamaguchi, S. K. F., J. B. Moreira, J. A. V. Costa, C. K. Souza, S. L. Bertoli, and L. F. Carvalho. 2019. Evaluation of adding *Spirulina* to freeze-dried yogurts before fermentation and after freeze-drying. *Industrial Biotechnology* 15 (2):89–94. doi: 10.1089/ind.2018.0030.

Yang, Y.,. L. Du, M. Hosokawa, and K. Miyashita. 2020. Effect of *Spirulina* lipids on high-fat and high-sucrose diet induced obesity and hepatic lipid accumulation in C57BL/6J mice. *Journal of Functional Foods* 65:103741. doi: 10.1016/j.jff.2019.103741.

Yingying, S., W. Hui, G. Guo, P. Yinfang, and Y. Binlun. 2014. The isolation and antioxidant activity of polysaccharides from the marine microalgae *Isochrysis galbana*. *Carbohydrate Polymers* 113:22–31. doi: 10.1016/j.carbpol.2014.06.058.

Yu, M., M. Chen, J. Gui, S. Huang, Y. Liu, H. Shentu, J. He, Z. Fang, W. Wang, and Y. Zhang. 2019. Preparation of *Chlorella vulgaris* polysaccharides and their antioxidant activity *in vitro* and *in vivo*. *International Journal of Biological Macromolecules* 137:139–50. doi: 10.1016/j.ijbiomac.2019.06.222.

Zahran, W. E., and M. A. Emam. 2018. Renoprotective effect of *Spirulina platensis* extract against nicotine-induced oxidative stress-mediated inflammation in rats. *Phytomedicine : international Journal of Phytotherapy and Phytopharmacology* 49:106–10. doi: 10.1016/j.phymed.2018.06.042.

Zhang, R., N. Lebovka, L. Marchal, E. Vorobiev, and N. Grimi. 2020. Pulsed electric energy and ultrasonication assisted green solvent extraction of bio-molecules from different microalgal species. *Innovative Food Science & Emerging Technologies* 62:102358 doi:10.1016/j.ifset.2020.102358.

Zhang, J., L. Liu, Y. Ren, and F. Chen. 2019. Characterization of exopolysaccharides produced by microalgae with antitumor activity on human colon cancer cells. *International Journal of Biological Macromolecules* 128:761–7. doi: 10.1016/j.ijbiomac.2019.02.009.

Zouari, N., M. Abid, N. Fakhfakh, M. A. Ayadi, L. Zorgui, M. Ayadi, and H. Attia. 2011. Blue-green algae (*Arthrospira platensis*) as an ingredient in pasta: Free radical scavenging activity, sensory and cooking characteristics evaluation. *International Journal of Food Sciences and Nutrition* 62 (8):811–3. doi: 10.3109/09637486.2011.582461.

Žugčić, T., R. Abdelkebir, F. J. Barba, A. Rezek-Jambrak, F. Gálvez, S. Zamuz, D. Granato, and J. M. Lorenzo. 2018. Effects of pulses and microalgal proteins on quality traits of beef patties. *Journal of Food Science and Technology* 55 (11):4544–53. doi: 10.1007/s13197-018-3390-9.

2.2 TÓPICOS COMPLEMENTARES RELEVANTESÀ TEMÁTICA DA TESE

2.2.1 Microbiota intestinal e prebióticos

A microbiota intestinal humana é constituída por um ecossistema microbiano complexo que incluibactérias, fungos, arquéias, vírus e protozoários,responsáveis por promoverem interações dinâmicas e mutualísticas envolvendo a digestão e a absorção de nutrientes provenientes da dieta (LAVELLE; HILL, 2019; PEREDO-LOVILLO; ROMERO-LUNA; JIMÉNEZ-FERNÁNDEZ, 2020).Devido às atividades exercidas por grupos microbianos que a compõe, a microbiota intestinal éconsiderada um "órgão" metabolicamente ativo (HOLSCHER, 2017) e agente protetor da saúde intestinal e sistêmica, que desempenha papel importante na manutenção da saúde humana (COSME; INÊS; VILELA, 2022).

De modo geral, o perfil da microbiota intestinal de adultos saudáveis é composto predominantemente por *Firmicutes* e *Bacteroidetes*, enquanto os filos menos abundantes incluem *Proteobacteria*, *Actinobacteria* e *Verrucomicrobia*, *Fusobacteria* e *Cynobacteria* (DIXIT *et al*., 2021).A composição da microbiota intestinal de adultos é relativamente estável em filos em comparação com indivíduos de maior idade. No entanto, com o avançar da idade, podem ocorrer alterações na composição da microbiota intestinal devido à ação de fatores externos (condições de estresse, estilo de vida, uso de medicamentos, exposição a pesticidas, poluentes, hábitos alimentares) e internos (genética do hospedeiro e imunossenescência), resultando em um desequilíbrio da microbiota intestinal relacionado ao aumento da incidência de diversas patologias (COMAN; VODNAR, 2020).

A dieta exerce um papel relevante na iniciação, formação e modulação do perfil populacional dos membros da microbiota intestinal, uma vez que fornece os nutrientes indispensáveis ao metabolismo de diversos grupos de bactérias, atuando como substrato direto por meio de seus nutrientes não digeridose de forma indireta, com os subprodutos resultantes da digestão (ÖZGÜN; UMU; DIEP, 2017).As bactérias colônicasbiotransformam os componentes dietéticos e produzem uma série de metabólitos responsáveis por modular o sistema imunológico e respostas metabólicas do hospedeiro. De maneira recíproca, a dieta modula a composição e a capacidade funcional da microbiota intestinal, o que influencia os processos bioquímicos do hospedeiro, estabelecendo um sistema de interação e interdependência mútua (DANNESKIOLD-SAMSOE *et al*.,2019).

Diante do reconhecimento da importância da microbiota intestinal no estabelecimento e manutenção da saúde humana, o interesse por intervenções dietéticas que possam modular a composição da microbiota intestinal e suas interações com o hospedeiro tem aumentado(HURTADO-ROMERO *et al*., 2020). Neste sentido, a administração de probióticos e prebióticos representam abordagens promissoras para manter ou restauraro equilíbrio da microbiota quando a homeostase é interrompida (disbiose) em consequência de processos patológicos ou da atuação de fatores externos nas diferentes fases da vida(QUIGLEY, 2019).

Probióticos são definidos como microrganismos vivos que quando administrados em quantidades adequadas, conferem benefícios à saúde do hospedeiro (HILL *et al*., 2014). Microrganismos pertencentes aos gêneros *Bifidobacterium*, *Lactobacillus* e gêneros associadoscomumente atendem aos requisitos de segurança para consumo humano como probióticos,sendo considerados GRAS (*Generally Recognized as Safe* - *GRAS*- status)e são amplamente usados na produção de alimentos e comercializados como suplementos alimentares (ZUO; MARCOTTE, 2021).

Lactobacillus sp são microrganimos Gram-positivos, em forma de bastonete, anaeróbico facultativo, não esporulante, tolerante a acidez e catalase-negativo. As espécies mais frequentemente isoladas do intestino humano são a *L. acidophilus*, *Lactobacillus salivarius*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus reuteri* e *Lactobacillus brevis* (BATT; TORTORELLO, 2014). A presença de *Lactobacillus* no colón humano melhora digestibilidade de alimentos e a biodisponibilidade de nutrientes, diminui a má absorção e os efeitos colaterais da intolerância a lactose e promove a liberação de moléculas bioativas com propriedades funcionais que não podem ser acessadas pelo metabolismo humano (MASKE *et al*., 2021).

Particularmente, acolonização de *L. acidophilus*05 está associada a sua capacidade de sobrevivência nas secreções do hospedeiro, resistência contra a atividade antagônica de outros microrganismos e por meio da capacidade de inibir o crescimento e adesão a células epiteliais intestinais hospedeiras, secreção de mucina e degradação dos nutrientes endógenos do hospedeiro (BATT; TORTORELLO, 2014).O seu consumo está associado à regulação do equilíbrio da microbiota intestinal, aumento da imunidade e melhora à resistência contra patógenos (ZHU *et al*., 2021), por meio de mecanismos que envolvem o aumento da abundância relativa de bactérias benéficas e proteção contra infecção intestinal (VEMURI *et*

al., 2018). Por essa razão, tem sido sugerida como uma estratégia promissora para modulação da composição e atividade metabólica da microbiota intestinal humana.

Os prebióticos, por sua vez, são definidos como substratos utilizados seletivamente por microrganismos, que conferem benefícios à saúde do hospedeiro (GIBSON *et al*., 2017). São naturalmente encontrados em frutas, verduras, alimentos fermentados e, assim como os probióticos, podem ser ingeridos através da suplementação (MARKOWIA; ŚLIŻEWSKA, 2017). Para estar de acordo com essa classificação, o prebiótico para uso em alimentos ou como suplemento alimentar deve atender alguns critérios específicos que incluem: ser resistente à acidez gástrica, hidrólise enzimática e a absorção pelo gastrointestinal; ser fermentado seletivamente por microrganismos intestinais; promover a proliferação e atividade metabólica de bactérias benéficas; melhorar a saúde e o bem-estar do hospedeiroe ser estável a condições de processamento (GIBSON *et al*., 2017; WLODARCZYK; ŚLIŻEWSKA, 2021).

A definição atual de prebiótico reconhece que os alvose efeitos dos prebióticos vão além do estímulo de *Bifidobacterium* e *Lactobacillus*,microrganismos frequentemente empregados durante a avaliação de novos ingredientes prebióticos (ZHANG *et al*., 2018).Gêneros bacterianos presentes na microbiota intestinalcomo *Bacteroides*, *Eubacterium*, *Faecalibacterium*, *Roseburia*, *Akkermansia muciniphila* e algumas espécies de *Clostridium*,também estão envolvidos na fermentação de prebióticos e têm sido descritos como microrganismos que apresentam efeitos benéficosno metabolismo dohospedeiro(VERBEKE *et al*., 2015; GIBSON *et al*., 2017; PEREDO LOVILLO; ROMERO-LUNA; JIMÉNEZ-FERNÁNDEZ, 2020).

Atualmente, diversas substâncias são reconhecidas como prebióticos, incluindo os carboidratos não digeríveis, como polissacarídeos (amido resistente, pectina e dextrina) e oligossacarídeos, como frutooligossacarídeos (FOS), oligossacarídeos resistentes como oligossacarídeos do leite humano (HMO), galacto-oligossacarídeos (GOS); frutooligossacarídeos (FOS) e inulina; mananoligossacarídeos, lactulose, polidextrose, entre outros (NASCIMENTO; JUNIOR, 2021;REZENDE; LIMA; NAVES, 2021).

Os frutooligossacarídeos (FOS) são compostos reconhecidamente seguros para o consumo humano (*Generally Recognized as Safe - GRAS*) que estimulam o crescimento e atividade metabólica de grupos de microrganismos benéficos presentes no cólon. Os efeitos do consumo de FOSincluem redução da incidência de infecções gastrointestinais (FLORES-MALTOS *et al*., 2016) e atenuação de efeitos de doenças crônico-degenerativas (FARIAS *et*

al., 2019). Além dos prebióticos supracitados, polidextrose, xilo-oligossacarídeos, ácidos graxos poliinsaturados, peptídeos e compostos fenólicostêm sido descritos como potenciais prebióticos (VALCHEVA; DIELEMAN; 2016; CUNNINGHAM *et al*., 2021).

Os prebióticos podem ser administrados como suplementos alimentares ou incorporadoscomo ingredientes funcionais em diversos produtos alimentícios, incluindo pão (MOLLAKHALILI-MEYBODI *et al*., 2021), biscoitos (DESAI *et al*., 2020a), massas (FICCO *et al*., 2019), leite (DANTAS *et al*., 2021), sorvete (SILVA *et al*., 2020), kefir (ATIK *et al*., 2021), iogurte (YU *et al*., 2021), massas, xaropes,suco de frutas (suspensões de pó em água ou em outras bebidas (CUNNINGHAM *et al*., 2021).

Características estruturais dos substratos prebióticos como grau de polimerização, comprimento da cadeia, composição e tipo de ligação glicosídica determinam sua fermentabilidade via microbiota colônica (TIAN *et al*., 2017; FERREIRA-LAZARTE *et al*., 2019). O metabolismo deprebióticos como fibras dietéticas e oligossacarídeos depende da ação de glicosidases celularese extracelulares e dos sistemas de transporte presentes em espécies bacterianas, que exercem função complementar na degradação de substâncias complexas durante o metabolismo humano (PEREDO-LOVILLO*;* ROMERO-LUNA; JIMÉNEZ-FERNÁNDEZ, 2020; MASKE *et al*., 2021).

O consumo de prebióticos está associado à modulação da resposta imunológica, aumento da absorção de minerais, melhora na função intestinal, efeitos positivos no metabolismo energético, redução do risco de infecções intestinais e estão diretamente relacionados ao seu impacto na composição da microbiota intestinal e síntese de metabólitos derivados (SANDERS *et al*., 2019; FARIAS *et al.*, 2019)..

A via de fermentação primária dos prebióticos fornece o piruvato a partir de hexoses (OLIPHANT; ALLEN-VERCOE, 2019) que é posteriormente utilizado por bactérias do colônicas para produção de CO_2, AGCC e outros compostos (SLAVIN, 2013). Os principais metabólitos finais da fermentação bacteriana de prebióticos são os ácidos graxos de cadeia curta(AGCC), incluindo o acetato, propionato e butirato, relacionados a diversos benefícios à saúde humana (CAMPOS *et al*., 2020).

As funções biológicas dos AGCCincluem a manutenção da integridade do epitélio intestinal, regulação do pH colônico, síntese de muco, fornecimento de energia para os colonócitos, melhora do metabolismo da glicose (SNELSO *et al*., 2019), da função imunológica e estresse oxidativo (LIU *et al*., 2021), bem como da resistência contra a invasão por patógenos (BLAACK *et al*., 2021). A inibição do crescimento de bactérias patogênicas no

ambiente intestinal ocorre devido a liberação de íons H^+ e consequente redução do pH intestinal, pela competição de energiae através da síntese de peptídeos antimicrobianos (MA *et al*., 2021).

O acetato é considerado o AGCC mais abundante (60%-75%) do total produzido, embora a quantidade e a proporção relativa de cada AGCC dependam da dieta, da composição da microbiota intestinal eexpressão de transportadores (MACFARLANE; MACFARLANE, 2003).O butirato é predominantemente utilizado como fonte de energia pelos colonócitos, desempenhando papel crucial na manutenção do crescimento e diferenciação celular (FELIZARDO *et al*., 2019), enquanto o propionato e o acetato são absorvidos pela veia porta e metabolizados por hepatócitos (SNELSON *et al*., 2019).Além da produção de AGCC, os prebióticos promovem a síntese de ácidos orgânicos (ácido lático, succínico e málico) egases (H_2, H_2S, CO_2 e CH_4)que contribuem para redução do pH colônico (VERBEKE *et al*., 2015; NSOR-ATINDANA *et al*., 2020).

Os compostos fenólicos são comumente classificados em dois grupos principais, que incluem flavonóides e não flavonóides (MOSELE; MACIÀ; MOTILVA).Devido à sua abundância nos alimentos, esses compostos fazem parte da dieta humana e evidências científicas recentes sugerem que o consumo dessas moléculas atua como importante modulador da microbiota intestinal (SAYERS;WIJEYESEKERA; GIBSON, 2021).A biotransformação de compostos fenólicos derivados da dieta também pode exercer efeitos benéficos sobre a modulação da microbiota intestinal, posicionando estes comopotenciaisprebióticos, uma vez que influenciam positivamente na adesão, crescimento, sobrevivência e funcionalidade de microrganismos probióticos (SOUZA *et al*., 2019; SANDERS *et al*., 2019;MOORTHY *et al*., 2020). Os compostos fenólicos são fitoquímicos encontrados em uma diversidade de frutas, verduras, derivados de plantas e microalgas, caracterizados por apresentar um ou mais grupos hidroxila eanéis aromáticos em sua estrutura química,variando desde a uma molécula fenólica simples até um polímero complexo de altopeso molecular (LIU *et al*., 2019; SANTHAKUMAR;AYYAPPAN; RAY, 2020).

O efeito prebiótico desempenhado por compostos fenólicos dietéticos pode sofrer influência de fatores como fonte alimentar, estrutura química do composto associado,assim comodas diferenças individuais na composição da microbiota intestinal do hospedeiro (SERRELI; DEIANA, 2019).Particularmente, a complexidade estrutural e o grau de polimerização dos compostos fenólicos dietéticos afetam sua biodisponibilidade no intestino delgado. Estima-se que apenas 5-10% da ingestão total de compostos fenólicos sejam

absorvidos no intestino delgado, sobretudo conjugados a açúcares ou ácidos orgânicos, liberando agliconas que entram no revestimento das células intestinais, sofrem biotransformação e os produtos metabólicos resultantes são difundidos pelo corpo ou excretados (D'ARCHIVIO*et al*., 2010).

Em contrapartida, as estruturas mais complexas restantes,que compreendemcerca de 90-95% podem alcançar o lúmen do intestino grosso de forma intacta,tornando-se expostos às atividades enzimáticas(α-ramnosidase, β-glucosidase e β-glucuronidase)e interações com microrganismos intestinais, que por sua vez, sintetizam metabólitosde melhor absorçãoassociados a benefícios à saúde humana (KAWABATA *et al*., 2019; BIAN *et al*., 2020; SAYERS;WIJEYESEKERA; GIBSON, 2021).

Embora os compostos fenólicos estejam presentes nos alimentos na forma livre, eles também são encontrados ligados a outros compostos, como a matriz da fibra alimentar (JACKSON; JEWELL, 2017). Considerando o efeito prebiótico de fibras dietéticas, pesquisas sugerem que as fibras presentes nos vegetais podem facilitar o transporte dos compostos fenólicos para o colón humano (SAYERS;WIJEYESEKERA; GIBSON, 2021). As principais biotransformações dos compostos fenólicos realizadas por bactérias intestinais são a fissão do anel, oxidação alfa ou beta, desidrogenação,desglicosilação, desidroxilação e desmetilação, resultando em compostos de menor peso molecular como ácidos benzóicos e ácidos fenil alcanóicos (JOHNSON *et al*., 2019).Os metabólitos fenólicos modificados serão absorvidos pelo corpo ou excretados na urina e nas fezes. Aqueles que são absorvidos podem sofrer metabolismo na fase II antes de serem circulados pelo corpo (MOSELE; MACIÀ; MOTILVA, 2015).

Os mecanismos que envolvem a interação entre os compostos fenólicos e o metabolismo intestinal podem estar associados à interferência com a expressão e atividade enzimática, modificações nas características do ambiente intestinal, mudanças na modulação das vias de sinalização responsáveis pelos efeitos pró-oxidantes, inflamatórios e carcinogênicos, aumento na proteção do epitélio intestinal contra os efeitos negativos de substâncias tóxicas, e /ou a modulação da população microbiana (MOSELE; MACIÀ; MOTILVA, 2015).No entanto, devido ao grande número de compostos fenólicos existentes, o elevado número de produtos derivados da bioconversão e sua interação com a microbiota intestinal, tem sido desafiador associar os efeitos benéficos de compostos fenólicos específicos à saúde humana (DANNESKIOLD-SAMSØE *et al*., 2019).

Avia que revela como os compostos fenólicos estimulam a síntese de AGCC ainda não foi completamente esclarecida, no entanto, a hipótese sugerida é que a proliferação de microrganismos anaeróbios como *Lactobacillus* promova o aumento destes compostos, sobretudo de butirato (LI *et al*., 2019a; ALVES-SANTOS *et al*., 2020). Recentemente, Zheng *et al*. (2021) sugeriram que os AGCC e fenólicos intermediários, produzidos a partir da atividade microbiana, atuam de maneira sinérgica para o alívio da inflamação e melhoria da saúde intestinal, através da supressão de citocinas pró-inflamatórias induzidas em modelo de célula Caco-2.

Embora haja reconhecimento crescente que compostos fenólicos podem modular a microbiota intestinal, ainda não há evidências conclusivas de seu efeito prebiótico especialmente devido a falta de ensaios em humanos que confirmem os achados obtidos pelos estudos *in vitro* e *in vivo*. No entanto, já é consenso que os compostos fenólicos podem estimular o crescimento de microrganismos alvos de prebióticos como *Lactobacillus* spp., *Bifidobacterium* spp., *Akkermansia* spp., *Roseburia* spp. e *Faecalibacterium* spp. e aumentar a produção de AGCC(MOSELE; MACIÀ; MOTILVA, 2015; CUEVA *et al*., 2020).

Considerando a importância da manutenção da microbiota intestinal equilibrada à saúde humanae a presença de fibras, oligossacarídeos, monossacarídeos e compostos fenólicos presentes nas biomassas, as microalgas revelam-se como fortes candidatas a desempenharem efeito prebiótico quando consumidas.

2.2.2Potencial prebiótico de microalgas

Devido à crescente demanda por produtos naturais, alimentos funcionais e ao aumento da conscientização do consumidor sobre os benefícios à saúde, a demanda do mercado global por prebióticos tem expandidonos últimos anos, sendo registrado cerca de 620 mil toneladas no ano de 2015, com estimativa de crescimento anual de aproximadamente 8% até 2024 (GRAND VIEW RESEARCH, 2018). Diante da expansão do conceito de prebióticos para substratos que não se limitam apenas aos prebióticos clássicos estabelecidos (carboidratos não digeríveis), pesquisas recentes têm buscado investigar o potencial prebiótico de diversos alimentos e compostos bioativos isolados, a exemplo de méis (MELO *et al*., 2020), subprodutos do processamento de frutas(MENEZES *et al*., 2020; CAMPOS *et al*., 2020; PRADO *et al*., 2021), farinhas de batata doce (ALBUQUERQUE *et al*., 2021), oligossacarídeos isolados de café (DESAI *et al*., 2020b), B-glucanas de cogumelos (RUTHES

et al., 2021), polissacarídeos e oligossacarídeos isolados de macroalgas marinhas e de microalgas (LEAL *et al*., 2017; CUI *et al*., 2021).

Deacordo com um relatório recente, o mercado global de produtos de algas é estimado em maisde 5,38 bilhões de dólares em 2025.As microalgas são consideradas o grupo mais diversificado de organismos fotossintéticos do planeta. O termo microalgas não possui conotação filogenética, visto que engloba diferentes grupos de organismos eucariontes como as clorofíceas (microalgas verdes) e procariontes como as cianobactérias, que pertencem ao domínio das bactérias, as quais são consideradas microalgas por serem organismos fotossintetizantes (ENZING *et al*., 2014). Esses microrganismos podem sobreviver fotoautotroficamente por meio da fotossíntese, heterotroficamente via o fornecimento de nutrientes ou mixotroficamente, por meio da combinação entre luz e nutrientes fontes de energia (VERMA *et al*., 2020).

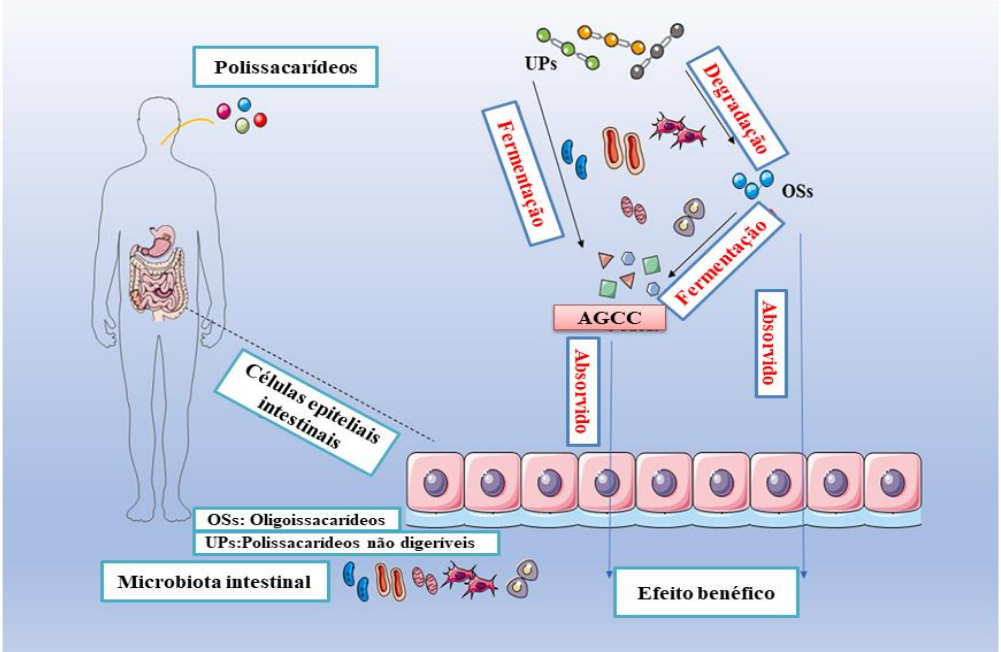
As microalgas são reconhecidas por serem fontes naturais de diversos nutrientes e compostos bioativos, incluindo proteínas, aminoácidos essenciais, lipídios, ácidos graxos poliinsaturados, carboidratos, polissacarídeos, compostos fenólicos, pigmentos, entre outros compostos (FERNÁNDES *et al*., 2021), razão pela qual têm sido amplamente empregadas na formulação de uma variedade de alimentos com propriedades tecnológicas e funcionais atrativas aos consumidores (COLLA *et al*., 2020; MEDEIROS *et al*., 2021). Os produtos alimentares podem ser classificados em dois grupos distintos (COLLA *et al*., 2020).

O primeiro envolve a utilização de biomassas de microalgas desidratadas, especialmente os gêneros*Chlorella* e *Spirulina*, e o segundo diz respeito aos produtos enriquecidos com biomassa microalgal, ou com compostos bioativos derivados. A biomassa de microalgas é comercializada em suplementos dietéticos ou na forma de pós, enquanto os produtos formulados com biomassa microalagal incluem produtos enriquecidos com pigmentos (astaxantina e ficocianina), antioxidantes e ácidos graxos essenciais (ômega-3, ácido docosahexaenóico e eicosapentaenóico) (LAFARGA, 2020).

A biomassa de microalgas é constituída por uma parede celular rígida composta por polissacarídeos estruturais (LEAL *et al*., 2017) que incluem celulose, hemicelulose, substâncias pécticas e glicoproteínas. Estes componentes estão sujeitos à variação em sua composição e/ ou estrutura entre os gêneros, espécies e cepas, que frequentemente ocorre em virtude das diferenças nas condições de cultivo empregadas e/ou estágio de vida celular (BAUDELET *et al*., 2017).

Em média, os polissacarídeos da parede celular (heteropolissacarídeos) de microalgas podem corresponder a 10% do peso da biomassa (BERNAERTS *et al*., 2018). Estes compostos podem conferir resistência à digestão por enzimas presentes no trato gastrointestinal superior, alcançar o colón humano, estimular seletivamente o crescimento de bactérias intestinais benéficas e promover a síntese de metabólitos bacterianos, como AGCC(RAPOSO *et al*., 2016; SONG *et al*., 2021), conforme apresentado na Figura 1.

Figura 1. Degradação de polissacarídeos na microbiota intestinal.



Fonte: Adaptado de Song *et al*. (2021).

Devido à resistência durante a digestão gastrointestinal, os polissacarídeos de microalgas ou seus derivados, incluindo as fibras dietéticas e oligossacarídeos, são potenciais candidatos a desempenharem efeito prebiótico, podendo ser empregados de forma direta (biomassa seca) ou após a extração do composto bioativo a partir da biomassa ou do meio de cultura (nutracêutico) (RAPOSO *et al*., 2016; MORENO *et al*., 2017; LEAL *et al*., 2017). Neste sentido, pesquisas publicadas recentemente têm revelado o potencial prebiótico de compostos isolados de espécies de microalgais ou mesmo de seus extratos.

Leal *et al*. (2017) investigaram o efeito prebiótico de oligossacarídeos isolados da biomassa microalgal das espécies *C. vulgaris* e *S. platensis*pormeio da hidrólise fosfórica. Os oligossacarídeos foram usados como fontes de carbono para o cultivo de*Bifidobacterium animalis*e*Lacticaseibacillus casei*durante 15 dias. Os achadosda pesquisa indicaram que os

oligossacarídeospromoveram crescimento das espécies avaliadas e contribuíram para síntese de ácido lático e ácido acético.

De modo similar, um oligossacarídeo isolado de *S. platensis* foi avaliado quanto ao crescimento de *Lacticaseibacillus paracasei*e *B. animalis* e seu efeito na microbiota intestinal humana *in vitro*. Os resultados obtidos demonstraram queo oligossacarídeo promoveu aumento do consumo de carboidratos e da produção deAGCC (ácidosbutírico evalérico). Além disso, promoveu aumento na abundância, diversidade e composição da microbiota intestinal, estimulando o crescimento dos gêneros *Bacteroides, Escherichia-Shigella* e *Megamonas*(CAI *et al*.,2022).Em outro estudo, a biomassa de *Euglena gracilis* e do seu polissacarídeo isolado promoveuindividualmente ou combinadosaumento do número de células de *Lactobacillus* spp, exibindo atividades prebióticas, enquanto a microalga *Chlamydomonas* spp não promoveu aumentono crescimento de *Lactobacillus* spp. mesmo com tempo de incubação prolongado (BHATTAD; KORADIYA; PRAKASH, 2021).

Pesquisas utlizando modelos experimentais *in vivo* têm avaliado o impacto da administração oral de biomassa de microalgas ou de seus componentes isolados, sobretudo os polissacarídeos, na composição da microbiota intestinal de animais. Guo *et al*. (2021) investigaram os efeitos da administração oral de polissacarídeosisolados das microalgas*Chlorella pyrenoidosa* e *S. platensis* (400 mg/ kg/ dia) durante dez semanas e observaram efeito efeito anti-obesogênico. Esses efeitos foram relacionados ao aumento da lipólise, diminuição da lipogênese hepática, bem como da significativa correção da disbiose, promovida através do estímulo de bactérias benéficas,diminuição de bactérias patogênicas e síntese de AGCC.

Ma *et al*. (2019) avaliaram o efeito da administração de polissacarídeos isolados da *S. platensis* na constipação e microbiota intestinal de camundongos. Os achados obtidos demonstraram significativa redução da constipação, melhora no peristaltismo intestinal, recuperação das vilosidades intestinais danificadas e modução da microbiota intestinal dos animais com umento na proporção de gêneros bactérias benéficos (*Lactobacillus*, *Akkermansia*, *Arthromitus*, *Butyricimonas*, *Candidatus* e *Prevotella*) e redução de gêneros nocivos (*Dorea* e *Clostridium*).

Em ratos idosos, a suplementação dadieta padrão comde 5,0 % biomassa seca de*S. platensis* durante o período de seis semanas promoveu modificações na composição da microbiota intestinal, como aumento nas contagens de *Roseburia* e *Lactobacillus* eredução de

marcadores hepáticos de estresse oxidativo e inflamatórios relacionados à imunidade intestinal e a outros sítios sistêmicos (NEYRINCK *et al*., 2017).

Li *et al*. (2019c) avaliaram o efeito da administração de extrato etanólico de *S. platensis* 55%no metabolismo lipídico de ratos hiperlipidêmicos. Os resultados da pesquisa indicaram aumento do metabolismo bioenergético, redução nos níveis de lipídios séricos e hepáticos e diminuição das abundâncias de *Turicibacter*, *Clostridium*_XlV e *Romboutsia*, microrganismos que apresentam correlação positiva com os indicadores do metabolismo lipídico, enquanto aumentou a abudância dos gêneros bacterianos*Alloprevotella*, *Prevotella*e *Barnesiella*. Os achados descritos acima foram atribuídos à presença de ácidos graxos poliinsaturados identificados no extrato em associação com a regulação da microbiota intestinal.

Uma proposta semelhante foi desenvolvida porWan *et al*. (2018), que investigaram a administração oral de umextrato etanólico a 55% obtido da microalga *Chlorella pyrenoidosa* no metabolismo lipídico e regulação damicrobiota intestinal em ratos hiperlipidêmicos. De maneira geral, os resultados da pesquisa indicaram que o extrato etanólico atenuou a dislipidemia através regulação do perfil lipídico sanguíneo e da inibição do acúmulo de lípidos hepáticos e da esteatose. Além disso,modulou a microbiota intestinal dos animais através do aumento da abundância dos gêneros bacterianos*Alistipes*, *Prevotella*, *Alloprevotella* e *Ruminococcus* e diminuição da abundância dos gêneros*Turicibacter* e *Lachnospira*.

Além do extrato lipídico de microalgas, peptídeos isolados também já foram investigados. O estudo desenvolvido por Hua *et al*. (2018)avaliou os efeitos de um hidrolisado protéico sobre o metabolismo lipídico e composição da microbiota intestinal de ratos alimentados com dieta hiperlipídica. Os resultados apresentados mostraram que ohidrolisado protéico reduziu os níveis de triglicerídeos, colesterol total, lipoproteína de baixa densidade (LDL-c), alanina aminotransaminase (ALT), aspartato aminotransaminase (AST) e aumentou a concentração da lipoproteína de alta densidade (HDL-c) no soro e no fígado dos animais após oito semanas de intervenção. Além disso, o tratamento com SPPHpromoveu aumentona abundância de bactérias benéficas.

Embora a maioria dos estudos evidenciem o potencial prebiótico de polissacarídeos, oligossacarídeos, ácidos graxos e peptídeos isolados de biomassas microalgais, o potencial efeito prebiótico da biomassa de microalgas não deve ser restrito a presença destes compostos, podendo também estar associado à presença de compostos fenólicos e de outros

constituintes existentes na biomassa integral (RAPOSO *et al*., 2016). Até o presente, permanece escasso o conhecimento acerca do efeito prebiótico de biomassas microalgaisintegrais, associados ou não a probióticos, na composição, diversidade e atividade metabólica de grupos bacterianos presentes na microbiota intestinal de indivíduos saudáveis, quando submetidos à fermentação *in vitro*.

2.2.3Microalgas inseridas no estudo

A microalga *Chlorella vulgaris*, reconhecida como segura para consumo humano [*Generally Recognized as Safe* (GRAS, *United States Food and Drug Administration* GRN 000396) FDA, 2011], é frequentemente isolada de ambientes de água doce (dulcícola). Os organismos pertencentes à espécie *C. vulgaris* são unicelulares, verdes e estão incluídas na classe Chlorophyceae. A morfologia celular da *C. vulgaris* é globular (3–8 µm em diâmetro) ou colonial (Figura 2A) (WILSON; HUNER, 2000) e ao longo do seu crescimento podem acumular diversos compostos bioativos, incluindo polissacarídeos sulfatados, proteínas, ácidos graxos, carotenoides, compostos fenólicos, vitaminas e outros compostos, o que a torna fonte de ingredientes naturais relevantes para formulação de alimentos funcionais e extração de compostos bioativos (SYLWIA; LŻBIETA, 2020; FERNÁNDEZ *et al*., 2021)

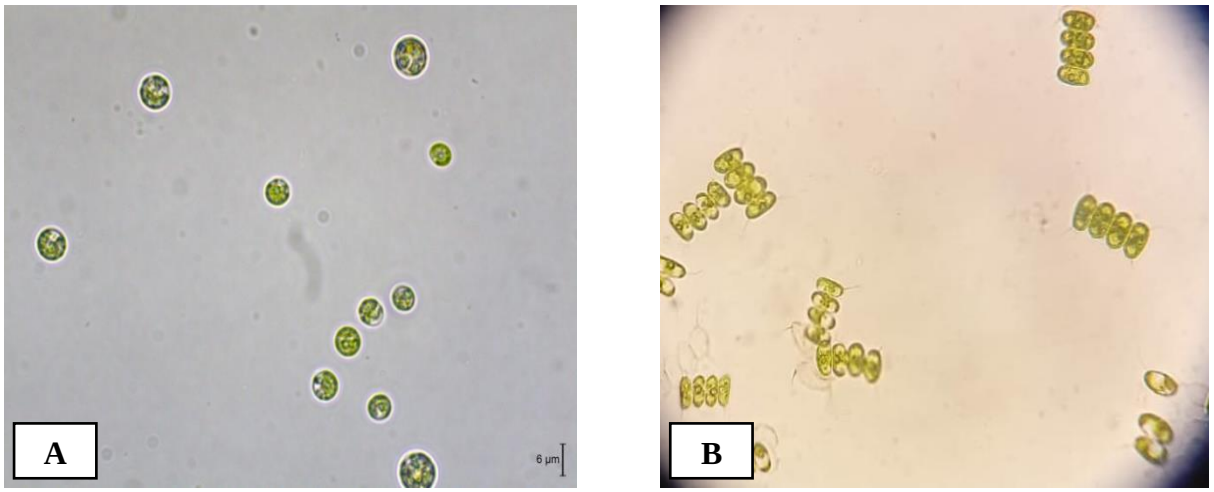
Particularmente, os polissacarídeos da parede celular de *Chlorella* spp são predominantemente constituídos por amido que apresentam baixa digestibilidade. Além do amido, as espécies apresentam uma parede celular rígida embutida em uma matriz polimérica mais flexível e hidrolisável. A espécie *C. vulgaris*, contém a glucosamina, como um glicano semelhante à quitina em sua composição. A matriz de açúcar é dominada por resíduos de açúcar neutros como ramnose(FERREIRA *et al*., 2020).Recentemente, os polissacarídeos têm recebido considerável atenção devido aos de promoção à saúde, associados à imunomodulação, atividade antioxidante, antitumoral e neuroprotetor (YUAN et al., 2020).

O gênero *Desmodesmus*, considerado importante componente do fitoplanctôn (HENTSCHKE; TORGAN, 2010), faz parte da família *Scenedesmaceae* e foi elevado à categoria de gênero devido a sua plasticidade morfológica (ROSINI; SANT'ANNA; TUCCI, 2013). A biomassa de *Desmodesmus* spp. tem sido bastante utilizada na extração de lipídeos para a produção de biocombustíveis (GONZÁLEZ-BALDERAS *et al*., 2020) e na síntese de bioetanol, devido a presença de açúcares fermentáveis (RIZZA *et al*., 2017).

Embora o conhecimento sobre a aplicação de *Desmodesmus* spp. em alimentos seja limitado, informações sobre seu potencial uso foram descritas por Pozzobon *et al*. (2020), que

apontaram a síntese de ácidos graxos essenciais (oléico, linoléico e linolênico) e luteína, em sistemas de cultivo elaborados com privação de nitrogênio. A espécie *Desmodesmus maximus*,considerada de importância industrial, é constituída por quatro células oblongas que apresentam disposição linear (Figura 2 B), extremidades arredondadas e um espinho em cada polo celular (SOUZA; FELISBERTO, 2014).

Figura 2. Micrografias de espécies de microalgas dulcícolas (clorofíceas).



Legenda: (A)*Chlorella vulgaris*; (B)*Desmodesmus maximus*.
Fonte: Laboratório de Ambientes Recifais e Biotecnologia com Microalgas. As espécies apresentadas foram isoladas de um filtro de água doce localizado no município de João Pessoa-PB e de um balneário localizado no estado do Rio Grande do Norte, respectivamente.

A espécie *S. platensis*, também conhecida como *Arthrospira platensis*, é considerada uma cianobactéria unicelular filamentosa, com coloração verde azulada e frequentemente isolada de água doce (Figura 3A). Os organismos pertencentes a estas espécies são procariontes, com metabolismo fotoautotrófico e suas semelhanças com plantas superiores e bactérias fizeram destes microrganismos modelos ideais para pesquisas em várias áreas da biologia (GUPTA *et al.*, 2017). Embora mais de 50 espécies de *Spirulina* tenham sido identificadas, apenas duas espécies (*S. platensis* e *S. maxima*) são cultivadas em grande escala no mundo (SONIA; SUDHAKAR; RANAB, 2017).

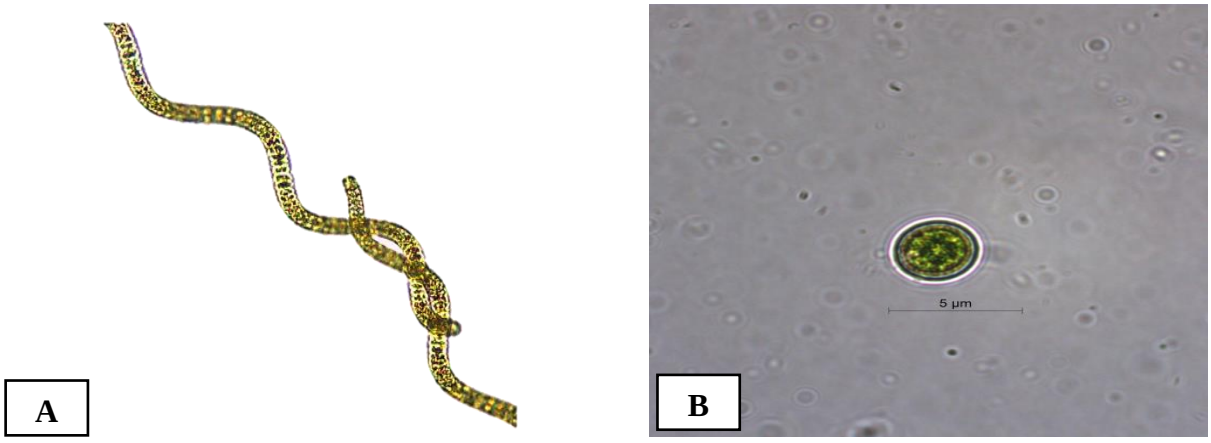
Spirulina spp. é classificada como segura para o consumo humano [Generally Recognized as Safe (GRAS, United States Food and Drug Administration GRN 000127) FDA, 2011] e tem longo histórico de uso na alimentação humana. A biomassa de*Spirulina* spp. contém aproximadamente 55–70% de proteínas com elevado índice de digestibilidade (≥ 89% em relação à caseína pura) e distribuição equilibrada de aminoácidos essenciais (REBOLEIRA *et al*., 2019). Além desses constituintes, a biomassa integral de *Spirulina* spp.

apresenta cerca de 18% de lipídeos (ácidos graxos poliinsaturados), 25% de carboidratos, entre outros compostos bioativos que incluem pigmentos, vitaminas, minerais e compostos fenólicos (ANVARA; NOWRUZI, 2021).

Devido a composição nutricional e ao seu consumo estar associado à prevenção de doenças crônicas não transmissíveis (GRIFFITHS *et al*., 2016), além das propriedades terapêuticas diversas (BOHÓRQUEZ-MEDINA *et al*., 2021), a biomassa de *S. platensis*tem sido reconhecida pela Organização Mundial de Saúde (OMS) como "superalimento", comercializado como suplemento nutricional na forma de pó seco, comprimidos, cápsulas ou incorporada a matrizes alimentares (LIESTIANTY *et al*., 2019, LAFARGA, 2019; MEDEIROS *et al*., 2021).

Pertencente ao grupo das clorofíceas, a *Chlorococcum* spp é uma microalga unicelular, esférica (cerca de 10 μm de diâmetro) da família Chlorococcaceae (Figura 3 B) (BERBEROGLU; GOMEZ; PILON, 2009). A microalga *Chlorococcum* spp possui a capacidade de acumular nutrientes, sobretudo carboidratos e lipídios, podendo ser empregada para a obtenção de compostos bioativos derivados aplicáveis em alimentos ou como nutracêuticos (SASSI *et al*., 2019). A biomassa desse gênero é considerada fonte de pigmentos (clorofilas e carotenoides) e de compostos fenólicos (BABADI *et al*., 2020), que quando extraídos podem apresentaratividade antioxidante e antimicrobiana frente patógenos bacterianos (CHOOCHOTE *et al*,. 2014; ELSHOBARY *et al*., 2020).

Figura 3. Micrografias de espécies de microalgas dulcícolas(cianobactéria e clorofícea).



Legenda: (A)*Spirulina platensis*; **(**B) *Chlorococcum sp cf hypnosporum*.
Fonte: Laboratório de Ambientes Recifais e Biotecnologia com Microalgas. A microalga S. *platensis* foi cedida da coleção brasileira de microalgas da Universidade Federal Fluminense, enquanto a *C.* sp cf *hypnosporum* foi isolada de um açude localizado no município de Cuité-PB.

Chlorella e *Spirulina* são os gêneros mais estudados e empregados para o desenvolvimento de alimentos funcionais e nutracêuticos (ANDRADE *et al*., 2018; KOYANDE *et al*., 2019). Neste sentido, o presente estudo buscou, diante da diversidade microalgal existente, contribuir para elucidar lacunas acerca da funcionalidade destas duas espécies e ampliar o potencial funcional de novas espécies de microalgas.

3MATERIAL E MÉTODOS

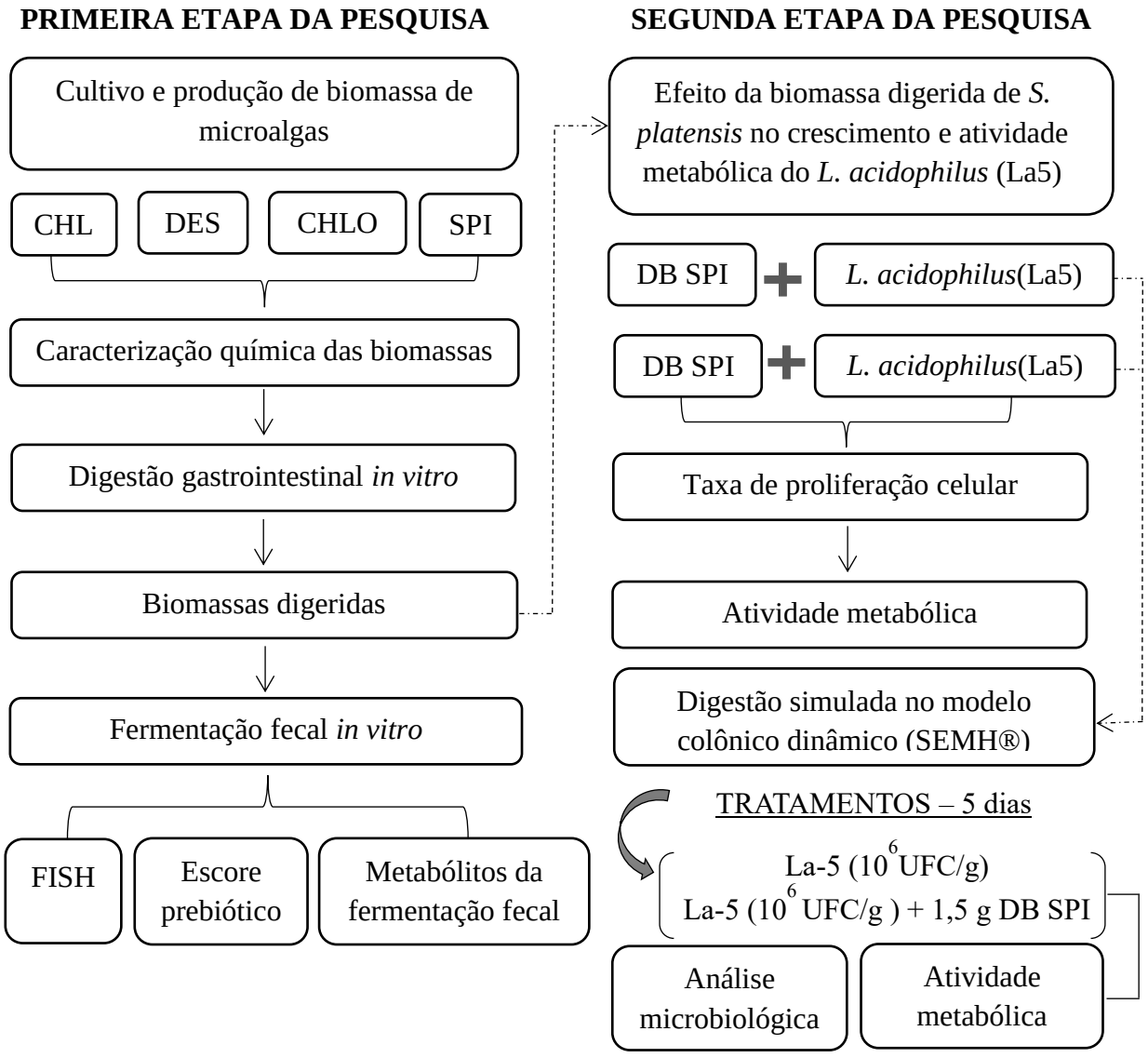
3.1 LOCAIS DE DESENVOLVIMENTO DA PESQUISA E DELINEAMENTO EXPERIMENTAL

Os experimentos que compõe a primeira etapa deste estudo foram desenvolvidos no Laboratório de Ambientes de Recifais e Biotecnologia com Microalgas (LARBIM/UFPB) e compreendem o cultivo de microalgas, o processamento do inóculo ea caracterização química das biomassas microalgais obtidas. Asanálises de digestão gastrointestinal *in vitro*das biomassas microalgais foram executadas no Laboratório de Processos Microbianos em Alimentos (LPMA/UFPB). Após a obtenção da biomassa digerida, realizou-se a avaliação do efeito prebiótico em sistema de fermentação *in vitro*, através deanálises de fluorescência de hibridização *in situ* (FISH) acoplada a citometria de fluxo multiparamétrica (CFM) e de metabólitos da fermentação fecal, no Laboratório de Microbiologia e Bioquímica de Alimentos (LMBA/UFPB). As análisesde determinação de perfil cromatográficoforam desenvolvidas em parceria com o Laboratório Experimental de Alimentos (LEA), localizado no Departamento de Tecnologia de Alimentos, do Instituto Federal do Sertão Pernambucano (IF Sertão-PE), Petrolina – PE.

Considerando os resultados obtidos nos ensaios experimentaisdescritos na primeira etapa desta pesquisa, foi delineado um novo desenho experimental que consiste na segunda etapadeste estudo, desenvolvido em duas fases distintas. A primeira foi executada no LPMA/UFPB e correspondeà avaliação do efeito da biomassa digerida de *S. platensis* na taxa de proliferação de *L. acidophilus*(La5)e síntese de metabólitos, quando comparado ao FOSdurante a fermentação em meio de cultura. Asegunda etapafoi desenvolvida em parceria com o Laboratório de Microbiologia em Alimentos, localizado na Faculdade de Ciências Farmacêuticas (Departamento de Ciência dos Alimentos e Nutrição, UNESP, Araraquara) ecorrespondeàavaliação dos efeitos da administração da biomassa de *S. platensis* em associação com *L. acidophilus*(La5)na composição da microbiota intestinal de indivíduos

saudáveis de meia idade e síntese de metabólitos, através do Simulador de Ecossistema Microbiano Intestinal Humano (SEMH®).A figura 5 representa o delineamento experimental descrito acima.

Figura 4. Delineamento experimental da pesquisa.



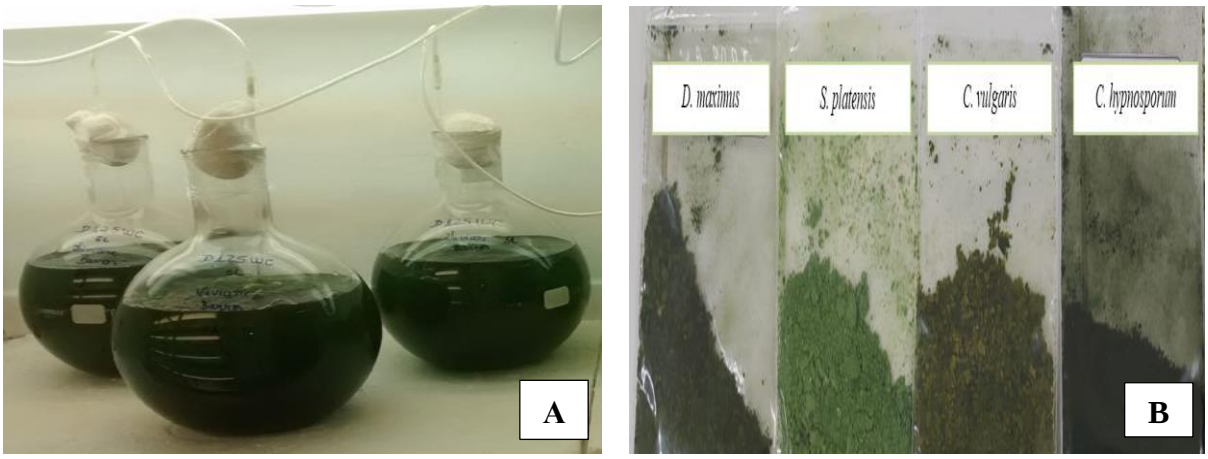
Fonte: Autor, 2022. Legenda: CHL: *Chlorella vulgaris*;DES: *Desmodesmus maximus*; CHLO: *Chlorococcum sp cf hypnosporum*;SPI: *Spirulina platensis*; FISH:Fluorescência de hibridização *in situ*; DB SPI: biomassa de *S. platensis* digerida; SEMH®: Simulador de Ecossistema Microbiano Intestinal.

3.2SELEÇÃO DAS ESPÉCIES DE MICROALGAS, CONDIÇÕES DE CULTIVO E PRODUÇÃO DE BIOMASSA

Foramestudadas quatro espécies de microalgasdulcícolas,nomeadamente*Chlorella vulgaris*(CHL),*Desmodesmus maximus*(DES),*Chlorococcum sp cf hypnosporum*(CHLO) e *Spirulina platensis* (SPI),pertencentes à coleção de culturas do Laboratório de Ambientes de Recifais e Biotecnologia com Microalgas (LARBIM/UFPB).Essas espécies foram previamente selecionadas devido ao status GRAS (*Generally recognized as Safe*) e aspectos de segurança relatados (não toxigênicas), além de serem fontes relevantes de compostos bioativos,sobretudo carboidratos (oligossacarídeos) e compostos fenólicos, que poderiam exercer efeitos prebióticos (ENZING*et al*., 2014; SASSI *et al*., 2019).

Os cultivos foram realizados em triplicatas em balões de fundo chato com capacidade de seis litros, contendo cinco litros de meios sintéticos(Figura 5 A). As microalgas CHL e DES foram cultivadas em meio WC (GUILLARD; LORENZEN, 1972), enquanto CHLO e SPI foram cultivados em meio Zarrouk (ZARROUK, 1966). As condições de cultivo foram temperatura de 25 ± 1 °C, iluminação de 4,5 ± 0,5 K lux usando lâmpadas fluorescentes de 40 W sob fotoperíodo de 12 h e aeração contínua de 0,12 L /h.Os cultivosforam acompanhados diariamente por contagens celulares em câmaras Fuchs-Rosenthal e Sedgewick-Rafter (para a espécie filamentosa *S. platensis*) e analisados em microscópio binocular Leica. Similarmente, foram realizadas análises de fluorescência "*in vivo*" do inóculo microalgal a cada 24 h, em Fluorômetro Turner Design 10005R.

Figura 5. Cultivo e produção de biomassa de microalgas em escala laboratorial.



Fonte: Autor, 2022. Legenda: (A) Cultivo de microalgas em meio sintético; (B)Biomassa de microalgas desidratadas.

Após atingir a fase estacionária (aproximadamente 11-14 dias), os cultivos foram interrompidos e em seguida as biomassas de CHL, DES e CHLO foram concentradas em centrífuga refrigerada (18 ºC) para obtenção da biomassa úmida. A biomassa de *S. platensis*

foi obtida por meio da filtração em um filtro de malha de 60 μm, devido ao seu diâmetro celular. As biomassas úmidas concentradas foram individualmente congeladas em ultrafreezer (-30ºC), liofilizadas utilizando um liofilizador de bancada (modelo Terroni, LD 1500, São Carlos, SP, Brasil) sob (temperatura de -55 ± 2 °C, pressão do vácuo <138 μHg, velocidade 1 mm/h), por aproximadamente 12 horas para alcançar uma umidade residual de 8%. Posteriormente, as biomassas desidratadas foram armazenadas a -18 °C em sacos de polietileno hermeticamente fechados (Figura 5 B).

Os parâmetros relacionados à cinética de crescimento microalgal foram calculados e incluem o tempo de cultivo (dias);a velocidade de crescimento celular (*k*),calculada conforme a equação descritaporStein (1973);a produtividade diária de biomassa microalgal (PB) que se refere à concentração de biomassa acumulada no cultivo(g/ L), determinada pela razão entre o total de biomassa desidratada obtida e os dias de cultivo; e orendimento máximo de células, que corresponde à densidade celular calculada através da diferença entre a densidade celular obtida no final do cultivo e a densidade celular do início do cultivo (inóculo) (CALIXTO *et al*.,2016).

3.3DETERMINAÇÃO DE FIBRA ALIMENTAR, AÇÚCARES, OLIGOSSACARÍDEOS E COMPOSTOS FENÓLICOS NA BIOMASSA DE MICROALGAS

Os teores de fibra alimentar total, solúvel einsolúvel foram quantificados pelo método enzimático-gravimétrico (Sigma-Aldrich; St. Louis, EUA) (método 991.43; AOAC, 2016).Os oligossacarídeos foram obtidos por sonicaçãoe hidrólise enzimática,conforme os procedimentos descritos por Leal *et al*. (2017), com pequenas adaptações. Resumidamente, 500 mg de biomassa de microalgas liofilizadas foram misturadas em uma solução tampão de acetato (pH 6; 20 g /L) e sonicadas a 40 kHz (Lavadora Ultrassônica Soniclean 6; Sanders Medical, Brasil) por 10 min. As amostras sonicadas foram adicionadas 0,4 mg/ mL de endo-1,3-β-Glucanase (EC: 3.2.1.39, Megazyme) a 45 °C e mantidas em agitador orbital a 200 rpm durante 6 h. Em seguida, as amostras foram centrifugadas (4500 × g, 15 min, 4 °C), o sobrenadante foi coletado e filtrado emfiltro de membrana Millex-HA de 0,22 μm (Millipore Co., Bedford, MA).

Os oligossacarídeos foram identificados por análise de Cromatografia Líquida de Alta Eficiência (HPLC) usando um cromatógrafo (1260, Agilent Technologies, EUA) acoplado a um detector de arranjo de diodos (DAD) (Agilent G1315D) e detector de índice de refração

(RID) (Agilent G1362A). As condições analíticas adotadas foram uma coluna Synergi Hydro-RP C18 com *endcapping* polar (150 × 4,6 mm, 4 μm) (Phenomenex, Torrance, CA, EUA) a 35 ° C. O fluxo de solvente foi de 0,7 mL/ min usando um gradiente de água ultrapura e acetonitrila em um tempo total de execução de 21 min (LIMA *et al*., 2019).

Os monossacarídeos foram obtidos por hidrólise ácida. Para tanto, a biomassa liofilizada (25 mg) foi adicionada de 72% (p/ p) de ácido sulfúrico (1:10; p / v) e mantida por 1 h a 30 °C em banho-maria (SL-150; Solab, São Paulo, Brasil). Posteriormente, foram adicionados 7 mL de água 18,2 MΩ, e a mistura foi submetida a uma nova extração a 121 °C em autoclave por 1 h. No final das extrações, as amostras foram neutralizadas com carbonato de cálcio (pH 6–8) e filtradas com um filtro Millex-HA de membrana de 0,22 μm (Millipore Co., Bedford, MA, EUA) (DONG*et al*., 2016). Os filtrados obtidos foram analisados por HPLC-DAD-RID usando uma coluna Agilent Hi-Plex H (7,7 × 300 mm, 8 μm) e 4 M H_2SO_4 em água ultrapura como fase móvel (taxa de fluxo 0,5 mL/min) (COELHO *et al*., 2018).

Para extraçãodos compostos fenólicos, 1,5 g de biomassa liofilizada foram homogeneizadas com etanol 80% (P.A) [1: 3 (m /V)] em um agitador orbital a 300 rpm por 20 min. Posteriormente, a mistura foi centrifugada (4500 × g, 15 min, 4 ° C), e o sobrenadante (extrato fenólico) foi coletado. Este procedimento foi repetido duas vezes e os sobrenadantes homogeneizados. A amostra foi filtrada em papel de filtro (Whatman n°1; Sigma-Aldrich, St. Louis, MA, EUA), evaporada a 60 °C, ressuspensa em água destilada estéril e filtrada em filtro Millex-HA de membrana de 0,22 μm (SCAGLIONI*et al*., 2018).

Os extratos obtidos foram injetadosem cromatógrafo de acordo com as condições descritas por Padilha *et al*. (2017). A separação cromatográfica dos compostos fenólicos foi realizada utilizando uma coluna Zorbax Eclipse Plus RP-C18 (100 × 4,6 mm, 3,5 μm) e a pré-coluna Zorbax C18 (12,6 × 4,6 mm, 5 μm). A temperatura da coluna foi ajustada para 35 °C e as fases móveis foram compostas por água acidificada (pH 2) com ácido fosfórico 0,1 mM / L (fase A) e metanol acidificado com ácido fosfórico 0,5% (fase B). A taxa de fluxo foi mantida a 0,8 mL/min. As aquisições de dados do DAD foram processadas usando o software OpenLAB CDS ChemStation Edition (Agilent Technologies).

Os picos de oligossacarídeos, monossacarídeos e compostos fenólicos foram identificados pela comparação de seus tempos de retenção com os de padrões externos (Sigma Aldrich). A quantificação foi realizada por meio de curvas de calibração do padrão externo de acordo com os métodos validados descritos por Coelho *et al*. (2018) para açúcares, Lima *et al*. (2019) para oligossacarídeos e Padilha *et al*. (2017) para compostos fenólicos. Todos os

compostos analisados apresentaram curvas de calibração com R2 ≥ 0,998. A pureza espectral de pico foi verificada usando a ferramenta Limiar para garantir a precisão da identificação em comparação com o padrão externo. Os compostos apresentaram os seguintes tempos de retenção (min): procianidina B1 (6,49), catequina (7,42), procianidina B2 (7,89), ácido clorogênico (8,45), glicose (8,78), galato de epigalocatequina (8,88), frutose (9,33), ramnose (9,79), ácido siríngico (9,84), 1-kestose (4,38), nistose (7,95), rafinose (4,04), epicatequina (9,86), epicatequina galato (13,08), procianidina A2 (14,83), miricetina (17,60), quercetina 3-glicosídeo (22,39), rutina (23,03), hesperidina (23,04), cis-resveratrol (25,60), kaempferol 3-glucosideo (25,60) e naringenina (28,29).

3.4DIGESTÃO GASTROINTESTINAL *IN VITRO* DE BIOMASSAS DE MICROALGAS

As biomassas de microalgas foram submetidas à digestão *in vitro*, simulando a fase oral, gástrica e intestinal, seguindo o protocolo da INFOGEST (MINEKUS *et al*., 2014) para obtenção de material com características semelhantes àqueles que atingem o cólonhumano (GUERGOLETTO *et al*., 2016). Estudos anteriores recomendam a digestão da biomassa antes de serem submetidas à fermentação colônica para avaliação de suas propriedades prebióticas (JIN *et al*., 2020). Após a digestão, as soluções contendo a fase final da digestão (entérica) foram dialisadas por 24 h contra NaCl 0,01 M em5 ± 0,5°C (GUERGOLETTO *et al*., 2016). A biomassa digerida foi congelada(-18 °C), liofilizada e armazenada (-20 ± 0,2 °C) em sacos de polietileno hermeticamente fechados. A membrana de diálise, enzimas (α-amilase, lipase, pepsina, pancreatina), sais biliares e todos outros reagentes foram adquiridos da Sigma-Aldrich.

3.5ELABORAÇÃO DO INÓCULO FECAL HUMANO E SISTEMA DE FERMENTAÇÃOUTILIZADO NA PRIMEIRA ETAPA DA PESQUISA

3.5.1 Procedimentos éticos

Em conformidade com a Resolução 466/12 (CNS-MS, 2012), responsável por aprovar as diretrizes e normas regulamentadoras de pesquisas envolvendo seres humanos, esse estudo foi submetido à apreciação pelo Comitê de Ética e Pesquisa em Seres Humanos do Centro de Ciências daSaúde da Universidade Federal da Paraíba – CEP/CCS, e obteve parecer favorável

ao seu desenvolvimento (CAAE n° 4.563.908), conforme apresentado no Anexo A. Os indivíduosque voluntariamente participaram da pesquisa foram esclarecidos a respeito do tema da pesquisae devidamente orientados para assinatura do Termo de Consentimento Livre e Esclarecido (Anexo B).

3.5.2 Coleta das amostras fecais

Amostras fecais frescas doadas por quatro voluntários adultos saudáveis (dois homens e duas mulheres, com idades entre 19 e 36 anos) foram utilizadas neste estudo após a aprovação do Comitê de Ética e Pesquisa em Seres Humanos do Centro de Ciências da Saúde da Universidade Federal da Paraíba. Os critérios de inclusão estabelecidos foram pessoas que não sofressem de nenhuma doença do gastrointestinal ou do cólon, seguissem dieta onívora regular sem o uso de alimentos probióticos, probióticos concentrados ou prebióticos e sem uso de antibióticos ou medicamentos durante os seis meses anteriores ao estudo. Os doadores receberam instruções específicas para a coleta/ armazenamento adequado das amostras, além de um kit higiênico contendo frascos coletores estéreis, álcool 70%, espátulas eluvas descartáveis. Os doadores foram instruídos sobre a correta higienização das mãos e manuseio asséptico das amostras. As fezes coletadas foram dispostas em frasco com sistema gerador de anaerobiose (AnaeroGen, Oxoid, Basingstoke, Inglaterra). Osexperimentos foram iniciados imediatamente após a chegada das fezes para garantia de uma microbiota fecal fresca.

3.5.3 Elaboração do inóculo fecal humano

Para os experimentos, a mesma quantidade de fezes de cada doador foram reunidas(1:1:1:1),para formar um único inóculo (120 g) e misturadas com solução salina tamponada com fosfato estéril (PBS; 0,1 M; pH 7,4; 1:10, p / v) sob agitação (200 rpm) por 2 min (RODRIGUES *et al*., 2016). A mistura fecal obtida foi pré-cultivada (37 ± 0,5 °C) em 1 L de meio estéril (121 °C, 1 atm, 15 min), elaborado com 10 g de triptona, 5 g de extrato de levedura, 10 g de NaCl, 5 g de glicose, 6 g de lactose e água destilada.Após 18 horas de cultivo, aproximadamente 120 mL da pré-cultura foi filtrado utilizando uma camada tripla de gazes estéreis para remoção de partículas maiores, seguindo por armazenamento refrigerado (37 ± 0,5 °C) em frascos anaeróbios estéreis.

3.5.4 Preparação do meio de fermentação colônica*in vitro*

Para a etapa de fermentação foi utilizado 1 L de meio de crescimento estéril, com a seguinte composição: 4,5 g de NaCl, 4,5 g de KCl, 1,5 g de $NaHCO_3$, 0,69 g de $MgSO_4$, 0,8 g de L-cisteína HCl, 0,5 g de KH_2PO_4, 0,5 g K_2HPO_4, 0,4 g de sais biliares, 0,08 g $CaCl_2$, 0,005 g de $FeSO_4$, 1 mL de Tween 80 e 4 mL de solução de resazurina (0,025%, p/v) como indicador anaeróbio. As biomassas microalgais foram submetidas à fermentação *in vitro* com o pré-cultivo do inóculo fecal.Cada lote foi composto por 40% do meio de fermentação (v/v), 40% de inóculo fecal humano combinado (v/v) e 20% da biomassa digerida de CHL, DES, CHLO ou SPI (m/v). A fermentação foi realizada a 37 °C por 48 h em condições anaeróbias (AnaeroGen). Um lote de controle positivo foi utilizado em substituição da biomassa de microalgas digeridas por FOS (20%, p /v), um dos compostos prebióticos mais reconhecidos. O lote sem adição de biomassa de microalgas digeridas ou FOS constituiu o controle (MENEZES*et al*., 2021). Os sais e reagentes usados no meio de fermentação foram adquiridos da Sigma-Aldrich.

3.5.5 Fluorescência de hibridização *in situ*(FISH) acoplada com citometria de fluxo multiparamétrica (CFM)

As modificações promovidas por cada biomassa microalgal digerida ou FOS nos grupos da microbiota fecal durante a fermentação foram avaliadas por FISH acoplado a CFM. As análises de FISH foram realizadas com cinco sondas marcadas com Cy3 fluorescentes (oligonucleotídeos) capazes de hibridizar a região específica do gene 16S rRNA de grupos bacterianos selecionados, como segue: *Bifidobacterium*: Bif164 (BIF, estritamente anaeróbios), *Lactobacillus* - *Enterococcus*: Lab 158 (LAB, anaeróbios facultativos), *Bacteroides* - *Prevotella*: Bac 303 (BAC, estritamente anaeróbios), *Eubacterium rectale-Clostridium coccoides*: Erec 482 (EREC, estritamente anaeróbios) e *Clostridium histolyticum*: Chis 150 (CHIS, estritamente anaeróbios) (Sigma-Aldrich, St. Louis, EUA (RODRIGUES *et al*., 2016). A população bacteriana total foi quantificada usando SYBR Green (Invitrogen, Carlsbad, CA, EUA) (CONTERNO*et al*., 2019). Os grupos bacterianos foram escolhidos porque representam membros da microbiota fecal que, uma vez modulados, são associados a respostas metabólicas positivas e negativas (RODRIGUEs *et al*., 2016; MENEZES *et al*., 2021; ALBUQUERQUE *et al*., 2021). Além disso, a utilização desses grupos proporcionou

resultados de alta precisão em investigações para avaliar os efeitos dos candidatos a prebióticos na microbiota intestinal (MENEZES *et al*., 2021).

No tempo zero e após 24 e 48h de fermentação, alíquotas de cada lote foram coletadas (375 µL), fixadas com solução de paraformaldeído a 4% (4 ± 0,5 °C) e lavadas duas vezes por centrifugação (10.000 × g 5 min, 4 °C) com PBS 1 M. estéril. As células fixadas foram ressuspensas em solução de PBS: etanol 99% (1: 1 v /v), filtradas através de um filtro Millex-HA de membrana de 0,2 µm (Millipore Co. Bedford, MA) e armazenadas (-20 °C). Para experimentos de hibridização, as alíquotas de células fixas foram ressuspensas em PBS 1X estéril (1:10, Gibco, Gaithersburg, EUA; pH 7,2), centrifugadas (4.000 × g. 15 min, 4 °C), ressuspensas em tampão Tris-EDTA (Tris-HCl 100 mM e EDTA 50 mM; pH 8) e centrifugadas (4.000 x g, 15 min, 4 °C). As amostras utilizadas para hibridização com as sondas Lab 158 e Bif 164 também foram previamente permeabilizadas por incubação com lisozima (1 mg / mL Tris-EDTA) por 10 min no escuro a 25 ± 0,5 °C (Anexo C).

As amostras foram ressuspensas em 45 µL de tampão de hibridização [(NaCl 0,9 M, Tris-HCl 20 mM (pH 7,5) e 0,1% (p / v) dodecil sulfato de sódio (SDS)], adicionadas de 5 µL da sonda testada (50 ng / µL), e incubadas a 46 ou 50 °C por 4 h no escuro (Anexo C). Após nova centrifugação (4.000 × g, 15 min, 25 °C), as amostras foram ressuspensas em 200 µL de tampão de hibridização sem SDS para lavagem a 48 ou 50 °C (Anexo C) por 30 min no escuro. Finalmente, as amostras foram centrifugadas usando as condições anteriormente descritas e ressuspensas com 200 µL de PBS 1X e 20 µL de SYBR Green (estoque 1: 1000 diluído em dimetilsulfóxido ≥ 99,9% Sigma-Aldrich, St. Louis, EUA), incubadas por 10 min no escuro em temperatura ambiente (25 ± 0,5 °C), centrifugadas (4.000 × g, 15 min, 25 °C) e ressuspensas em 200 µL de PBS 1X.

As medições foram feitas em um citômetro de fluxo BD Accuri C6 (New Jersey, EUA) usando excitação de 488 nm a partir de um laser de estado sólido azul. Os sinais de células individuais através da zona de laser foram coletados como sinais logarítmicos. Os sinais de fluorescência foram registrados nos canais FL1 (SYBR Green) e FL2 (Lab 158, Bif 164, Bac 303, Chis 150 e Erec 482) como citogramas pelo software BD Accuri C6. Os resultados foram expressos como abundância relativa, calculados considerando a porcentagem de células hibridizadas com cada sonda em relação ao total de bactérias coradas (CONTERNO *et al*., 2019).

3.5.6Determinação do índice prebiótico

O índice prebiótico de cada biomassa de microalga e FOS foi determinado usando a equação descrita por Albuquerque *et al*. (2021).

Índice prebiótico =% Lab +% Bif -% Bac -% Chis -% Erec (Eq. 1)

Onde:% Lab é a porcentagem hibridizada para Lab após 24 ou 48 h - porcentagem hibridizada no tempo zero; % Bif é a porcentagem hibridizada para Bif após 24 ou 48 h - a porcentagem hibridizada para Bif no tempo zero; % Bac é a porcentagem hibridizada para Bac após 24 ou 48 h - a porcentagem hibridizada para Bac no tempo zero; % Chis é a porcentagem hibridizada para Chis após 24 ou 48 h - a porcentagem hibridizada para Chis no tempo zero; e% Erec é a porcentagem de hibridização de Erec após 24 ou 48 h - a porcentagem de hibridização de Erec no tempo zero.

A equação assume como efeito positivo o aumento das populações de *Lactobacillus* - *Enterococcus* e/ou *Bifidobacterium*, enquanto considera um aumento das populações de *Bacteroides* - *Prevotella*, *C. histolyticum* e *E. rectale* - *C. coccoides*um efeito negativo. De acordo com Albuquerque *et al*. (2021), o valor do índice prebiótico positivo indica um equilíbrio benéfico dos grupos bacterianos identificados na fermentação colônica promovida pelo substrato examinado e, consequentemente, potencial atividade prebiótica. Em contrapartida, a obtenção de um valor de índice prebiótico negativo reporta uma modulação indesejável da microbiota pelo substrato investigado.

3.5.7Metabólitos da fermentação fecal

Metabólitos resultantes da fermentação do inóculo fecal humano em meios com diferentes biomassas de microalgas digeridas, FOS e controle foram avaliados no momento da incubação (tempo zero - logo após a homogeneização), após 24 e 48 h de fermentação, através da determinação de valores de pH (método 981.12) em potenciômetro digital (Quimis, Diadema, São Paulo, Brasil) (AOAC, 2016). Açúcares, ácidos orgânicos e compostos fenólicos foram analisados de acordo com a metodologiadescritas na seção 3.3.

3.6EFEITOS DA BIOMASSA DIGERIDA DE *S. PLATENSIS* NO CRESCIMENTO DE *L. acidophilus* (LA5)

Cultura liofilizada de *L. acidophilus* - La5 obtida de Chr. Hansen S.A. (Valinhos, São

Paulo, Brasil) foi previamente selecionada para uso neste estudo devido aos resultados promissores apresentados por (MEDEIROS *et al*., 2021), que revelaram o aumento da abundância relativade *Lactobacillus-Enterococcus* após administração da biomassa de *S. platensis* digerida em sistestema de fermentação *in vitro*. Inicialmente, a cultura liofilizada de *L. acidophilus* - La5 foi cultivada em caldo de Man, Rogosa e Sharpe (MRS) (Hi-Media, Mumbai, Índia) a 37 °C, sob condições anaeróbicas (Sistema Anaeróbico Anaerógeno, Oxoid) por 24 h. As células foram coletadas por centrifugação (4500 × g, 15 min, 4°C), lavadas duas vezes, ressuspensas em solução salina estéril (NaCl 0,85 g/ 100mL), e homogeneizadas por 30 s usando um vórtex para obter o inóculo inicial. Suspensões de células com densidade óptica (OD) a 660 nm (OD_{660})de 0,8 forneceram contagens viáveis de aproximadamente 6 log UFC/mL. Posteriormente, 1% (v/v) da suspensão de células foram inoculadas individualmente em meio MRS (10 mL) contendo a biomassa de *S. platensis* digerida (DB SPI) ou frutooligossacarídeos (FOS) (controle positivo) na concentração final de 0,5 mg/mL.Ocontrole foi elaborado sem adição de ingredientes prebióticos. A DB SPI foi obtida seguindo o protocolo da INFOGEST's reportado na seção 3.4.

Imediatamente após a homogeneização e após 24, 48 e 72 h de incubação, uma alíquota de 100 µL de cada ensaio foram diluídos em série em solução salina estéril e semeados em ágar MRS para realização da contagem celular usando a técnica de microgotas (HERIGSTAD*et al*., 2001). As células viáveis foram contadas após a incubação anaeróbica a 37°C e os resultados foram expressos como log UFC/mL. A taxa de proliferação celular foi calculada de acordo com a equação descrita por Li *et al*. (2019b), que considera o crescimento bacteriano no meio de cultivo (ausência da substância avaliada) e no meio de cultivo adicionado da substância sob avaliação. O número de colônias no meio MRS sem o ingrediente prebiótico é considerado 100%, e a taxa de proliferação foi calculada usando a Equação 2.

$$Taxa\ de\ proliferação = \frac{número\ de\ colônias\ em\ meio\ MRS\ com\ biomassa\ digerida}{número\ de\ colônias\ no\ meio\ MRS\ x\ 100\%}$$

(Eq. 2).

3.6.1 Avaliação da atividade metabólica de *L. acidophilus*(La5)

A atividade metabólica da microbiota fecal foi avaliada durante a fermentação (tempo zero - logo após a homogeneização), após 24, 48 e 72 h de incubação. A medição dos valores de pH (método 981.12) foi realizada em um potenciômetro digital (Quimis, Diadema, São Paulo, Brasil) (AOAC, 2016). Além disso, foram avaliados os teores de açúcares (glicose e frutose), ácidos orgânicos (ácido lático, ácido cítrico, ácido succínico, ácido málico, ácido acético, e ácido propiônico) e o teor de compostos fenólicos.

A cada intervalo de tempo, alíquotas do meio MRS contendo FOS, biomassa de *S.platensis* digerida (DB SPI) e controle negativo foram coletados, centrifugados (4500 x g, 5 min, 4°C) e os sobrenadantes foram filtrados usando um 0,45- Filtro Millex-HA de membrana de μm (Millipore Co., Bedford, MA, EUA). Em seguida, 10 μL foram injetados e avaliados por Cromatografia Líquida de AltaEficiência (HPLC) usando uma cromatógrafo (modelo 1260 Infinity LC, Agilent Technologies, EUA) acoplado a um detector de arranjo de diodos (DAD) (Agilent 1315D) e detector de índice de refração (RID) (Agilent G1362A).

A quantificação e identificação do perfil de açúcares eácidos orgânicos seguiu as condições descritas por Coelho *et al*. (2018), empregando HPLC-DAD-RID com coluna Agilent Hi-Plex H (7,7 × 300 mm, 8 μm) e 4 M H_2SO_4 em água ultrapura como fase móvel (vazão 0,5 mL/min). Os compostos fenólicos foram analisados por cromatografia de acordo com as condições referidas por Padilha *et al*. (2017). A separação cromatográfica dos compostos fenólicos foi realizada usando uma coluna Zorbax Eclipse Plus RP-C18 (100 × 4,6 mm, 3,5 μm) e a pré-coluna Zorbax C18 (12,6 × 4,6 mm, 5 μm). A temperatura da coluna foi ajustada para 35 °C, e as fases móveis foram compostas por água acidificada (pH 2) com 0,1 mM/L de ácidos fosfóricos (fase A) e metanol acidificado com ácido fosfórico a 0,5% (fase B). A vazão foi mantida a 0,8 mL/min. As aquisições de dados do DAD foram processadas usando OpenLAB CDS ChemStation Edição do software (Agilent Technologies).

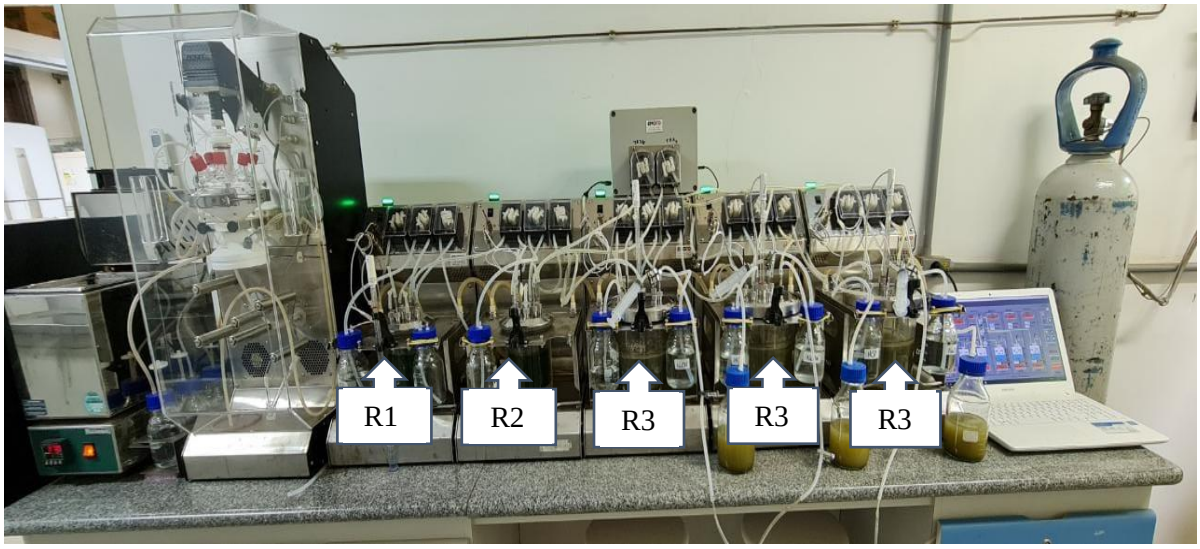
A quantificação foi realizada por meio de curvas de calibração do padrão externo de acordo com os métodos validados (PADILHA *et al*., 2017; COELHO *et al*., 2018). Todos compostos analisados apresentaram curvas de calibração com R2 ≥ 0,998. Além disso, a pureza espectral de pico foi verificada usando a ferramenta Threshold para garantir a precisão da identificação em comparação com o externo padrão (Sigma Aldrich, St. Louis, EUA).

3.7DIGESTÃO SIMULADA NO MODELO COLÔNICO DINÂMICO

O SEMH® é controlado por um computador e consiste em 5 compartimentos fechados representando o estômago, intestino delgado, cólon ascendente, cólon transversoe cólon descendente (MOLLY*et al*., 1994). Neste experimento, o SEMH® (Figura 7) foi adaptado para a triplicação do cólon ascendente, onde o cólon transverso e descendente foram substituídos (Figura 7), de acordo com da Cruz Rodrigues *et al*. (2020).

A capacidade volumétrica, pH, temperatura (37 °C) e tempo de retenção (24 h) foram controlados (POSSEMIERS *et al*., 2004). A anaerobiose do sistema foi conseguida pela adição de nitrogênio e o valor de pH corrigido em cada recipiente usando ácido clorídrico ou hidróxido de sódio (pH 5,6 - 5,9) (MOLLY *et al*., 1994; POSSEMIERS *et al*., 2004). Os compartimentos foram colonizados com fezes de cinco voluntários saudáveis de meia idade. Os critérios de inclusão foram indivíduos com idades entre 40 e 50 anos que não usaram antibióticos, probióticos ou prebióticos nos 6 meses anteriores ao estudo. As fezes dos doadores foram homogeneizadas na mesma porção, armazenadas em tubo plástico estéril (10 g) e mantidas a -80 °C até a realização das análises.

Figura 6. Fotografia do Simulador do Ecossistema Microbiano Humano (SEMH®).



R1- reator 1 (estômago); R2- reator 2 (intestino delgado); R3- reator 3 (cólon ascendente).
Fonte: Autor, 2022.

Uma porção de 40 g de fezes foi homogeneizada e diluída em 200 mL de tampão fosfato (pH 6.5). O tampão era composto por 7,08 g/L de fosfato monossódico (Synth, SP, Brasil), 5,98 g/Lde fosfato dissódico (Synth, SP, Brasil) e 1 g/L de tioglicolato de sódio (Merck, Brasil) (POSSEMIERS*et al*., 2010). Essa mistura foi então homogeneizada em um stomacher e centrifugada a 3000g por 15 min.O sobrenadante foi coletado e 10 mL

adicionados a cada um dos últimos 3 compartimentosjuntamente com 500 mL de meio de alimentação estéril (VAN DEN ABBEELE *et al*., 2019). O meio de alimentação foi constituído a base de carboidratos [4,0 g/L de amido (Maizena, São Paulo, BR), 4,0 g/L de mucina (Sigma, St. Louis,178 EUA), 3,0 g/L de extrato de levedura (Difco, Becton Dickinson Co., Sparks, MD, EUA), 1,0 g/Larabinogalactano (Sigma, St. Louis, EUA), 1,0 g/L de xilano (Sigma, St. Louis, EUA), 1,0 g/L de peptona (Himedia, Mumbai, Índia), 0,5 g/L de cisteína (Sigma, St. Louis, EUA) e 0,4 g/L de glicose (Synth,Diadema, BR].

Um meio de alimentação deve conter fontes de carboidratos complexos, proteínas com a adição de mucinas e uma mistura de minerais e vitaminas para manter a sobrevivência da microbiota em um período de tempo mais longo (VAN DE WIELE *et al*., 2015). A concentração dos compostos utilizados no presente estudo foi empregada em estudos anteriores com SEMH ® (BIANCHI *et al*., 2018; ROSELINO*et al*., 2020; SALGAÇO *et al*., 2021).As condições estomacais foram simuladas no reator 1 adicionando HCl para regular o pH, juntamente com o meio à base de carboidratos. A passagem pelo duodeno foi simulada com 60 mL de suco pancreático artificial (12,5 g/L de $NaHCO^3$, 6 g/L de Oxgall e 1,9 g/L de pancreatina, Sigma- Aldrich, St. Louis, EUA) que possuem papel essencial na adaptação ambiental e no crescimento do inóculo, com a formação de uma comunidade estável e representativa (MOLLY *et al*., 1994).

3.7.1 Protocolo experimental

O projeto de pesquisa foi submetido ao Comitê de Ética em Pesquisa (CEP) pertencente a Faculdade de Ciências Farmacêuticas do Campus de Araraquara da Universidade Estadual Paulista e obteve parecer favorável à sua execução (CAAE n° 30156020.8.0000.5426). O período experimental no reator SEMH®durou 4 semanas. Inicialmente, o meio de alimentação (240 mL) foi inserido no sistema e deixado para estabilizar por 14 dias (BIANCHI *et al*., 2018). Após estas duas semanas, durante uma semana, diariamente, 210 mL demeio de alimentação e 60 mL de suco pancreático foram inseridos no sistema (tratamento de controle). Em seguida, o tratamento foi administrado duas vezes por 5 dias. Os tratamentos empregados foram: *L. acidophilus* (La5, 10^6 UFC/g) ou 1,5 g de biomassa de *S. platensis* com *L. acidophilus* - La5 (Spi-La5, 10^6 UFC/g).

Os experimentos foram realizados em triplicata biológica.A concentração de *S. platensis*utilizada foi estabelecida com base em diretrizes para consumo diário seguro (FDA,

2011, HUANG *et al*., 2018).Além disso, foi considerado a dosagem recomendada de *S. platensis*pelo FDA para aplicação em produtos alimentícios (1,5 g/100 g), como substitutos e misturas de refeições, bebidas esportivas, refrigerantes energéticos bebidas, sucos de frutas, leite de soja com baixo teor de gordura, entre outros (FDA, 2011). Um estudo anterior reportou que concentrações semelhantes de *S. platensis* (1,5 e 3,0 g/kg) promoveram um aumento na proporção de *Firmicutes* e *Bacteroidetes* após os 21 dias de administração a camundongos (HU*et al*., 2019). Desta forma, na perspectiva de agregar mais benefícios à saúde do hospedeiro, é sugerido que a combinação de biomassa de *S.platensis*(1,5 g, duas vezes ao dia) e *L. acidophilus* (10^6 UFC/g) possa ser uma estratégia de avaliação adequada.

3.7.2 Atividade metabólica: análise de amônia (NH^{+4}) e ácidos graxos de cadeia curta (AGCCs)

Após a digestão simulada, as amostras do compartimento do cólon (n = 3) foram coletadas e armazenadas a -20°C. Inicialmente, os íons amônia (NH^{+4}) foram quantificados de acordo com Bianchi *et al*. (2014) usando um medidor de íons específico (Modelo 710A, Orion) acoplado a um eletrodo de íons de amônia seletivo (Modelo 95-12, Orion). Em seguida, os ácidos graxos de cadeia curta (AGCCs) foram analisados de acordo com o protocolo adotado por Duque *et al*. (2016), com modificações. As amostras (n = 3, segunda semana dos reatores do cólon) foram centrifugadas (14.000 *x g*, 5 min), seguidas pela diluição de 100 μL do sobrenadante em 1900 μL de água ultrapura. Após esse procedimento, foram adicionados NaCl (1g), ácido crotônico (100 μL), isobutanol (70 μL) e 2 M H_2SO_4 (200 μL).

As curvas analíticas foram construídas a partir de soluções estoque dos ácidos de interesse (acético, propiônico e butírico). A análise dos AGCCs foi realizada usando um cromatógrafo de gás modelo HP-5975 (Agilent Technologies, Santa Clara CA, EUA) equipado com um injetor split/splitless, um detector seletivo de massa e um amostrador automatizado HP-7683B. Os AGCCs foram separados por uma coluna ZB-WAX (60 m × 0,25 mm × 0,25 μm) (Phenomenex, Torrance-CA, EUA). A cromatografia gasosa foi realizada usando a coluna ajustada a 35°C, 2°C/min, 38°C; 10°C/min, 75°C; 35°C/min, 120°C (1 minuto); 10°C/min, 170°C (2 min); 40°C/min, 170°C (2 min), e a temperatura do injetor e do detector foram mantidas a 220°C e 250°C, respectivamente. O hélio foi utilizado como gás de arraste, com taxa de fluxo fixada em 1 mL/min (HOVING *et al*., 2018)

3.7.3 Análise microbiológica empregando sequenciamento do gene 16S RNA

A análise microbiológica empregando o sequenciamento do gene 16S rRNA (n = 2) foi realizada por Next-generation Sequencing (Neoprospecta Microbiome Technologies, Brasil) utilizando primers específicos que amplificaram a região V3–V4 do 16S rRNA, 341F e 806R de 1 ng de DNA (CAPORASO *et al*., 2011; WANG; QIAN, 2009). A análise das sequências e a identificação das unidades taxonômicas foram avaliadas conforme as metodologias propostas por Giongo *et al.* (2010); Hong *et al*. (2006). A PCR foi realizada em triplicata com Platinum Taq (Invitrogen USA) nas seguintes condições: desnaturação inicial a 94°C por 3 min; vinte ciclos de desnaturação a 94°C por 45s, anelamento a 50°C por 30s, extensão a 65°C por 90s e extensão final a 65°C por 10 min. Os produtos da PCR foram purificados usando esferas AMPure (Beckman coulter) (CHRISTOFF *et al*., 2017) e quantificados por fluorometria usando Qubit® 2.0 Fluorometer (Invitrogen, EUA) (FAGEN *et al*., 2012).

As bibliotecas de sequenciamento foram preparadas de acordo com a Neoprospecta ® Microbiome Technologies e o sequenciamento foi realizado usando a plataforma MiSeq (Illumina). As análises de bioinformática foram realizadas utilizando o programa QIIME (Quantitative Insights Into Microbial Ecology) de acordo com os procedimentos descritos por Caporaso *et al*. (2011). As descrições taxonômicas foram geradas com base na taxonomia do banco de dados NCBI (http://www.ncbi.nl m.nih.gov) para as entradas do banco de dados RDP usando o conjunto de scripts listados no pipeline TaxCollector (GIONGO *et al*., 2010). As strings foram filtradas e classificadas de acordo com a similaridade. Foi considerado um mínimo de 80% de similaridade para identificação de domínio e filo, 90% para identificação de ordem e classe, 90% para família, 95% para gênero e 99% de similaridade para identificação de espécie (SAVY *et al*., 2018).

As análises de sequenciamento do gene 16S rRNA foram realizadas no RStudio, versão 3.2.4 (R Core Team, 2018), utilizando o pacote phyloseq (MCMURDIE; HOLMES, 2013) para importar os dados de amostra e calcular a diversidade alfa e beta. A diversidade alfa foi calculada usando o índice Chao1, índice Shannon com QIIME (V1.7.0) e exibido com software R (V2.15.3). A diversidade beta foi medida usando métricas de distância unifrac ponderadas e não ponderadas pelo software QIIME (V1 7.0).

3.8 ANÁLISES ESTATÍSTICAS

As análises foram conduzidas em triplicata em três experimentos independentes, e os resultados computados e expressos em média ± desvio padrão. Inicialmente, os dados foram submetidos ao Teste t de Student ou análise de variância unilateral (ANOVA), seguido do teste de comparações múltiplas de Tukey, em um nível de significância de 5% ($p < 0,05$), utilizando o software computacional GraphPad Prism versão 8.0 (La Jolla, EUA).

A Análise de Componentes Principais (PCA) foi executada com uma matriz de 15 linhas (4com a biomassa de diferentes espécies microalgas e FOS, em três momentos diferentes) e 13 colunas (grupos microbianos, açúcares e ácidos orgânicos). PCA é uma técnica multivariada amplamente utilizada na busca de padrões de comportamento e pode auxiliar na interpretação de informações de uma grande quantidade de dados. Desta forma, as tendências e informações relevantes podem ser reveladas com o PCA, o que não seria imediatamente observado nas tabelas (MEIRA *et al*., 2020). Além disso, elipses de confiança foram construídas usando a técnica de *bootstrap* e 500 reamostragens. A análise de agrupamento hierárquico (HCA) foi realizada usando as coordenadas da primeira e segunda dimensão do mapa PCA. Foram utilizados o método de Ward (método de aglomeração), distâncias euclidianas (dissimilaridade) e truncamento automático. A qualidade do dendrograma foi avaliada usando o coeficiente de correlação cofenética. O Software computacional GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, EUA) e o XLSTAT para Windows versão 2020.1.2 (Addinsoft, Nova York, EUA) foram usados.

Para a análise microbiológica usando dados de sequenciamento do gene 16S rRNA, as bibliotecas de sequenciamento foram preparadas de acordo com Neoprospecta® Microbiome Technologies, e o sequenciamento foi realizado na plataforma MiSeq (Illumina). Inicialmente, as análises de bioinformática foram realizadas usando o programa QIIME (Quantitative Insights Into Microbial Ecologia) de acordo com os procedimentos descritos por CAPORASO *et al*. (2011). Posteriormente, as descrições taxonômicas foram geradas com base no banco de dados de taxonomia do NCBI (http://www.ncbi.nlm.nih.gov) para as entradas do banco de dados RDP usando o conjunto de scripts listados no TaxCollector pipeline Giongo *et al*. (2010).

A significância das variáveis categóricas foi determinada usando o teste não paramétrico de Wilcoxon para duas comparações de categorias ou o teste de Kruskal-Wallis ao comparar três ou mais categorias. Gráficos de coordenadas principais contaram com o teste PERMANOVA para estimar os valores de p (FIERER *et al*., 2010). Os valores de p foram

ajustados para comparações múltiplas usando o algoritmo FDR (BENJAMINI *et al*., 2001). Para comparar a abundância relativa dos diferentes táxons entre os grupos microbianos, foi realizado a Análise de Discriminante Linear (LDA), implementado no LEfSe (DEI-CAS *et al.*, 2020). A análise de correlação entre os grupos microbianos e os produtos do metabolismo foi realizada usando o teste de correlação de Spearman usando a função cor.mtest das estatísticas corrplot pacote (WEI; SIMKO, 2021).

4RESULTADOS

Os resultados da tese sãodescritos em dois artigos originais, dispostos em Apêndices (A e B). O primeiroartigointitulado como “Freshwater microalgae biomasses exert a prebiotic effect on human colonic microbiota”, foi publicado no periódico *Algal Research*(Apêndice A) e trata-se do cultivo e obtenção de biomassas de quatro espécies de microalgas dulcícolas (*C. vulgaris*, *D. maximus*, *C.*sp cf *hypnosporum* e *S. platensis*), caracterização química das biomassas obtidas e da avaliação do efeito prebiótico destas biomassas digeridas sobre a microbiota colônica humana e atividade metabólicadurante 48 h de fermentação *in vitro*, em comparação com o FOS.

Os resultados demonstraram que as microalgas cultivadas apresentaramcrescimento e produtividade da biomassa variáveis, sendo *C.*sp cf *hypnosporum* e *S.platensis*as espécies que obtiveram maior produtividade diária/total, valores *k*e rendimento máximo de células. A composição química das biomassas também diferiu entre as espécies e esse aspecto pode estar associado aos seus efeitos moduladores sobre a microbiota intestinal e consequente síntese de metabólitos. A biomassa de *S. platensis*apresentou maiores teores de nistose, rafinose e compostos fenólicos, enquanto a biomassa de *D. maximus*continha maiores teores fibras insolúveis ekestose. Diferentemente, a biomassa de *C. vulgaris*mostrou maiores concentrações de glicose, frutose e ramnosequando comparado à biomassa de*C.* cf *hypnosporum*,que apresentou maiores concentrações decompostos fenólicos, incluindo aprocianidina A2, hesperidina e kaempferol 3-glicosídeo.

As biomassas das microalgas digeridas e FOS promoveram aumento nas abundâncias relativas de *Lactobacillus*, *Enterococcus* e *Bifidobacterium*, enquanto reduziram a abundância dos grupos *Bacteroides*spp./*Prevotella*spp, *Clostridium histolyticum* e *Eubacterium rectale-Clostridium coccoides*. A biomassa de *S. platensis*promoveu aumento na abundância relativa de *Lactobacillus-Enterococcus* e*Bifidobacterium*, enquanto reduziu a abundância de*Eubacterium rectale-Clostridium coccoides*, *Clostridium histolyticum* e *Prevotellaceae-*

Bacteroidaceae.Após 24h de incubação, o meio fermentado com *S. platensis* promoveu aumentona concentração de ácidos acético e propiônico. Por outro lado, a biomassa de *D. maximus*promoveu maior abundância relativa de *Lactobacillus-Enterococcus* e aumento nas concentrações de ácido butírico e propiônicoapós 48 h, enquanto a biomassa de *C. vulgaris*aumentou a abundância relativa de *Bifidobacterium* após 48 h. Considerando os resultados descritos, este estudo forneceu evidências que as biomassas de microalgas dulcícolas exercemefeito prebiótico sobre a microbiota intestinal humana e sugere o uso de novas espécies de microalgas para fins alimentares.

O segundo artigointitulado como "*Spirulina platensis* biomass enhances the proliferation rate of *Lactobacillus acidophilus* 5 (La-5) and combined with La-5 impact the gut microbiota of medium-age healthy individuals through an in vitro gut microbiome model", foi publicado no periódico *Food Research International* (Apêndice B)e descreve oefeito da biomassa digerida de *S. platensis* sobre o crescimento e atividade metabólica de *L. acidophilus* La-5, durante a fermentação em meio de cultura sintético, em comparação ao FOS. Além disso, aborda os efeitos da administração da biomassa digerida de *S. platensis* em associação com *L. acidophilus*(Spi-La5) na composição da microbiota intestinal de indivíduos saudáveis de meia idade e o impacto na síntese de metabólitos, através do Simulador de Ecossistema Microbiano Intestinal (SEMH ®).

Os resultados deste estudo demonstraram que a biomassa digerida de *S. platensis* promoveu maior impacto na taxa de proliferação celular e na atividade metabólica de *L. acidophilus*(La5) (valores de pH mais baixos e maiores concentrações de ácidos acético, lático e propiônico), em comparação com FOS. Além disso, a administração de Spi-La5 resultou no aumento da abundância dos gêneros *Erysipelatoclostridium*, *Roseburia*, *Enterococcus*, *Bifidobacterium*, *Coriobacteriaceae* UCG-003, *Enterobacter* e *Paraclostridium*, enquanto a administração do La5 aumentou a abundância dos gêneros*Bacteroides*, *Megasphaera*, *Lactobacillu*s, e *Parabacteroide* e reduziua concentração dos íons amônia. Com base nestes achados, é possível considerar que a administração de *L. acidophilus* (La5) e Spi-La5 modifica a microbiota intestinal de forma distinta e que ambas asintervenções podem contribuirpara melhorar a microbiota intestinal de indivíduos saudáveis de meia-idade.

O artigo de revisão intitulado como "Microalgae in the meat processing chain: feed for animal production or source of techno-functional ingredientes",publicado no periódico*Current Opinion in Food Science*(Apêndice C), aborda informações relevantes sobre

a aplicação de biomassas de microalgas como fonte de nutrientes na alimentação animal, assim como o uso de seus compostos isolados como fonte de ingredientes com propriedades tecno-funcionais relevantes para aplicação em produtos cárneos processados. Os resultados descritos no artigo apontam que as biomassas de microalgas, assim como os seus compostos bioativos isolados, contribuem satisfatoriamente para a produção de alimentos cárneos mais saudáveis, representando, portanto, um novo nicho de produtos a serem explorados pela indústria de alimentos.

O desenvolvimento desta tese forneceu informações relevantes sobre os aspectos nutricionais e funcionais de biomassas microalgais dulcícolas obtidas por meio de cultivos em meio de crescimento sintético. De maneira geral, as biomassas microalgais avaliadas apresentaram conteúdos distintos de fibra alimentar total (solúvel e insolúvel), oligossacarídeos, monossacarídeos e compostos fenólicos e foram capazes demodular (isoladamente ou em associação com o LA-5) a composição e atividade metabólica da microbiota intestinal de indivíduos adultos e de meia idade saudáveis, quando submetidas à digestão gastrointestinal em modelo estático e dinâmico, respectivamente. Assim, aliada aos conhecimentos já difundidos, a presente pesquisa reforça o potencial nutricional e funcional de biomassas microalgas e estabelece uma nova e promissora aplicação destes ingredientes como prebióticoscom potenciais aplicações tecnológicas e funcionais.

O seguimento de pesquisas futuras sobre o potencial prebiótico de microalgas dulcícolas deve atentar-se para algumas limitações identificadas neste estudo, como por exemplo, o baixo rendimento da biomassa microalgal seca. Portanto sugere-se o emprego de cultivos em larga escala a fim de minimizar o tempo necessário para obtenção da biomassa e aumentar o rendimento da produção. Além disso, o uso de meios de cultivo alternativos aos sintéticos também são encorajados para redução dos custos e aumento da viabilidade da produção de biomassa microalgal em larga escala. Pesquisas futuras também poderão fazer uso de compostos extraídos da miomassa microalgal (polissacarídeos, oligossacarídeos, compostos fenólicos) para avaliação da ação destes componentes isolados sobre o perfil da microbiota intestinal de adultos e/ou de indivíduos pertencentes a faixas etárias distintas.

REFERÊNCIAS

ABU-GHOSH, S. *et al*. Dietary fiber from Indian edible seaweeds and its in-vitro prebiotic effect on the gut microbiota. **Bioresource Technology**, v. 329, n. 124895, p.1–12, 2021.

ADAK, A., KHAN, M. R. An insight into gut microbiota and its functionalities. **Cellular and Molecular Life Sciences**, v.76, p. 473–493, 2019.

AL-RASHIDI, H. E. Gut microbiota and immunity relevance in eubiosis and dysbiosis. *Saudi Journal of Biological Sciences*, 2021.

ALBUQUERQUE, T. M. R. *et al*. Potential prebiotic properties of flours from different varieties of sweet potato (*Ipomoea batatasL*.) roots cultivated in Northeastern Brazil. **Food Bioscience**, v. 36, n. 100614, p. 1–13, 2020.

ALBUQUERQUE, T. M. R. *et al*. Effects of digested flours from four different sweet potato (*Ipomoea batatas L*.) root varieties on the composition and metabolic activity of human colonic microbiota in vitro. **Journal of Food Science**, v. 86, p. 3707-3719, 2021.

ALVES-SANTOS, A. M.*et al*. Prebiotic effect of dietary polyphenols: A systematic review.**Journal of Functional Foods**, v. 74, n. 04169, p. 1–11, 2020.

ANDRADE, L. M. *et al*. *Chlorella* and *Spirulina* microalgae as sources of functional foods, nutraceuticals, and food supplements: an overview. **Food Processing & Technology**, v. 6, n. 2, p. 1–15, 2018.

ANDRADE, D. S., TELLES, T. S., CASTRO, G. H. L. The Brazilian microalgae production chain and alternatives for its consolidation. **Journal of Cleaner Production**, v. 250, n. 119526, p. 1–13, 2020.

ANVARA, A. A, NOWRUZI, B. Bioactive properties of Spirulina: A Review. **Microbial Bioactives**, v. 4, n. 1, p. 134–142, 2021.

AOAC – ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. **Official Methods of Analysis of AOAC International**, 20th Edition, Washington, DC – USA, 2016.

ARABI, S. M.*et al*. Impact of synbiotic supplementation on cardiometabolic and anthropometric indices in patients with metabolic syndrome: A systematic review and meta-analysis of randomized controlled trials. **Pharmacological Research**, v.176, n. 106061, p. 1-12, 2022

BATT, C., TORTORELLO, M. L. Microflora of the intestine | Biology of *Lactobacillus acidophilus.* Reference Module in Food Science. In:AIMUTIS *et al*., 2014. **Encyclopedia of Food Microbiology**, 2 ed, Elsevier, p. 646–651, 2014.

BARSANTI, L., GUALTIERI, P. Is exploitation of microalgae economically and energetically sustainable? **Algal Research**,v. 31, p. 107–115, 2018.

BABADI, F. E. *et al*. Identification of carotenoids and chlorophylls from green algae *Chlorococcum humicola* and extractionby liquefied dimethyl ether. **Food and Bioproducts Processing**,v. 123, p. 296–303, 2020.

BAUDELET, P.*et al*.A new insight into cell walls of Chlorophyta. **Algal Research**, v. 25, p. 333–371, 2017.

BENJAMINI, Y. *et al*. Controlling the false discovery rate in behavior genetics research. **Behavioural Brain Research**, v. 125, p. 279–84, 2001.

BERBEROGLU., GOMEZ, P. S., PILON, L. Radiation characteristics of *Botryococcus braunii*, *Chlorococcum littorale*, and *Chlorella* sp. used for CO_2 fixation and biofue production. **Journal of Quantitative Spectroscopy & Radiative Transfer**, v, 110, p. 1879–1893, 2009.

BERNAERTS, T. M. M. *et al*. Comparison of microalgal biomasses as functional food ingredients: Focus on the composition of cell wall related polysaccharides. **Algal Research**, v. 32, p.150–161, 2018.

BHATTAD, T., KORADIYA, A., PRAKASH, G. Prebiotic activity of paramylon isolated from heterotrophically grown *Euglena gracilis.* **Heliyon**, v.7, n. e07884, p. 1–6, 2021.

BIAN, Y. *et al*. Natural polyphenols targeting senescence: A novel prevention and therapy strategy for cancer.**International Journal of Molecular Sciences**, v. 21, n. 684, p. 1–17, 2020.

BIANCHI, F. *et al*. Beneficial effects of fermented vegetal beverages on human gastrointestinal microbial ecosystem in a simulator. **Food Research International**, v. 64, p. 43–52, 2014.

BIANCHI, F. *et al.* Modulation of gut microbiota from obese individuals by in vitro fermentation of citrus pectin in combination with *Bifidobacterium longum* BB-46. **Applied Microbiology and Biotechnology**, v. 102, n. 20, p. 8827–8840, 2018.

BLAAK, E.E. *et al*. Short chain fatty acids in human gut and metabolic health. **Beneficial Microbes**, v. 11, n. 5, p. 411– 455, 2020.

BOHÓRQUEZ-MEDINA, S. L. *et al*. Impact of Spirulina supplementation on obesity-related metabolic disorders: A systematic review and meta-analysis of randomized controlled trials.**NFS Journal**, v. 25, p. 21–30, 2021.

BRASIL. Ministério da Saúde. Conselho Nacional de Saúde. **Aprova diretrizes e normas regulamentadoras de pesquisas envolvendo seres humanos**. 240° Reunião Ordinária.

Resolução n° 466, de 12 de dezembro de 2012. Disponível em: <http://www.conselho.saude.gov.br/resolucoes/2012/Reso466.pdf> Acesso em: 15 de outubro de 2019.

CAI, B. *et al*. Structural characterization of oligosaccharide from *Spirulina platensis* and its effect on the faecal microbiota in vitro. **Food Science and Human Wellness**, v. 11, p. 109–118, 2022.

CAPORASO, J. G. *et al*. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. **Proceedings of the National Academy of Sciences**, v. 108(Supplement_1), p. 4516–4522, 2011.

CALIXTO, C.D. *et al*. Biochemical compositions and fatty acid profiles in four species of microalgae cultivated on household sewage and agro-industrial residues. **Bioresource Technology**, v. 221, p. 1–36, 2016.

CAMACHO, F., MACEDO, A., MALCATA, F. Potential industrial applications and commercialization of microalgae in the functional food and feed industries: A short review. **Marine Drugs**, v. 17, n. 6, p. 1–25, 2019.

CAMPOS, D. A. *et al*. Impact of functional flours from pineapple by-products on human intestinal microbiota. **Journal of Functional Foods**, v. 67,n. 103830, p. 1–13, 2020.

ÇELEKLI, A., ALSLIBI, Z. A., BOZKURT, H. Influence of incorporated *Spirulina platensis* on the growth of microflora and physicochemical properties of ayran as a functional food. **Algal Research**, v. 44, n. 101710, p. 1–6, 2019.

CHOOCHOTE, W., SUKLAMPOO, L., OCHAIKUL, D. Evaluation of antioxidant capacities of green microalgae.**Journal of Applied Phycology**, v. 26, n. 1, p. 1–6, 2014.

CHRISTOFF, A. P. *et al*. Bacterial identification through accurate library preparation and highthroughput sequencing. **Neoprospecta Microbiome Technologies**, v. 25, p. 1–5, 2017.

COELHO, E. M. *et al*. Simultaneous analysis of sugars and organic acids in wine and grape juices by HPLC: Method validation and characterization of products from northeast Brazil. **Journal of Food Composition and Analysis**, v. 66, p. 160–167, 2018.

CONTERNO, L. *et al*. Measuring the impact of olive pomace enriched biscuits on the gut microbiota and its metabolic activity in mildly hypercholesterolaemic subjects. **European Journal of Nutrition**, v. 58, p. 63–81, 2019.

COLLA, E.*et al*. Microalgae: **A new and promising source of food**.Handbook of Algal Science, Technology and Medicine, p. 507–518, 2020.

COSME, F., INÊS, A., VILELA, A. Consumer's acceptability and health consciousness of probiotic and prebiotic of non-dairy products. **Food Research International**, v. 151, n. 110842, p. 1–20, 2022.

CUEVA, C. *et al*. Interplay between dietary polyphenols and oral and gut microbiota in the development of colorectal cancer. **Nutrients**, v. 12, n. 625, p.1–19, 2020.

CUI, Y. *et al*. Structure of a laminarin-type β-(1→3)-glucan from brown algae *Sargassum henslowianum* and its potential on regulating gut microbiota. **Carbohydrate Polymers**, v. 255, n. 117389, p. 1-12, 2021.

CUNNINGHAM, M., AZCARATE-PERIL, A. M., BARNARD, A. shaping the future of probiotics and prebiotics.**Trends in Microbiology**,v. 29, n. 8, p. 667-685, 2021.

DANNESKIOLD-SAMSØE, N. B.*et al*. Interplay between food and gut microbiota in health and disease. **Food Research International**, v. 115, p. 23–31, 2019.

DANTAS, A. *et al*. Current knowledge about physical properties of innovative probioticspray-dried powders produced with lactose-free milk and prebiotics. **LWT - Food Science and Technology**, v. 151,n. 112175, 1 –11, 2021.

da CRUZ RODRIGUES, V. C., *et al*. Modulation of the intestinal microbiota and the metabolites produced by the administration of ice cream and a dietary supplement containing the same probiotics. **British Journal of Nutrition**, v. 124, p. 57–68, 2020.

D'ARCHIVIO, M. *et al*. Bioavailability of the Polyphenols: Status and controversies. **International Journal of Molecular Sciences**, v.11, n. 4, p.1321–1342, 2010.

DESAI, N. M. *et al*. Prebiotic oligosaccharide enriched green coffee spent cookies and their nutritional, physicochemical and sensory properties. **LWT - Food Science and Technology**, v. 134, n. 109924, p. 1 –7, 2020a.

DESAI, N. M. *et al*. Non-digestible oligosaccharidesof green coffee spent and their prebiotic efficiency. **LWT - Food Science and Technology**,v. 118, n.108784, p. 1–7, 2020b.

DEI-CAS, I. *et al*. Metagenomic analysis of gut microbiota in non-treated plaque psoriasis patients stratified by disease severity: development of a new Psoriasis-Microbiome Index. **Scientific Report**, v. 10, n. 12754, p. 1–11, 2020.

DIXIT, K. *et al*. Restoration of dysbiotic human gut microbiome for homeostasis. **Life Sciences**, v. 278, n. 119622, p. 1–12, 2021.

DONG, S.T. *et al*. Impact of biochemical composition on susceptibility of algal biomass to acid-catalyzed pretreatment for sugar and lipid recovery. **Algal Research**, n. 18, p. 69–77, 2016.

DUQUE, A. L. R. F. *et al*. An exploratory study on the influence of orange juice on gut microbiota using a dynamic colonic model. **Food Research International**, v. 84, p. 160–169, 2016.

ELSHOBARY, M. E. *et al*. Antimicrobial and antioxidant characterization of bioactive components from *Chlorococcum minutum*. **Food Bioscience**, v. 35, 100567, p. 1–10, 2020.

ENZING, C. *et al*. **Microalgae-based products for the food and feed sector: an outlook for Europe**. In JRC Scientific and Policy Reports. European Comission, 2014.

FAGEN, J. R. *et al*. Characterization of the relative abundance of the citrus pathogen

Ca. Liberibacter asiaticus in the microbiome of its insect vector, Diaphorina citri, using high throughput 16s rRNA sequencing. **Open Microbiology Journal**, v. 6, p. 29–33, 2012.

FARIAS, P. D *et al*. Prebiotics: Trends in food, health and technological applications. **Trends in Food Science & Technology**, v. 93, p. 23–35, 2019.

FERNÁNDEZ, F. G. A.*et al*. The role of microalgae in the bioeconomy. **New Biotechnology**,v.61, p. 99–107, 2021.

FERREIRA-LAZARTE, A. *et al*. In vitro digestibility of galactooligosaccharides: effect of the structural features on their intestinal degradation. **Journal Agricultural Food Chemistry**, v. 67, p. 4662−4670, 2019.

FERREIRA, A. S. *et al*. Reserve, structural and extracellular polysaccharides of Chlorella vulgaris: Aholistic approach. **Algal Research**, v. 45, n.101757, p. 1-11, 2020.

FELIZARDO, R. J. F. *et al*. The interplay among gut microbiota, hypertension and kidney diseases: The role of short-chain fatty acids. **Pharmacological Research**, v. 141, p. 366–377, 2019.

FIERER, N. *et al*. Forensic identification using skin bacterial communities. **Proceedings of the National Academy of Sciences**, v. 107, n. 6477–6481, 2010.

FICCO, D. B. M. *et al*. Comparison of gluten peptides and potential prebiotic carbohydrates in oldand modern *Triticum turgidum* ssp. genotypes, **Food Research International**, v. 120, p.568 –576, 2019.

FOOD AND DRUG ADMINISTRATION. *Spirulina platensis* GRAS self affirmation 7-1-**Executive**, p. 1- 42, 2011.

FOOD AND DRUG ADMINISTRATION. *Chlorella vulgaris* GRAS Self affirmation. **Executive**, p. 1- 57, 2011.

FLORES-MALTOS, D.A. *et al*. Biotechnological production and application of fructooligosaccharides. **Critical Reviews Biotechnology**, v. 36, n. 2, p. 259–267, 2016.

FRANKS, A. H. *et al*. Variations of bacterial populations in human feces measured by fluorescent in situ hybridization with group-specific 16S rRNA targeted oligonucleotide probes. **Applied Environmental Microbiology**, v. 64, n. 535, p. 3336–45, 1998.

GIBSON, R. G.*et al*. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. **Nature Reviews Gastroenterology & Hepatology**, v. 14, p. 491–502, 2017.

GIONGO, A. *et al*. TaxCollector: Modifying current 16S rRNA databases for the rapid classification at six taxonomic levels. **Diversity**, v. 2, n. 7, p. 1015–1025, 2010.

GONZÁLEZ-BALDERAS, R.M. *et al*. Intensified recovery of lipids, proteins, and carbohydrates from waste water grown microalgae *Desmodesmus* sp. by using ultrasound or ozone. **Ultrasonics – Sonochemistry**, v. 62, n. 104852, p.1–11, 2020.

GRAND VIEW RESEARCH. **Prebiotics market size, share & trends analysis by ingredients (FOS, INULIN, GOS, MOS), by application (food and beverages, dietary supplements, animal feed), by region, and segment forecasts**, 2014- 2024. Disponível em <https://www.grandviewresearch.com/industry-analysis/prebiotics-market>, 2018. Acesso em: 01 de junho de 2021.
GRIFFITHS M. *et al*. **Major commercial products from micro- and macroalgae**. In: Bux F., Chisti Y. (eds) Algae Biotechnology. Energia e tecnologia verdes. Springer, Cham, 2016.

GUO, W. *et al*. Microalgae polysaccharides ameliorates obesity in association with modulation of lipid metabolism and gut microbiota in high-fat-diet fed C57BL/6 mice. **International Journal of Biological Macromolecules**, v. 182, p. 1371–1383, 2021.

GUPTA, S. *et al*. Prebiotic efficiency of blue green algae on probiotics microorganisms. **Journal of Microbiology & Experimentation**, v. 4, n. 4, p. 1–4, 2017.

GUILLARD, R.R.L.; LORENZEN, C.J. Yellow-green algae with chlorophyllidc. Journal of Phycology, v.8, p.10-14, 1972.

GUERGOLETTO, K.B. et al. In vitro fermentation of juçara pulp (Euterpe edulis) by human colonic microbiota. **Food Chemistry**, v.196, p. 251 - 258, 2016.

HARMSEN, H. J. M. *et al*. A 16S rRNA-targeted probe for detection of lactobacilli and enterococci in faecal samples by fluorescent in situ hybridization.**Microbial Ecology in Health and Disease**, v. 11, p. 3–12, 1999.

HERIGSTAD, B. *et al*. How to optimize the drop plate method for enumerating bacteria. **Journal of Microbiological Methods**, v. 44, n. 2, p. 121–129, 2001.

HENTSCHKE, G. S., TORGAN, L. C. *Desmodesmus* e *Scenedesmus* (Scenedesmaceae, Sphaeropleales, Chlorophyceae) em ambientes aquáticos na Planície Costeira do Rio Grande do Sul, Brasil. **Rodriguésia**, v. 61, p. 585–601, 2010.

HESHMATI, J. *et al*. A systematic review and meta-analysis of the probiotics and synbiotics effects on oxidative stress. **Journal of Functional Foods**, v. 46, p. 66–84, 2018.

HILL, C. *et al*. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. **Nature Reviews Gastroenterology & Hepatology**, v. 11, p. 506–514, 2014.

HOLSCHER, H. D. Dietary fiber and prebiotics and thegastrointestinal microbiota. Gut **Microbes**, v. 8, n. 2, p. 172–184, 2017.

HONG, S. H. *et al*. Predicting microbial species richness. **Proceedings of the National Academy of Sciences of the United States of America**, v. 103, n. 1, p. 117–122, 2006.

HOVING, L. R. *et al*.GC-MS analysis of short-chain fatty acids in feces, cecum content, and blood samples. **Clinical Metabolomics**, v. 1730, p. 247–256, 2018.

HU, J.*et al*. Dose effects of orally administered Spirulina suspension on colonic

microbiota in healthy mice. **Frontiers in Cellular and Infection Microbiology**, v. 9, p. 1–13, 2019.

HUA, P. *et al*. Regulatory efficacy of *Spirulina platensis* protease hydrolyzate on lipid metabolism and gut microbiota in high-fat diet-fed rats. **International Journal of Molecular Sciences**, v. 19, n. 4023, p. 1–18, 2018.
HUANG, H. *et al*. Quantifying the effects of Spirulina supplementation 599 on plasma lipid and glucose concentrations, body weight, and blood pressure. **Diabetes, metabolic syndrome and obesity: targets and therapy**, v. 11, n. 729, p. 729–742, 2018.

HURTADO-ROMERO, A.*et al*. Innovative technologies for the production of food ingredients with prebiotic potential: Modifications, applications, and validation methods. **Trends in Food Science & Technology**, v. 104, p.117–131, 2020.

JACKSON, M. I., JEWELL, M. I. Impact of fiber-bound polyphenols on gut microbiome metabolism is influenced by background diet. **The FASEB Journal**, v. 31, p. 792–23, 2017.

JOHNSON, S. L. *et al*. Polyphenol microbial metabolites exhibit gut and blood–brain barrier permeability and protect murine microglia against LPS-induced inflammation **Metabolites**, v. 9, n. 78, p. 1–13, 2019.

KAWABATA, K., YOSHIOKA, Y., TERAO, J. Role of intestinal microbiota in the bioavailability and physiological functions of dietary polyphenols. **Molecules**, v. 24, n. 370, p. 1–25, 2019.

KAZEMI, A. *et al*. Effect of probiotic and synbiotic supplementation on inflammatory markers in health and disease status: A systematic review and meta-analysis of clinical trials. **Clinical Nutrition**, v. 39, n. 3, p. 789-819, 2020.

KHALIL, N. A. *et al*. Prospective evaluation of probiotic and prebiotic supplementation ondiabetic health associated with gut microbiota. **Food Bioscience**, v. 42, n. 101149, p. 1–10, 2021.

KOYANDE, A. K. *et al*. Microalgae: A potential alternative to health supp lementation for humans. **Food Science Human Wellness**, v. 8, n. 1, p. 16–24, 2019.

KUSMAYADI, A. *et al*. Microalgae as sustainable food and feed sources for animals and humans - Biotechnological and environmental aspects. **Chemosphere**, v. 271, n. 129800, p. 1–9, 2021.

LAFARGA, T.Effect of microalgal biomass incorporation into foods: Nutritional and sensorial attributes of the end products. **Algal Research**, v. 41, n. p. 1–10, 2019.

LAFARGA, T. Cultured microalgae and compounds derived thereof for food applications: strain selection and cultivation, drying, and processing strategies. **Food Reviews International**, p. 559-583, 2020.

LANGENDIJK. P, S. *et al*. Quantitative fluorescence in situ hybridization of *Bifidobacterium* spp. with genus-specific 16S rRNA-targeted probes and its application in fecal samples. **Applied and Environmental Microbiology**,v. 61, p. 3069–3075, 1995.

LAVELLE, A., HILL, C. Gut microbiome in health and disease: Emerging diagnostic opportunities. **Gastroenterology Clinics of North America**, v. 48, n. 2, p. 221–235, 2019.

LEAL, B. E. S. *et al*. Potential prebiotic oligosaccharides from aqueous thermopressurized phosphoric acid hydrolysates ofmicroalgae used in treatment of gaseous steakhouse waste. **Algal Research**,v. 24, p. 138–147, 2017.

LEVASSEUR, W., PERRÉ, P., POZZOBON, V. A review of high value-added molecules production by microalgae in light of the classification. **Biotechnology Advances**, v. 41, n. 107545, p. 1–21, 2020.

LI, Q. *et al*. Mulberry leaf polyphenols and fiber induce synergistic antiobesity and display a modulation effect on gut microbiota and metabolites. **Nutrients**, v. 11, n. 1017, p. 1–19, 2019a.

LI, E. *et al*. In-vitro digestion by simulated gastrointestinal juices of *Lactobacillus rhamnosus* cultured with mulberry oligosaccharides and subsequent fermentation with human fecal inocula. LWT- **Food Science and Technology**, v. 101, n. 133, p. 61–68, 2019b.

LI, T. T. *et al*. Polyunsaturated fatty acids from microalgae *Spirulina platensis* modulates lipid metabolism disorders and gut microbiota in high-fat diet rats. **Food and Chemical Toxicology**, v. 131, n. 110558, p. 1–9, 2019c.

LIESTIANTY, D. *et al*. Nutritional analysis of Spirulina sp to promote as superfood candidate. **IOP Conf. Series: Materials Science and Engineering**, v. 509, p.1–6, 2019.

LIMA, M. S. *et al*. Determining 1-kestose, nystose and raffinose oligosaccharides in grape juices and wines using HPLC: Method validation and characterization of products from northeast Brazil. **Journal of Food Science & Technology**, v. 56, p. 4575–4584, 2019.

LIU, S. *et al*. Removal of bound polyphenols and its effect on antioxidant and prebiotics properties of carrot dietary fiber. **Food Hydrocolloids**, v. 93, p. 284–292, 2019.

LIU, P. *et al*. The role of short–chain fatty acids in intestinal barrier function, inflammation, oxidative stress, and colonic carcinogenesis. **Pharmacological Research**, v. 165, n.105420, p.1–11, 2021.

LYNCH, S., PEDERSEN, O. The human intestinal microbiome in health and disease. **The New England Journal of Medicine**, v. 15, n.375, p. 2369–2379, 2016.

LYNN, A. M. *et al*. The composition of the gut microbiota following early-life antibiotic exposure affects host health and longevity in later life.**Cell Reports**, v. 36, n. 8, p. 1-21, 2021.

MA, H. *et al*. Polysaccharide from *Spirulina platensis* ameliorates diphenoxylate-induced constipation symptoms in mice. **International Journal of Biological Macromolecules**, v. 133, p. 1090–1101, 2019.

MACFARLANE, S., MACFARLANE, G. T. Regulation of short-chain fatty acid production. **Proceedings of the Nutrition Society**, v. 62, n. 1, p. 67–72, 2003.

MANO, M. C. R.*et al*. Oligosaccharide biotechnology: an approach of prebiotic revolution on the industry. **Applied Microbiology and Biotechnology**, v. 102, n. 1, p. 1–21, 2018.
MANZ, W. *et al*. Application of a suite of 16S rRNA-specific oligo nucleotide probes designed to investigate bacteria of the phylum cytophaga-flavobacter-bacteroides in the natural environment. **Microbiology**, v. 142, p. 1097-1106, 1996.

MASKE, B. L. *et al*. A review on enzyme-producing lactobacilli associated with the human digestive process: From metabolism to application. **Enzyme and Microbial Technology**, v. 149, n. 109836, p. 1–18, 2021.

MCMURDIE, P. J., HOLMES, S. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. **PloS One**, v. 8, n. e61217, 2013.

MARKOWIAK, P.; ŚLIŻEWSKA, K. Effects of probiotics, prebiotics, and synbiotics on human health. **Nutrients**, v. 9, n. 1021, p. 1–30, 2017

MASKE, B. L. *et al*.A review on enzyme-producing lactobacilli associated with the human digestive process: From metabolism to application. **Enzyme and Microbial Technology**, v. 149, n. 109836, p. 1–18, 2021.

MEDEIROS, V. P. B. *et al*. Microalgae as source of functional ingredients in new-generation foods: challenges, technological effects, biological activity, and regulatory issues. **Critical Reviews in Food Science and Nutrition**, p. 1–22, 2021.

MEIRA, C. L. C. *et al*. Application of principal component analysis for the evaluation of the chemical constituents of *Mimosa tenuiflora* methanolic extract by DLLME/GC–MS. **Microchemical Journal**, v. 152, n. 104284, p. 1-6, 2020.

MELO, F. H. C. *et al*. Prebiotic activity of monofloral honeys produced by stingless bees in the semi-arid region of Brazilian Northeastern toward *Lactobacillus acidophilus* LA-05 and *Bifidobacterium lactis* BB-12.**Food Research International**, v. 128, n. 108809, p.1–11, 2020.

MENEZES, F. N. D. D. *et al*. Acerola (*Malpighia glabra* L.) and guava (*Psidium guayaba* L.) industrial processing by-products stimulate probiotic Lactobacillus and Bifidobacterium growth and induce beneficial changes in colonic microbiota. **Journal of Applied Microbiology**, v. 130, n. 4, p. 1–14, 2020.

MENEZES, F.N.D.D. *et al*. Impact of cashew (*Anacardium occidentale* L.) by-product on composition and metabolic activity of human colonic microbiota in vitro indicates prebiotic properties. **Current Microbiology**, v. 78, n.6, p. 2264–2274, 2021.

MOHANTY, D. *et al*. Prebiotics and synbiotics: Recent concepts in nutrition. **Food Bioscience**, v. 26, p. 152–160, 2018.

MOLLY, K., *et al*. Validation of the simulator of the human intestinal microbial ecosystem (SHIME) reactor using microorganism-associated activities. **Microbial Ecology in Health and Disease**, v. 7, n. 4, p. 191–200, 1994.

MORENO, F. J.*et al*. Current state and latest advances in the concept, production and functionality of prebiotic oligosaccharides. **Current Opinion in Food Science**, v. 13, p. 50–55, 2017.
MOSELE, J. I., MACIÀ, A., MOTILVA, M. J.Metabolic and microbial modulation of the large intestine ecosystem by non-absorbed diet phenolic compounds: a review. **Molecules**, v. 20, p. 17429–17468, 2015.

MOORTHY, M. Prebiotic potential of polyphenols, its effect on gut microbiota and anthropometric/clinical markers: A systematic review of randomized controlled trials. **Trends in Food Science & Technology**, v. 99, p. 634–649, 2020.

MOLLAKHALILI-MEYBODI, M. N. *et al*. Prebiotic wheat bread: Technological, sensorial and nutritionalperspectives and challenges. **LWT - Food Science and Technology**, v. 149, n. 111823, p. 1 –8, 2021.

NAJARIAN, A.; SHARIF, S.; GRIFFITHS, M.W. Evaluation of protective effect of *Lactobacillus acidophilu*s La-5 on toxicity and colonization of *Clostridium difficile* in human epithelial cells in vitro. **Anaerobe**,v. 55, p. 142-151, 2019.

NASCIMENTO, P. R., JUNIOR, M. R. M. Probiotics and Prebiotics in Foods. **Challenges, Innovations, and Advances**, p. 13–46, 2021.

NEYRINCK, A. M.*et al*. *Spirulina* protects against hepatic inflammation in aging: an effect related to the modulation of the gut microbiota? **Nutrients**, v. 9, n. 6, p. 1–13, 2017.

NSOR-ATINDANA, J. *et al*. Enhancing the prebiotic effect of cellulose biopolymer in the gut by physical structuring via particle size manipulation. **Food Research International**, v. 131, n. 108935, p.1–11, 2020.

OLIPHANT, K., ALLEN-VERCOE, E. Macronutrient metabolism by the human gut microbiome: major fermentation byproducts and their impact on host health. **Microbiome,** v, 7, n. 91, p. 1–15, 2019.

ÖZGÜN, C. O., UMU, K, R., DIEP, D. B. Modulation of the gut microbiota by prebiotic fibres and bacteriocins. **Microbial ecology in health and disease**, v. 28, n. 1348886, p. 1-12, 2017.

PADILHA, C. V. S. *et al*. Rapid determination of flavonoids and phenolic acids in grape juices and wines by RP-HPLC/DAD: Method validation and characterization of commercial products of the new Brazilian varieties of grape. **Food Chemistry**, v. 228, p. 106–115, 2017.

PATEL, A. K. *et al*. Emerging prospects of macro and microalgae as prebiotic. **Microbial Cell Factories**, v. 20, n.112, p. 1–16, 2021.

PEREDO-LOVILLO, A., ROMERO-LUNA, H. E., JIMÉNEZ-FERNÁNDEZ, M. Health promoting microbial metabolites produced by gut microbiota after prebiotics metabolism. **Food Research International**, v. 136, n. 109473, p 1–13, 2020.

PRADO, S. B. R. *et al*. Modulation of human gut microbiota by dietary fibers from unripe and ripe papayas: Distinct polysaccharide degradation using a colonic in vitro fermentation model. **Food Chemistry**, v. 348, 129071, p. 1–11, 2021.

POSSEMIERS, SAM. *et al*. PCR-DGGE based quantification of stability of the microbial community in a simulator of the human intestinal microbial ecosystem. **FEMS Microbiology Ecology**, v. 49, n. 3, p. 495–507, 2004.

POSSEMIERS, S. *et al*. Bacteria and chocolate: A successful combination for probiotic delivery. **International Journal of Food Microbiology**, v. 141, n. 2, p. 97–103, 2010.

POZZOBON, V.*et al*. *Desmodesmus* sp. pigment and FAME profiles under different illuminations and nitrogen status. **Bioresource Technology Reports**, v. 10, n. 100409, p. 1–8, 2020.

QUIGLEY, E. M. M. Prebiotics and probiotics in digestive health. **Clinical Gastroenterology and Hepatology**, v. 17, p. 333–344, 2019.

RAPOSO, M. F. J., MORAIS, A. M. M. B., MORAIS, R. M. S. C. Emergent Sources of Prebiotics: Seaweeds and Microalgae. **Marine Drugs**, v. 14, n. 27, p. 1–27, 2016.

REBOLEIRA, J. *et al.*Chapter 3.39 – Spirulina. In: NABAVI, S. M., SILVA, A. S. **Nonvitamin and Nonmineral Nutritional Supplements**:Elsevier, p. 409–413, 2019.

REZENDE E. S. V., LIMA, G. C., NAVES, M. M. V. Dietary fibers as beneficial microbiota modulators: A proposal classification by prebiotic categories. **Nutrition**, v. 89, n. 111217, p. 1–9,2021.

RIZZA,L. S. *et al*. Bioprospecting for native microalgae as an alternative source of sugars for the production of bioethanol. **Algal Research**, v. 22, p. 140–147, 2017.

RODRIGUES, D. *et al*. In vitro fermentation and prebiotic potential of selected extracts from seaweeds and mushrooms. **LWT - Food Science and Technology**, v. 73, p. 131–139, 2016.

RODRIGUES, V. C. D. C. *et al*. Modulation of the intestinal microbiota and the metabolites produced by the administration of ice cream and a dietary supplement containing the same probiotics. **British Journal of Nutrition**, v. 124, n. 1, p. 57–68, 2020.

ROSINI. E. F., SANT'ANNA, C. L., TUCCI, A. Scenedesmaceae (Chlorococcales, Chlorophyceae) de pesqueiros da Região Metropolitana de São Paulo, SP, Brasil: levantamento florístico. **Hoehnea**, v. 40, n. 4, p. 661–678, 2013.

ROSELINO, M. N. *et al*. Effect of fermented sausages with probiotic *Enterococcus faecium* CRL 183 on gut microbiota using dynamic colonic model. **LWT- Food Science and Technology**, v. 132, p. 1–8, 2020.

RUTHES, A. C. *et al*. Prebiotic potential of mushroom D-glucans: implications of physicochemical properties and structural features. **Carbohydrate Polymers**, v. 262, n. 117940, p.1–8, 2021.

SANDERS, M. E. *et al*. Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. **Nature Reviews Gastroenterology & Hepatology**, v. 16, p. 605–616, 2019.

SANTHAKUMAR, P., AYYAPPAN, S. M., RAY, J.G. Nutraceutical applications of twenty-five species of rapid-growing green microalgae as indicated by their antibacterial, antioxidant and mineral content. **Algal Research**, v. 47, n. 101878, p. 1–12, 2020.

SALGAÇO, M. K. *et al*. Probiotic infant cereal improves children's gut microbiota: Insights using the Simulator of Human Intestinal Microbial Ecosystem (SHIME®). **Food Research International**, v. 143, n. 110292, p. 1–9, 2021.

SAVY, N. *et al*. Acute pediatric hyperammonemia: Current diagnosis andmanagement strategies. **Hepatic Medicine: Evidence and Research**, v. 10,
p. 105–115, 2018.
SASSI, K. K. B.*et al*. Metabolites of interest for food technology produced by microalgae from the Northeast Brazil. **Revista Ciência Agronômica**, v. 50, n. 1, p. 54–65, 2019.

SATHASIVAM, R. *et al*. Microalgae metabolites: A rich source for food and medicine. **Saudi Journal of Biological Sciences**, v. 26, n. 4, p. 709–722, 2019.

SAYERS, B., WIJEYESEKERA, A., GIBSON, G. Exploring the potential of prebiotic and polyphenol-based dietary interventions for the alleviation of cognitive and gastrointestinal perturbations associated with military specific stressors. **Journal of Functional Foods**, v. 87, n. 104753, p. 1–15, 2021.

SCAGLIONI, P. T.*et al*. Inhibition of enzymatic and oxidative processes by phenolic extracts from *Spirulina* sp. and *Nannochloropsis* sp. **Food Technology and Biotechnology**, v. 56, n. 3, p. 344–353,2018.

SERRELI, G., DEIANA, M. In vivo formed metabolites of polyphenols and their biological efficacy. **Food & Function**, v. 10, 6999, p. 6999–7021, 2019.

SENÉS-GUERRERO, C. *et al*. Dietary fiber and gut microbiota. **In book: Science and Technology of Fibers in Food Systems**, p. 277–298, 2020.

SILVA, J. *et al*. *Chlorella*. In: NABAVI, S. M., SILVA, A. S. **Nonvitamin and Nonmineral Nutritional Supplements**:Elsevier, p. 187–193, 2019.

SILVA, J. M. Passion fruit-flavored ice cream processed with water-soluble extract of riceby-product: What is the impact of the addition of different prebioticcomponents?**LWT - Food Science and Technology**, v. 128, n. 109472, p. 1 –8, 2020.

SLAVIN, J. Fiber and Prebiotics: Mechanisms and health benefits. **Nutrients**, v. 5, 1417–1435, 2013.

SNELSON, M., KELLOW, N. J., COUGHLAN, M. T. Modulation of the gut microbiota by resistant starch as a treatment of chronic kidney diseases: evidence of efficacy and mechanistic insights. **Advances in Nutrition**, v. 10, p. 303–320, 2019.

SONIA, R. A., SUDHAKAR, K., RANA, R. S. *Spirulina* - From growth to nutritional product: A review. **Trends in Food Science & Technology**, v. 69, p. 157−171, 2017.

SONG, Q. *et al*. Review of the relationships among polysaccharides, gut microbiota, and human health. **Food Research International**, v. 140, n. 109858, p.1–10, 2021.

SOUZA, D. B. S., FELISBERTO, S. A. *Comasiella*, *Desmodesmus*, *Pectinodesmus* e *Scenedesmus* na comunidade perifítica em ecossistema lêntico tropical, Brasil Central. **Hoehnea**, v. 41, n. 1, p. 109–120, 2014.

SOUZA, E. L. *et al*. Potential interactions among phenolic compounds and probiotics for mutual boosting of their health promoting properties and food functionalities – A review. **Critical Reviews in Food Science and Nutrition**, v. 59, n. 10, p. 1645−1659, 2019.

STEIN, J.R. Handbook of phycological methods: culture methods and growth measurements. In: Handbook of phycological methods. Cambridge University Press, p.448, 1973

TIAN, T. *et al*. Chemical characterization of potentially prebiotic oligosaccharides in brewed coffee and spent coffee grounds.**Journal of Agricultural and Food Chemistry**, v. 65, p. 2784−2792, 2017.

VALCHEVA, R., DIELEMAN, L. A. Prebiotics: Definition and protective mechanisms.**Best Practice & Research Clinical Gastroenterology**, v. 30, p. 27–37, 2016.

VAN DE WIELE, T. *et al*. The simulator of the human intestinal microbial ecosystem (SHIME®). **The impact of food bioactives on health**, p. 305-317, 2015.

VAN DEN ABBEELE, P. *et al*. 2′ -Fucosyllactose alters the composition and activity of gut microbiota from formula-fed infants receiving complementary feeding in a validated intestinal model. **Journal of Functional Foods**, v. 61, p. 1–8, 2019.

VEMURI, R. *et al*. *Lactobacillus acidophilus* DDS-1 modulates the gut microbiota and improves metabolic profiles in aging mice. **Nutrients**, v. 10, n. 9, p. 1–19, 2018.

VERBEKE, K. A. Towards microbial fermentation metabolites as markers for health benefits of prebiotics. **Nutrition Research Reviews**, v. 28, 42–66, 2015.

VERMA, R. *et al*. Photoautotrophic, mixotrophic, and heterotrophic culture media optimization for enhanced microalgae production. **Journal of Environmental Chemical Engineering**, v. 8, n. 5, p. 1-13, 2020.

WAN, X. *et al*. Effect of marine microalga *Chlorella pyrenoidosa* ethanol extract on lipid metabolism and gut microbiota composition in high-fat diet-fed rats. **Marine Drugs**, v. 16, n. 498, p. 1–14, 2018.

WANG, Y., QIAN, P. Y. Conservative fragments in bacterial 16S rRNA genes and primer design for 16S ribosomal DNA amplicons in metagenomic studies. **PLoS ONE**, v. 4, n. 10, p. 1–9, 2009.

WEI, T., SIMKO, V. (2021). R package "corrplot": Visualization of a correlation matrix.

https://github.com/taiyun/corrplot.

WILSON, K. E., HUNER, N. P. A. The role of growth rate, redox-state of the plastoquinone pool and the trans-thylakoid Delta pH in photoacclimation of *Chlorella vulgaris* to growth irradiance and temperature. **Planta**, v. 212, p. 93–102, 2000.

WŁODARCZYK, M., ŚLIŻEWSKA, K. Obesity as the 21st Century's major disease: The role of probiotics and prebiotics in prevention and treatment.**Food Bioscience**, v. 42, n. 101115, p.1–24, 2021.

YO, D. *et al*. Influence of prebiotic biopolymers on physicochemical and sensorycharacteristics of yoghurt. **International Dairy Journal**, v. 115, n. 104915, 2021.

YUAN, Q. *et al*. Isolation, structures and biological activities of polysaccharides from Chlorella: A review. **International Journal of Biological Macromolecules**, v. 163, p. 2199–2209, 2020.

ZARROUK, C. Contribution a l'etude d'une cyanophycee: influence de divers facteurs physiques et chimiques sur la croissance et la photosynthese de *Spirulina maxima* (Setch et Gardner) Geitler. Theises. Faculty of Science. Universite des Paris; 1966

ZHAO, W. The immune regulatory role of *Lactobacillus acidophilus*: An updated meta-analysis of randomized controlled trials. **Food Bioscience**, v. 36, n. 100656, p. 1–6, 2020.

ZHANG, S. *et al*. Preparation and prebiotic potential of pectin oligosaccharides obtained from citrus peel pectin. **Food Chemistry**, v. 244, p. 232–237, 2018.

ZHENG, S. *et al*. Do short chain fatty acids and phenolic metabolites of the gut have synergistic anti-inflammatory effects? – New insights from a TNF-α-induced Caco-2 cell model. **Food Research International**, v. 139, 109833, p. 1–13, 2021.

ZUO, F., MARCOTTE, H. Advancing mechanistic understanding and bioengineering of probiotic lactobacilli and bifidobacteria by genome editing. **Current Opinion in Biotechnology**,v. 70, p. 75–82, 2021.

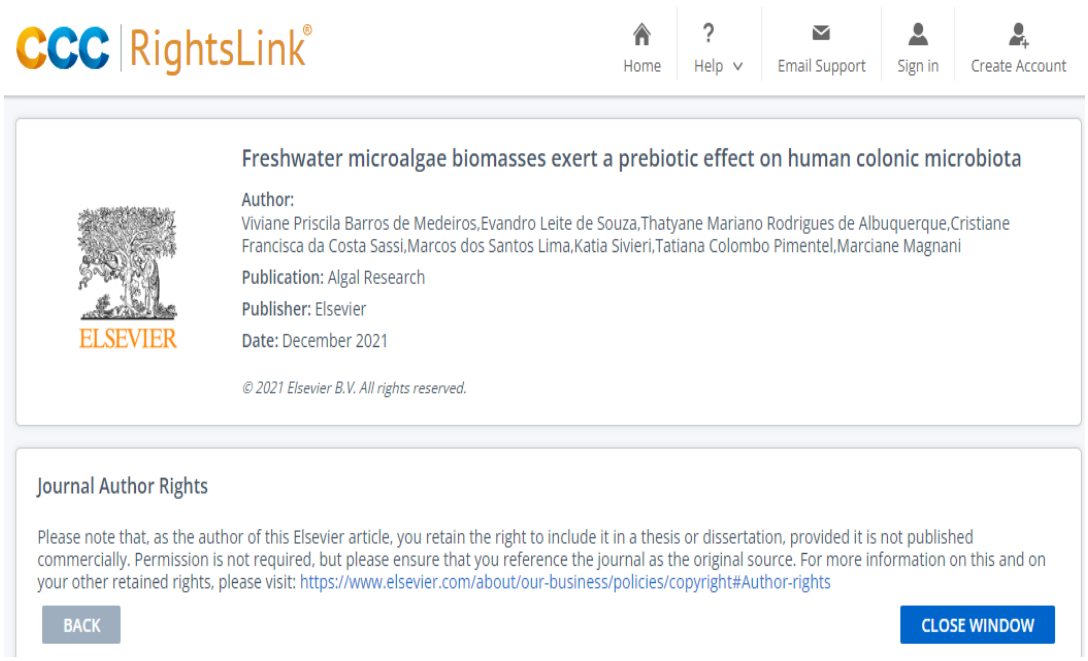
ZHU, L. *et al*. Nutritional composition, antioxidant activity, volatile compounds, and stability properties of sweet potato residues fermented with selected lactic acid bacteria and bifidobacteria. **Food Chemistry**,v. 374., n. 131500, p.1 –11, 2022.

APÊNDICES

APÊNDICE A – ARTIGO I

Freshwater microalgae biomasses exert a prebiotic effect on human colonic microbiota.

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Phenolics

ABSTRACT

The effects of digested microalgae biomass of *Chlorella vulgaris*, *Desmodesmus maximus*, *Chlorococcum* sp. cf *hypnosporum*, and *Spirulina platensis* or fructooligosaccharides on human colonic microbiota during in vitro fermentation (48 h) were evaluated. Fructooligosaccharides and digested microalgae biomass increased *Lactobacillus-Enterococcus* and *Bifidobacterium* relative abundances and decreased *Prevotellaceae-Bacteroidaceae*, *Clostridium histolyticum*, and *Eubacterium rectale-Clostridium coccoides*. The digested microalgae biomass s showed a higher prebiotic index than fructooligosaccharides, evidenced through the higher selective stimulation of beneficial microorganisms and inhibition of undesirable bacteria on colonic microbiota. *S. platensis* biomass contained nystose, raffinose, and phenolics, increased the relative abundance of *Lactobacillus-Enterococcus* and *Bifidobacterium*, and decreased *Eubacterium rectale-Clostridium coccoides*, *Clostridium histolyticum*, and *Prevotellaceae-Bacteroidaceae*. After 24 h of incubation, the medium fermented with *S. platensis* promoted an increase in the concentration of acetic and propionic acids. *D. maximus* biomass showed higher insoluble fibers and kestose, the greatest *Lactobacillus-Enterococcus* relative abundance, and increases in the butyric and propionic acids after 48 h. *C. vulgaris* biomass showed the highest glucose and fructose concentrations, some phenolics, and the highest *Bifidobacterium* relative abundance after 48 h.

1. Introduction

Microalgae form a diverse group of ubiquitous photosynthetic organisms comprising eukaryotic green algae and prokaryotic cyanobacteria. They can grow and reproduce in a wide variety of environments, especially in freshwater (lakes and rivers), brackish, marine waters (seas and oceans), soil, and wastewater. Moreover, they can be easily cultivated, providing rapid growth and biomass yield, being a sustainable production alternative compared to traditional foods [1].

The microalgae market is dominated by two microalgae genera, *Spirulina* sp., with more than 12,000 tons of biomass produced each year, and *Chlorella* sp., with about 5000 tons/year [2]. *Spirulina platensis* and *Chlorella vulgaris* have been the main microalgae species studied and they are classified as safe for human consumption (Generally recognized as safe (GRAS)) according to the Food and Drug Administration [2,3]. Furthermore, their biomass has a wide variety of nutrients and bioactive compounds, including polyunsaturated fatty acids (PUFAs), pigments (chlorophylls), carbohydrates (polysaccharides and oligosaccharides), proteins (peptides and amino acids), vitamins, polyphenols, and phytosterols [2]. As microalgae produce several primary and secondary metabolites, the possibility of their using as food supplements has been investigated [4].

However, nature has a diversity and abundance of microalgae species that are still poorly exploited. *Desmodesmus* sp. and *Chlorococcum* sp. are non-toxic microalgae, and they are found in poorly studied and isolated regions [5]. *Chlorococcum* sp. can accumulate nutrients,

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especially carbohydrates, lipids, and bioactive compounds of interest [6]. The *Desmodesmus* sp. biomass has been widely used to extract lipids to produce biofuels [7] and in the synthesis of bioethanol due to the presence of fermentable sugars [8]. *Spirulina platensis*, *Chlorella vulgaris*, *Desmodesmus maximus*, and *Chlorococcum* sp. cf *hypnosporum* are GRAS or non-toxic producing species with relevant bioactive compounds, mainly carbohydrates (oligosaccharides) and phenolic compounds, which could have a potential prebiotic effect.

Prebiotics are substrates that are selectively utilized by host microorganisms conferring a health benefit [9,10]. A prebiotic compound must be resistant to acid and enzyme digestion and absorption in the upper gastrointestinal tract. In this way, it is fermented by gut microbiota and stimulates their growth, improving their activity and host health and well-being [9]. In addition, prebiotic consumption may modulate bacterial composition and metabolism by producing short-chain fatty acids, tryptophan, and organic acids [11]. The beneficial effects of prebiotics encompass defense against pathogens, modulation of the immune response, mineral absorption, improvement in bowel function, metabolic effects, and satiety [12].

The main prebiotic compounds studied are inulin-type fructans, mainly fructooligosaccharides (FOS). However, the utilization of unconventional prebiotic ingredients has emerged as a new trend [10]. Microalgae biomass has received attention as a promising alternative source of prebiotic ingredients due to oligosaccharides [13] and phenolic compounds in its composition [14].

To evaluate the possible in vitro microalgae prebiotic effects on intestinal microbiota, it is advisable to use an in vitro digestion protocol (oral, gastric, and intestines) and further subject the digested biomass to colonic fermentation, where the microbiota modulation induced by the microalgae and produced metabolites can be quantified [15]. Studies considering the prebiotic effects of microalgae are still scarce, and they evaluated, mainly, the effects of microalgae isolated oligosaccharides on the growth of specific probiotic cultures [13]. A previous study reported that the administration of microalgae *Euglena gracilis* as a whole-cell increased the relative abundance of bacteria belonging to the genus *Faecalibacterium* with a consequent synthesis of butyrate in the human colonic microbiota of healthy individuals in an in vitro assay [16]. The prebiotic potential of *E. gracilis* or its isolated polysaccharide (Paramylon) was also demonstrated by Bhattad et al. [17]. Finally, one study reported a human microbiota modulation effect provided by microalgae. Still, it used the most common microalgae species (*Chlorella vulgaris*) in a dried form and evaluated only propionate-producing bacteria [15]. However, no prior studies reported the effect of different freshwater microalgae species biomass on human microbiota. Considering its safety aspects and nutritional and bioactive composition, microalgae biomass could represent a new source of prebiotic ingredients.

This study aimed to evaluate the effect of digested microalgae biomass (DMB, *Chlorella vulgaris*, *Desmodesmus maximus*, *Chlorococcum* sp. cf *hypnosporum*, and *Spirulina platensis*) on the relative abundance of specific gut microbiota bacterial groups using an in vitro colonic fermentation system. The microbial metabolites consumed or produced (organic acid and sugar contents, short-chain organic acids) and phenolics were quantified in the fermentation medium over 48 h. FOS was used as a positive control.

2. Materials and methods

2.1. Species, cultivation, and production of microalgae biomass

Four species of freshwater microalgae, namely *Chlorella vulgaris* (CHL), *Desmodesmus maximus* (DES), *Chlorococcum* sp. cf *hypnosporum* (CHLO), and *Spirulina platensis* (SPI), belonging to the culture collection of the Laboratory of Reef Environments and Biotechnology with Microalgae (Federal University of Paraíba, João Pessoa, Paraíba, Brazil) were used. These species were selected due to the GRAS status, reported safety aspects (non-toxigenic), and bioactive compounds concentration, mainly carbohydrates (oligosaccharides) and phenolic compounds, which could exert prebiotic effects [5].

The CHL and DES were cultivated in the WC medium, while CHLO and SPI were cultivated in the Zarrouk medium [18]. The composition of Zarrouk [19] and WC [20] media are provided in Tables S1 and S2, respectively. The cultivation conditions were 25 ± 1 °C, illumination of 4.5 ± 0.5 klx using 40 W fluorescent lamps under a 12 h photoperiod, and continuous aeration at 0.12 L/h. Cell counts and in vivo fluorescence measurements (using Turner-Design 10005R fluorometer) were performed every 24 h up to reaching the stationary phase (approximately 11–14 days). The biomass obtained for each species was centrifuged at refrigerated temperature, frozen at −30 °C, and lyophilized (8% moisture content). Lyophilized biomasses were hermetically sealed in polyethylene bags and stored at −18 °C. The biomass weight, daily biomass productivity, growth rate (k), and maximum cell numbers [21] were determined.

2.2. Determination of dietary fiber, sugars, oligosaccharides, and phenolic compounds in the microalgae biomass

The total, insoluble, and soluble dietary fiber contents were determined using the enzymatic-gravimetric method (Sigma-Aldrich, St. Louis, USA) (method 991.43) [22]. Oligosaccharides were obtained through sonication and enzymatic hydrolysis following the procedures described by Leal et al. [13], with minor adaptations, and analyzed according to dos Santos Lima [23]. According to Dong et al. [24], monosaccharides were obtained by acid hydrolysis and analyzed following Coelho et al. [25]. Finally, the phenolic compounds were extracted according to Scaglioni et al. [26] and analyzed following Padilha et al. [27]. The methodologies used to determine oligosaccharides, monosaccharides, and phenolic compounds are described in the Supplementary Material. The peaks of oligosaccharides, monosaccharides, and phenolic compounds were identified by comparing their retention times with external standards (Sigma Aldrich, St. Louis, USA). All analyzed compounds presented calibration curves with $R^2 \geq 0.998$. In addition, the peak spectral purity was verified using the Threshold tool to ensure the identification accuracy compared with the external standard.

2.3. In vitro gastrointestinal digestion of microalgae biomasses

The microalgae biomasses were submitted to in vitro gastrointestinal digestion following INFOGEST's protocol [28]. After the digestion, the solutions containing the final digestion phase were dialyzed for 24 h against 0.01 M NaCl at 5 ± 0.5 °C [29]. The digested microalgae biomass was frozen (−18 °C), freeze-dried, and stored (5 ± 0.2 °C) in hermetically sealed polyethylene bags. The dialysis membrane, enzymes (α-amylase, lipase, pepsin, pancreatin), bile salts, and all other reagents were purchased from Sigma-Aldrich (St. Louis, USA).

2.4. Human fecal inoculum and fermentation system

Fecal samples from 4 healthy adult volunteers (two men and two women, aged 19–36 years old) were used after approval from the Committee for Ethics in Research with Human Beings (Federal University of Paraíba, João Pessoa, Paraíba, Brazil, CAAE no. 4.563.908). The inclusion criteria were people not suffering from any gastrointestinal or colonic illness, following a regular omnivorous diet without using probiotic foods, concentrated probiotics, or prebiotics, and with no antibiotics or medicines during the six months before the study.

The donors received specific instructions in addition to a suitable collection kit. The collection kit of feces included sterilized collecting tubes, disposable spatulas, instructions for collecting/mailing the specimen, disposables gloves, and alcohol 70%. In addition, the instructions contained in the kit described the steps of hand hygiene and aseptic handling of samples. The feces were collected in sterile tubes. They were

arranged in a jar with an anaerobiosis generator system (AnaeroGen, Oxoid, Basingstoke, England) and transported to the laboratory. The experiments were initiated immediately after stool arriving to ensure a fresh fecal microbiota, and the feces were manipulated in an environment under anaerobic conditions. For the experiments, the same amount of feces from each donor were pooled and mixed with sterile phosphate-buffered saline (PBS; 0.1 M; pH 7.4; 1:10, w/v) under stirring (200 rpm) for 2 min [30].

Each batch was composed of 40% of the fermentation medium (v/v; Table S3), 40% of pooled human fecal inoculum (v/v), and 20% of the digested biomass of CHL, DES, CHLO, or SPI (w/v). The fermentation was performed at 37 °C for 48 h under anaerobic conditions (AnaeroGen). A positive control batch was performed, replacing the digested microalgae biomass with FOS (20%, w/v). The batch without the addition of digested microalgae biomass or FOS comprised the negative control [35]. The salts and reagents used in the fermentation medium were purchased from Sigma-Aldrich (St. Louis, USA).

2.5. Fluorescent in situ hybridization (FISH) coupled with flow cytometry (FC)

FISH analyses were performed with five fluorescent Cy3-labelled probes (oligonucleotides) able to hybridize the 16S rRNA gene-specific region of selected bacterial groups (Table 1) as follow: *Bifidobacterium*: Bif164 (BIF, strictly anaerobes), *Lactobacillus–Enterococcus*: Lab 158 (LAB, facultative anaerobes), *Bacteroides–Prevotella:* Bac 303 (BAC, strictly anaerobes), *Eubacterium rectale-Clostridium coccoides*: Erec 482 (EREC, strictly anaerobes), and *Clostridium histolyticum*: Chis 150 (CHIS, strictly anaerobes) (Sigma-Aldrich, St. Louis, USA) [30–34]. The total bacterial population was measured using SYBR Green (Invitrogen, Carlsbad, CA, USA) [36]. The bacterial groups were chosen because they represent groups of the fecal microbiota that, once modulated, have been associated with positive and negative metabolic responses [30,35,37]. Furthermore, the utilization of these groups has provided high accuracy results in investigations to assess the effects of prebiotic candidates on gut microbiota [38].

At zero time and after 24 and 48 h of fermentation, aliquots were collected from each batch (375 μL), overnight fixed with 4% paraformaldehyde solution at 4 ± 0.5 °C and washed two times by centrifugation (10,000 ×*g* 5 min 4 °C) with sterile PBS 1 M. The fixed cells were resuspended in PBS: ethanol 99% (1:1 v/v) solution and filtered through a 0.2-μm membrane Millex-HA filter (Millipore Co., Bedford, MA) and stored (−20 °C). For hybridization experiments, aliquots of fixed cells were resuspended in sterile PBS 1× (1:10, Gibco, Gaithersburg, USA; pH 7.2), centrifuged (4000 ×*g*, 15 min, 4 °C), resuspended in Tris-EDTA buffer (100 mM Tris-HCl and 50 mM EDTA; pH 8) and centrifuged (4000 ×*g*, 15 min, 4 °C). The samples used for hybridization with probes Lab 158 and Bif 164 were also previously permeabilized by incubation with lysozyme (1 mg/mL Tris-EDTA) for 10 min in the dark at 25 ± 0.5 °C.

Samples were resuspended in 45 μL of hybridization buffer [0.9 M NaCl, 20 mM Tris–HCl (pH 7.5) and 0.1% (w/v) sodium dodecyl sulfate (SDS)], added with 5 μL of the tested probe (50 ng/μL), and incubated at 46 or 50 °C (Table 1) for 4 h in the dark. After new centrifugation (4000 ×*g*, 15 min, 25 °C), samples were resuspended in 200 μL of hybridization buffer without SDS for washing at 48 or 50 °C (Table 1) for 30 min in the dark. Finally, samples were centrifuged using the previously described conditions and resuspended with 200 μL of PBS 1× and 20 μL of SYBR Green (1:1000 stock diluted in dimethyl sulfoxide ≥ 99.9% Sigma-Aldrich, St. Louis, USA), incubated for 10 min in the dark at room temperature (25 ± 0.5 °C), centrifuged (4000 ×*g*, 15 min, 25 °C), and resuspended in 200 μL of PBS 1×.

Measurements were done in a BD Accuri C6 flow cytometer (New Jersey, USA) using 488 nm excitation from a blue solid-state laser. Signals from individual cells through the laser zone were collected as logarithmic signals. Fluorescence signals were recorded on FL1 (SYBR Green) and FL2 (Lab 158, Bif 164, Bac 303, Chis 150, Erec 482) channels as cytograms by the BD Accuri C6 Software. Results were expressed as relative abundance calculated considering the percentage of cells hybridized with each probe (Table S4) concerning total bacteria stained [36].

2.6. Prebiotic index determination

The prebiotic index of each microalgae biomass and FOS was determined using the equation described by Albuquerque et al. [37]

$$\text{Prebiotic index} = \%\text{Lab} + \%\text{Bif} - \%\text{Bac} - \%\text{Chis} - \%\text{Erec} \quad (1)$$

where: %Lab is the percentage hybridized for Lab after 24 or 48 h - percentage hybridized at time zero; %Bif is the percentage hybridized for Bif after 24 or 48 h - the percentage hybridized for Bif at time zero; % Bac is the percentage hybridized for Bac after 24 or 48 h - the percentage hybridized for Bac at time zero; %Chis is the percentage hybridized for Chis after 24 or 48 h - the percentage hybridized for Chis at time zero; and %Erec is the percentage hybridized for Erec after 24 or 48 h - the percentage hybridized Erec at time zero.

The equation assumes as a positive effect the increase in *Lactobacillus-Enterococcus* and/or *Bifidobacterium* populations and as the negative effect the increase in *Bacteroides-Prevotella*, *C. histolyticum*, and *E. rectale-C. coccoides* populations.

2.7. Fermentation metabolites of fecal fermentation analyses

Metabolites resulting from the activity of human fecal inoculum in media with different digested microalgae biomass, FOS, and negative control were evaluated at the time of incubation (time zero - just after homogenization) and after 24 and 48 h of fermentation through the determination of pH values (method 981.12) in digital potentiometer (Quimis, Diadema, São Paulo, Brazil) [22] and sugars, organic acids, and phenolic compounds according to the methodologies described in Section 2.2.

Table 1
Oligonucleotide probes of 16S rRNA and hybridization conditions used in FISH analyses.

Probe name	Bacteria group	Sequence (5′-3′)	Hybridization pre-treatment	Temperature of Hybridization/washing (°C)	References
Bif 164	*Bifidobacterium* spp.	CATCCGGCATTACCACCC	Lysozyme	50/50	Langendijik et al. [31]
Lab 158	*Lactobacillus* spp. and *Enterococcus* spp.	GGTATTAGCAYCTGTTTCCA	Lysozyme	50/50	Harmsem et al. [32]
Bac 303	Most of *Prevotellaceae*, *Bacteroidaceae* and some *Porphyromonadaceae* (Bacteroides group)	CCAATGTGGGGGACCTT	None	46/48	Manz et al. [33]
Erec 482	Most of *Eubacterium rectale* group and *Clostridium coccoides* (Clostridium cluster XIVa and XIVb)	GCTTCTTAGTCARGTACCG	None	50/50	Franks et al. [34]
Chis150	Most of *Clostridium histolyticum* group (Clostridium clusters I and II)	TTATGCGGTATTAATCTYCCTTT	None	50–50	Franks et al. [34]

2.8. Statistical analysis

All analyses were conducted in triplicate in three different experiments, and the results were computed, submitted to a normality test, and expressed as means ± standard deviation. Data were submitted to a Student's *t*-test or one-way analysis of variance (ANOVA), followed by the Tukey's multiple comparisons test ($p < 0.05$). Principal component analysis (PCA) was run with a matrix of 15 lines (4 microalgae biomass and FOS at three different times) and 13 columns (microbial groups, sugars, and organic acids). PCA was used to observe the differences in microalgae biomass during fermentation, considering the microbial groups, sugars, and organic acids. PCA is a multivariate technique widely used in search of behavior patterns, and it can assist in interpreting information from a large amount of data. In this way, trends and relevant information may be revealed with PCA, which would not be immediately observed in tables [39]. Furthermore, confidence ellipses were constructed using the bootstrap technique and 500 resamplings. Hierarchical clustering analysis (HCA) was performed using the coordinates of the first and second dimensions of the PCA map. Ward's method (agglomeration method), Euclidean distances (dissimilarity), and automatic truncation were used. The dendrogram's quality was evaluated using the cophenetic correlation coefficient. The computational software GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA) and the XLSTAT software for Windows version 2020.1.2 (Addinsoft, New York, USA) were used.

3. Results

3.1. Production of microalgae biomass and cell growth parameters

The growth and biomass productivity of the microalgae cultivated on synthetic media varied with the microalgae species (Table S5). SPI and CHLO species showed higher total biomass productivity (500 and 450 mg/L), daily productivity (38.46 and 32.14 mg L^{-1} day^{-1}), k values (1.60 and 1.23), and cell number productivity (18.01 and 16.20 × 10^5 mL^{-1}) than DES and CHL species. The highest growth and biomass productivity were observed for SPI.

3.2. Dietary fiber and profile of sugars, oligosaccharides, and phenolic compounds of microalgae biomass

The microalgae biomass from all species presented a higher insoluble dietary fiber content (27–42.6 g/100 g) than soluble dietary fiber (1.5–2.08 g/100 g). Besides, they showed glucose (4–16 g/100 g), rhamnose (2.8–52 g/100 g), and fructose (4–10 g/100 g) as sugars. In addition, sixteen different phenolic compounds were identified, including flavonols, flavanone, and stilbenes (Table 2).

The type of microalgae species significantly impacted the type and concentration of dietary fiber, sugars, oligosaccharides, and phenolic compounds found in the biomass. DES biomass was characterized by the higher concentration of insoluble and total dietary fibers, kestose as oligosaccharide, and epigallocatechin gallate and naringenin as phenolic compounds. SPI biomass was characterized by nystose and raffinose as oligosaccharides, and higher concentrations of chlorogenic acid, epicatechin, and myricetin as phenolic compounds. CHL biomass was characterized by the higher concentration of sugars (glucose, rhamnose, and fructose) and syringic acid, catechin, epicatechin gallate, procyanidin B1, procyanidin B2, quercetin 3-glucoside, rutin, and cis-resveratrol as phenolic compounds. CHLO biomass was characterized by procyanidin A2, hesperidin, and kaempferol 3-glucoside as phenolic compounds.

3.3. Relative abundance of bacterial populations measured during in vitro colonic fermentation of digested microalgae biomass

The type of microalgae species biomass and the colonic fermentation

Table 2
Fibers, oligosaccharides, sugars and phenolics contents in microalgae biomass.

Components	Microalgae species			
	CHL	DES	CHLO	SPI
Fibers (g/100 g)				
Soluble dietary fiber	2.00 ± 0.02^{a}	2.08 ± 0.04^{a}	2.00 ± 0.02^{a}	1.50 ± 0.04^{a}
Insoluble dietary fiber	40.0 ± 0.06^{b}	42.60 ± 0.07^{a}	33.88 ± 0.03^{c}	27.00 ± 0.02^{d}
Total dietary fiber	42.0 ± 0.05^{b}	44.68 ± 0.06^{a}	35.88 ± 0.06^{c}	28.5 ± 0.05^{d}
Oligosaccharides (mg/100 g)				
Kestose	53.67 ± 0.05^{c}	110.62 ± 0.04^{a}	69.68 ± 0.04^{b}	<LOD
Nystose	<LOD	<LOD	<LOD	3.71 ± 0.02^{a}
Raffinose	<LOD	<LOD	<LOD	6.14 ± 0.03^{a}
Sugars (g/100 g)				
Glucose	16.00 ± 0.03^{a}	6.00 ± 0.05^{c}	10.00 ± 0.02^{b}	4.00 ± 0.03^{d}
Rhamnose	52.00 ± 0.05^{a}	2.80 ± 0.01^{c}	12.00 ± 0.04^{b}	12.00 ± 0.01^{b}
Fructose	10.00 ± 0.02^{a}	8.00 ± 0.03^{b}	8.00 ± 0.02^{b}	4.00 ± 0.04^{c}
Phenolic compounds (mg/100 g)				
Syringic acid	7.02 ± 0.01^{a}	< LOD	<LOD	<LOD
Chlorogenic acid	<LOD	< LOD	<LOD	387.46 ± 0.03^{a}
Catechin	280.16 ± 0.05^{a}	55.23 ± 0.04^{b}	34.21 ± 0.03^{c}	58.01 ± 0.02^{b}
Epicatechin	16.04 ± 0.04^{b}	< LOD	5.06 ± 0.04^{c}	33.10 ± 0.05^{a}
Epicatechin gallate	25.05 ± 0.03^{A}	20.31 ± 0.01^{B}	<LOD	<LOD
Epigallocatechin gallate	18.13 ± 0.02^{B}	27.21 ± 0.03^{A}	<LOD	<LOD
Procyanidin B1	54.05 ± 0.03^{a}	<LOD	<LOD	32.25 ± 0.02^{b}
Procyanidin B2	37.71 ± 0.03^{a}	13.07 ± 0.02^{c}	<LOD	25.34 ± 0.01^{b}
Procyanidin A2	39.06 ± 0.04^{b}	41.25 ± 0.03^{b}	56.17 ± 0.02^{a}	<LOD
Hesperidin	54.14 ± 0.03^{b}	<LOD	128.13 ± 0.02^{a}	51.13 ± 0.01^{c}
Myricetin	<LOD	<LOD	<LOD	29.04 ± 0.04
Naringenin	25.22 ± 0.02^{B}	26.81 ± 0.04^{A}	<LOD	<LOD
Quercetin 3-glucoside	118.20 ± 0.04^{a}	18.45 ± 0.05^{b}	19.14 ± 0.03^{b}	<LOD
Rutin	9.35 ± 0.01^{a}	2.19 ± 0.05^{b}	<LOD	2.26 ± 0.01^{b}
Kaempferol 3-glucoside	5.22 ± 0.03^{d}	6.14 ± 0.02^{c}	18.58 ± 0.01^{a}	11.21 ± 0.02^{b}
cis-Resveratrol	44.25 ± 0.02^{a}	20.14 ± 0.03^{b}	<LOD	15.10 ± 0.02^{c}

Data reported as mean ± standard deviation, n = 3 repetitions. CHL: *Chlorella vulgaris*; DES: *Desmodesmus maximus*; CHLO: *Chlorococcum* cf. *hypnosporum*; SPI: *Spirulina platensis*. A–B: Different superscript capital letters in the same row indicate a significant difference ($p < 0.05$) among the content of fibers, oligosaccharides, sugars and phenolics of the microalgae biomass, based on Student t-test. a–c: different superscript small letters in the same row denotes difference ($p < 0.05$) among the content of fibers, oligosaccharides, sugars and phenolics of the microalgae biomass, based on Tukey's test. LOD: limit of detection; LOD were as follow: Kestose 0.214 g/L, nystose 0.179 g/L, raffinose 0.192 g/L; glucose 0.02 g/L; fructose 0.04 g/L, and rhamnose 0.04 g/L; catechin 0.06 mg/L, epicatechin 0.06 mg/L, syringic acid 0.08 mg/L, epigacatechin-gallate 0.06 mg/L, epigallocatechin-gallate 0.04 mg/L, procyanidin A2 0.06 mg/L, procyanidin B1 0.01 mg/L, procyanidin B2 0.04 mg/L; *cis*-resveratrol 0.08 mg/L, hesperidin 0.12 mg/L, chlorogenic acid 0.11 mg/L, quercetin 3-glucoside 0.11 mg/L, rutin 0.08 mg/L, naringenin 0.16 mg/L, kaempferol 3-glucoside 0.10 mg/L, myricetin 0.05 mg/L.

time significantly impacted microbial diversity (Fig. 1, Table S6). A decrease in *Lactobacillus-Enterococcus*, *Bifidobacterium*, *Bacteroides-Prevotella*, and *C. histolyticum* relative abundance, and an increase in *E. rectale-C. coccoides* relative abundance in the microbiota of the negative control was observed during the colonic fermentation. On the contrary, increases in *Lactobacillus-Enterococcus* and *Bifidobacterium* and decreases in *Bacteroides-Prevotella*, *C. histolyticum*, and *E. rectale-C. coccoides* relative abundances were observed during colonic fermentation when FOS and the microalgae biomass (DES, SPI, CHL, and CHLO) were added to the medium. The increase in *Lactobacillus-Enterococcus*, and *Bifidobacterium* was faster in the medium with SPI, where the higher counts were observed after 24 h of fermentation.

At the end of the colonic fermentation (48 h), the highest *Lactobacillus–Enterococcus* relative abundance was observed for FOS, DES, and SPI, with similar increases in the relative abundance (approximately 30%) during the colonic fermentation. At the same time, the highest *Bifidobacterium* relative abundance was observed for CHL and SPI, with similar increases in the relative abundance (36–37%) during the fermentative process. The lowest *C. histolyticum* and *E. rectale-C. coccoides* relative abundances were observed for SPI. Finally, the lowest *Bacteroides–Prevotella* relative abundance was observed for FOS, SPI, and CHL. However, a higher reduction was observed for FOS and SPI (16–18%) than CHL (0.68%) during the colonic fermentation.

3.4. Determination of prebiotic index of digested microalgae biomass

The prebiotic index of control was negative (−1.35), and it decreased with the increase in the colonic fermentation time (−3.96, Table 3). On the other hand, the prebiotic indexes of FOS and microalgae biomasses (DES, SPI, CHL, CHLO) were positive and increased with the colonic fermentation time. After 24 h of colonic fermentation, SPI showed the highest prebiotic index (6.93), followed by CHLO (4.97), DES (4.90), FOS (2.75), and CHL (2.54). At the end of the fermentation time (48 h), the prebiotic index of all digested microalgae biomasses was higher than that of FOS. DES showed the highest prebiotic index (11.29), followed by SPI (10.10), CHL (8.51), CHLO (7.16), and FOS (4.67).

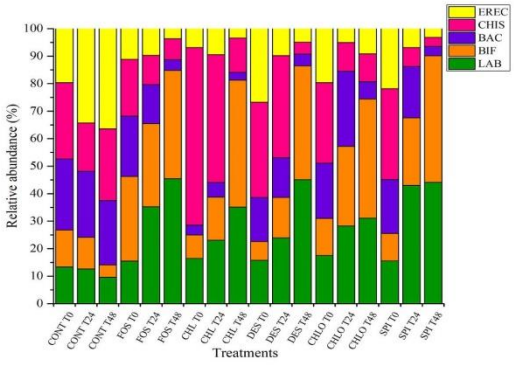


Fig. 1. Relative abundance of different bacterial groups in an in vitro human colonic fermentation system with digested biomass microalgae (CHL, DES, CHLO and SPI), fructooligosaccharides (FOS) and control (no added fermentable substrate) at time zero, 24 and 48 h of fermentation.
CHL: *Chlorella vulgaris*; DES: *Desmodesmus maximus*; CHLO: *Chlorococcum* cf. *hypnosporum*; SPI: *Spirulina platensis*. Lab 158 specific to *Lactobacillus–Enterococcus*, Bif 164 specific to *Bifidobacterium*, Bac 303 specific to *Bacteroides–Prevotella*, Chis 150 specific to *Clostridium histolyticum* and Erec 482 specific to *Eubacterium rectale–Clostridium coccoides*. Results are expressed as means ± standard deviation, n = 3 repetitions.

Table 3
Prebiotic score of different digested microalgae biomass based on the measurements of relative prevalence of distinct bacterial groups by fluorescence in situ hybridization (FISH) combined with multiparametric flow cytometry after 24 and 48 h of an in vitro colonic fermentation.

Samples	Prebiotic score	
	24 h	48 h
CONT	-1.35 ± 0.04^{Ae}	-3.96 ± 0.02^{Bf}
FOS	2.75 ± 0.05^{Bc}	4.67 ± 0.06^{Ae}
CHL	2.54 ± 0.02^{Bd}	8.51 ± 0.04^{Ac}
DES	4.90 ± 0.04^{Bb}	11.29 ± 0.05^{Aa}
CHLO	4.97 ± 0.07^{Bb}	7.16 ± 0.06^{Ad}
SPI	6.93 ± 0.05^{Ba}	10.10 ± 0.07^{Ab}

Data reported as mean ± standard deviation, n = 3 repetitions. CHL: *Chlorella vulgaris*; DES: *Desmodesmus maximus*; CHLO: *Chlorococcum* cf. *hypnosporum*; SPI: *Spirulina platensis*. A–B: Different superscript capital letters in the same row for the same cultivation media denotes differences ($p < 0.05$), based on Student's *t*-test. a–f: different superscript small letters in the same column at a same time interval denotes difference ($p < 0.05$) among cultivation media, based on Tukey's test.

3.5. Fermentation metabolites in media with digested microalgae biomass

A decrease in glucose, lactic acid, succinic acid, butyric acid, propionic acid, and pH values were observed in the negative control media during the colonic fermentation (Table 4). On the contrary, decreases in fructose and glucose contents and pH values and increases in lactic acid and acetic acid contents were observed during colonic fermentation when FOS and the microalgae biomass (DES, SPI, CHL, CHLO) were added to the medium (Table 4). The utilization of DES resulted in decreases in malic acid and succinic acid contents and increases in butyric acid and propionic acid contents during fermentation. The utilization of CHLO resulted in increases in malic, butyric, and propionic acid content, while the utilization of CHL increased the propionic acid content. Finally, the utilization of SPI increased the succinic and malic acid contents. At 24 h of fermentation, the medium with SPI showed the highest concentration of propionic acid and acetic acid. Then, after 48 h of fermentation, FOS showed the highest concentrations of fructose, glucose, lactic acid, and malic acid and the lowest pH value. At the same time, CHLO showed the highest concentration of butyric and propionic acids.

PCA map and confidence ellipses are shown in Fig. 2. The first principal component (F1) explained 36.26% of the variability of the original variables, whereas the second principal component (F2) explained 29.52%, totaling 65.78% of explanation (Fig. 2A). The variables that presented a correlation coefficient with each component greater than 0.6 (absolute values) were considered relevant (Table S7). Fructose, glucose, lactic acid, and malic acid were positively correlated with F1, while propionic acid was negatively correlated whit this axis. In F2, *Lactobacillus-Enterococcus*, *Bifidobacterium*, and acetic acid were positively correlated, and *C. histolyticum* and *E. rectale-C. coccoides* were negatively correlated. *Bacteroides-Prevotella*, butyric acid, and succinic acid did not correlate with any axis ($r < 0.6$).

The F1 component separated FOS from the microalgae biomass. When FOS was used, a higher concentration of sugars (fructose and glucose), lactic acid, and malic acid were observed (right side of the axis). In contrast, when the microalgae biomass (DES, SPI, CHL, and CHLO) were used, a higher concentration of propionic acid was observed (left side of the axis) (Fig. 2A). The F2 component separated the microalgae biomass based on the fermentation time. All microalgae biomasses from 0 h and DES and CHL from 24 h were located below the axis, together with FOS. They were characterized by the higher relative abundance of *C. histolyticum* and *E. rectale-C. coccoides*. On the other hand, all the microalgae biomass from 48 h of fermentation and SPI and CHLO from 24 h were located above the axis and characterized by the higher relative abundance of *Lactobacillus-Enterococcus* and

Table 4
pH values and sugar and organic acid contents (g/L) in media with digested microalgae biomass, fructooligosaccharides (FOS) and control at time zero and after 24 and 48 h of an in vitro colonic fermentation.

Parameter	Samples	Time of incubation		
		0 h	24 h	48 h
pH value	CONT	4.85 ± 0.04^{Ad}	4.64 ± 0.02^{Bc}	3.62 ± 0.03^{Ca}
	FOS	4.60 ± 0.02^{Ae}	3.29 ± 0.04^{Bf}	2.25 ± 0.04^{Cf}
	CHL	5.19 ± 0.05^{Ac}	3.55 ± 0.04^{Be}	3.35 ± 0.02^{Ccd}
	DES	5.20 ± 0.04^{Ac}	4.18 ± 0.02^{Bd}	3.51 ± 0.01^{Cb}
	CHLO	5.88 ± 0.02^{Aa}	4.85 ± 0.02^{Bb}	3.29 ± 0.05^{Ce}
	SPI	5.54 ± 0.04^{Ab}	4.98 ± 0.02^{Ba}	3.40 ± 0.02^{Cc}
Fructose	CONT	<LOD	<LOD	<LOD
	FOS	7.66 ± 0.05^{Aa}	5.35 ± 0.04^{Ba}	4.88 ± 0.04^{Ca}
	CHL	0.20 ± 0.02^{Ab}	0.12 ± 0.02^{Bb}	0.09 ± 0.03^{Cb}
	DES	0.14 ± 0.01^{Ac}	0.09 ± 0.01^{Bbc}	0.05 ± 0.01^{Cb}
	CHLO	0.16 ± 0.02^{Ac}	0.10 ± 0.02^{BCbc}	0.07 ± 0.02^{Cb}
	SPI	0.10 ± 0.01^{Ad}	0.04 ± 0.01^{Bc}	0.03 ± 0.01^{Bb}
Glucose	CONT	0.27 ± 0.01^{Af}	0.20 ± 0.01^{Bbc}	0.17 ± 0.01^{Cb}
	FOS	1.77 ± 0.05^{Aa}	1.54 ± 0.04^{Ba}	1.17 ± 0.04^{Ca}
	CHL	0.51 ± 0.02^{Ab}	0.17 ± 0.02^{Bc}	0.14 ± 0.03^{Bb}
	DES	0.36 ± 0.01^{Acd}	0.18 ± 0.01^{Bbc}	0.15 ± 0.02^{Bb}
	CHLO	0.42 ± 0.02^{Ac}	0.24 ± 0.03^{b}	0.19 ± 0.01^{Bb}
	SPI	0.33 ± 0.02^{Ade}	0.25 ± 0.02^{Bb}	0.22 ± 0.03^{Bb}
Lactic acid	CONT	1.63 ± 0.02^{Ac}	1.55 ± 0.04^{Ac}	1.28 ± 0.02^{Be}
	FOS	2.09 ± 0.03^{Cb}	3.93 ± 0.03^{Ba}	4.33 ± 0.02^{Aa}
	CHL	0.47 ± 0.02^{Ce}	0.94 ± 0.05^{Bd}	1.47 ± 0.01^{Ad}
	DES	1.00 ± 0.04^{Bd}	1.10 ± 0.04^{Bd}	1.82 ± 0.02^{Ac}
	CHLO	2.36 ± 0.01^{Ca}	2.51 ± 0.02^{Bb}	2.60 ± 0.04^{Ab}
	SPI	0.18 ± 0.02^{Af}	1.20 ± 0.03^{Ae}	1.27 ± 0.04^{Ae}
Succinic acid	CONT	0.03 ± 0.01^{Ac}	0.02 ± 0.00^{Ad}	<LOD
	FOS	0.05 ± 0.02^{Ac}	0.08 ± 0.02^{Ac}	0.03 ± 0.01^{Ab}
	CHL	0.05 ± 0.01^{Bc}	0.11 ± 0.01^{Ac}	0.04 ± 0.01^{Bb}
	DES	0.24 ± 0.04^{Ab}	0.18 ± 0.02^{Ab}	0.05 ± 0.01^{Bb}
	CHLO	0.07 ± 0.03^{Bc}	0.03 ± 0.01^{Bd}	0.02 ± 0.00^{Bb}
	SPI	1.12 ± 0.03^{Ba}	1.20 ± 0.01^{Aa}	1.23 ± 0.03^{Aa}
Malic acid	CONT	<LOD	<LOD	<LOD
	FOS	0.55 ± 0.03^{Ba}	0.81 ± 0.03^{Aa}	0.57 ± 0.03^{Ba}
	CHL	0.12 ± 0.01^{Bc}	0.20 ± 0.02^{Abc}	0.13 ± 0.03^{Bc}
	DES	0.20 ± 0.02^{Ab}	0.17 ± 0.02^{Ac}	0.09 ± 0.02^{Bc}
	CHLO	<LOD	0.02 ± 0.00^{Ad}	0.02 ± 0.00^{Ad}
	SPI	<LOD	0.24 ± 0.03^{Ab}	0.23 ± 0.02^{Ab}
Acetic acid	CONT	0.23 ± 0.03^{Ad}	0.23 ± 0.02^{Ae}	0.20 ± 0.02^{Ae}
	FOS	0.30 ± 0.02^{Bc}	0.46 ± 0.01^{Ad}	0.43 ± 0.01^{Ad}
	CHL	0.40 ± 0.01^{Bb}	0.46 ± 0.02^{Bd}	0.52 ± 0.02^{Ad}
	DES	0.24 ± 0.02^{Ccd}	0.61 ± 0.03^{Bc}	1.13 ± 0.04^{Ac}
	CHLO	0.44 ± 0.04^{Cb}	0.87 ± 0.02^{Bb}	1.23 ± 0.03^{Ab}
	SPI	0.87 ± 0.02^{Ba}	1.88 ± 0.03^{Aa}	1.93 ± 0.02^{Aa}
Butyric acid	CONT	0.38 ± 0.03^{Aa}	0.06 ± 0.02^{Bd}	0.03 ± 0.01^{Bd}
	FOS	0.10 ± 0.02^{Abc}	0.13 ± 0.02^{Ac}	0.10 ± 0.03^{Ac}
	CHL	0.02 ± 0.00^{Ac}	0.04 ± 0.01^{Ad}	0.01 ± 0.00^{Ad}
	DES	0.06 ± 0.02^{Bc}	0.04 ± 0.01^{Bd}	0.53 ± 0.02^{Ab}
	CHLO	0.36 ± 0.04^{Ca}	1.33 ± 0.02^{Aa}	1.03 ± 0.04^{Ba}
	SPI	0.15 ± 0.03^{ABb}	0.22 ± 0.03^{Ab}	0.14 ± 0.02^{BCc}
Propionic acid	CONT	0.90 ± 0.03^{Ad}	0.93 ± 0.02^{Ae}	0.78 ± 0.03^{Bd}
	FOS	0.57 ± 0.04^{Ae}	0.08 ± 0.02^{Bf}	0.10 ± 0.02^{Be}
	CHL	2.47 ± 0.01^{Bc}	2.45 ± 0.04^{Bb}	2.80 ± 0.04^{Ac}
	DES	2.54 ± 0.02^{Bc}	2.30 ± 0.04^{Cc}	2.82 ± 0.01^{Ac}
	CHLO	3.41 ± 0.01^{Bb}	1.79 ± 0.02^{Cd}	4.28 ± 0.02^{Aa}
	SPI	4.72 ± 0.02^{Aa}	2.83 ± 0.02^{Ca}	3.44 ± 0.02^{Bb}

Data reported as mean ± standard deviation, n = 3 repetitions. CHL: *Chlorella vulgaris*; DES: *Desmodesmus maximus*; CHLO: *Chlorococcum* cf. *hypnosporum*; SPI: *Spirulina platensis*. A–C: Different superscript capital letters in the same row for the same cultivation media denotes differences ($p < 0.05$), based on Tukey's test. a–f: different superscript small letters in the same column at a same time interval denotes difference ($p < 0.05$) among cultivation media, based on Tukey's test. LOD: limit of detection. LOD values were as follow: malic acid 0.007 g/L, lactic acid 0.02 g/L, acetic acid 0.001 g/L, succinic acid 0.02 g/L, propionic acid 0.024 g/L, and butyric acid 0.02 g/L.

Bifidobacterium and higher acetic acid concentration. From the map with confidence ellipses (Fig. 2B), it could be observed that the utilization of DMB resulted in differences compared to FOS, as the ellipses were well-separated. Furthermore, the fermentation time was an important factor, impacting the digested microalgae biomasses more than FOS. The HCA (Fig. S1) showed a cophenetic correlation of 0.87. The obtained dendrogram confirmed the existence of 4 groups: (1) FOS from 0 h, (2) FOS from 24 and 48 h, (3) all digested microalgae from 0 h and CHL and DES from 24 h, and (4) all digested microalgae from 48 h and SPI and CHLO from 24 h.

During the medium fermentation with digested microalgae biomass, 14 phenolic compounds have been identified. None of these phenolic compounds were detected in a medium with FOS or control (Table 5). SPI was characterized by the highest concentration of gallic acid, hesperidin, rutin, and myricetin after 24 h of fermentation, and catechin, epigallocatechin gallate, epicatechin gallate, procyanidin B2, procyanidin A2, hesperidin, rutin, and myricetin after 48 h of fermentation. At the same time, CHLO showed the highest concentration of gallic acid, epicatechin, procyanidin B1, and quercetin 3-glucoside after 48 h of fermentation.

In all microalgae biomasses, there was a reduction in gallic acid and increases in epicatechin gallate during fermentation compared to control. The effect on the other phenolic compounds was dependent on the microalgae species used. In DES, decreases in naringenin, catechin, epigallocatechin gallate, procyanidin A2, and kaempferol 3-glucoside, and increases in procyanidin B2 were observed. In CHL, decreases in naringenin, catechin, epigallocatechin gallate, procyanidin B2, procyanidin A2, kaempferol 3-glucoside, and increases in epicatechin and procyanidin B1 were observed. In CHLO, increases in catechin, epicatechin, procyanidin B1, procyanidin A2, and quercetin 3-glucoside, and decreases in procyanidin B2, hesperidin, and kaempferol 3-glucoside were observed. Increases in most of the phenolic compounds were observed in SPI.

4. Discussion

Our study demonstrated that microalgae biomass had a higher concentration of insoluble dietary fiber than soluble dietary fiber and presented some types of sugars, such as glucose, rhamnose, and fructose. Furthermore, oligosaccharides and sixteen phenolic compounds were identified. Finally, the type of microalgae species significantly impacted the type and concentration of dietary fiber, sugars, oligosaccharides, and phenolic compounds found in the biomass. Microalgae have been considered a rich source of fiber, protein, carbohydrate, and sugars for nutritional supplementation in livestock and humans [15]. The most common monosaccharides in microalgae are glucose, rhamnose, xylose, galactose, fucose, and fructose [2]. Furthermore, they contain oligosaccharides [13] and phenolic compounds [2], which may show prebiotic properties. Dong et al. [24] reported that the composition of microalgae is species-specific and depends on the growth conditions and environment.

In an overall view, increases in the relative abundance of *Lactobacillus-Enterococcus* and *Bifidobacterium* spp. and decreases in the relative abundance of *Prevotellaceae*, *Bacteroidaceae* and some *Porphyromonadaceae*, *Eubacterium rectale* group and *Clostridium coccoides*, and *Clostridium histolyticum* group were observed for FOS and DMBs. *Lactobacillus* and *Bifidobacterium* are considered probiotics that contribute directly or indirectly to beneficial functions in the human body. Thus, the increase in their relative abundance in the gut is desired [40]. Furthermore, some *Enterococcus* species (members of the healthy intestinal microbiota) are considered potentially probiotic. They have auto- and co-aggregation ability, resistance to low pH and bile salts, adherence to hydrocarbons, susceptibility to some antibiotics as well as exopolysaccharide synthesis [41].

On the contrary, studies have reported that increases in the relative abundance of *Prevotella* spp. populations within diverse microbial ecosystems have been associated with various infections [42]. In an animal model, intestinal colonization by *Prevotella* spp. promoted changes in microbiota composition and function, resulting in a reduction of SCFA content, decreased IL-18 production, and consequent elevation in intestinal inflammation [43]. Furthermore, *C. histolyticum* can negatively

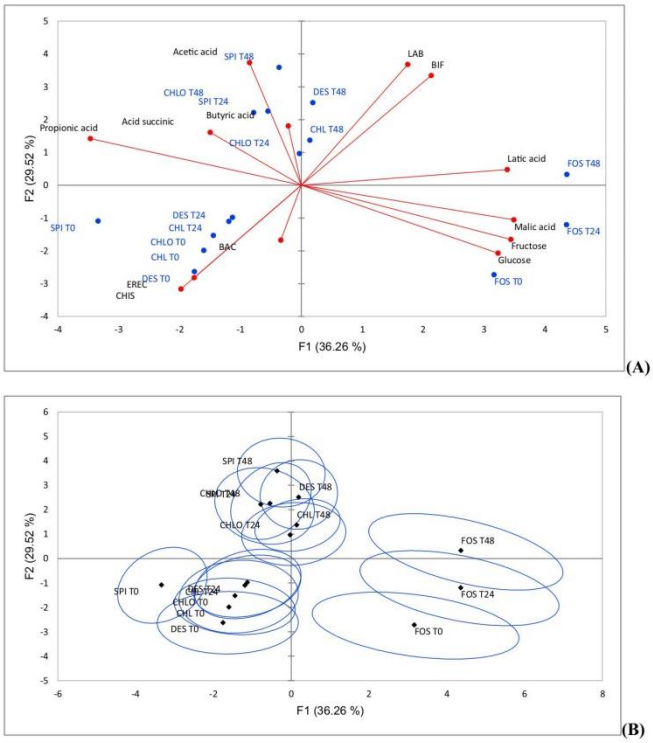


Fig. 2. Principal component analysis (A) and confidence ellipses (B) with the relative abundance of hybridized bacterial groups, sugars, organic acids, and short-chain fatty obtained during colonic fermentation in media with fructooligosaccharides (FOS) and digested biomass microalgae.
CHL: *Chlorella vulgaris*; DES: *Desmodesmus maximus*; CHLO: *Chlorococcum* cf. *hypnosporum*; SPI: *Spirulina platensis*. (F1, 36.26% and F2, 29.52%.)

affect colon health due to the production of detrimental metabolites [29]. Therefore, the decrease in *Eubacterium rectale* group and *Clostridium coccoides*, *Clostridium histolyticum*, *Prevotellaceae*, *Bacteroidaceae*, and some *Porphyromonadaceae* relative abundances in gut microbiota are desired in a host health perspective.

During the colonic fermentation, sugars were consumed (fructose and glucose), and organic acids were produced (lactic acid and acetic acid), with a consequent pH reduction. The phenolic compounds content was also changed, with decreases in gallic acid and increases in epicatechin gallate for the media with the DMB. Gut bacteria, mainly *Lactobacillus*, *Enterococcus*, and *Bifidobacterium* spp., can metabolize sugars and complex carbohydrates (non-digestible polysaccharides, oligosaccharides, and dietary fiber) [44,45]. Lactic acid is the main organic acid produced during *Lactobacillus* and *Enterococcus* metabolism, and it is an important end product of *Bifidobacterium* species [46]. Acetic acid is also produced during the colonic fermentation of carbohydrates by these microorganisms [47]. Gallic acid may have been consumed as a substrate to maintain bacterial fermentation of *Lactobacillus* spp. and *Bifidobacterium* spp. groups [48]. *Lactobacillus* shows gallate decarboxylase that can degrade gallic acid to other compounds used in the Krebs cycle [49]. The lowest pH values and the highest lactic acid content in the FOS medium are related to the higher concentration of fructose and glucose, as simpler carbohydrates are more available for metabolism [50]. Thus, they can be associated with the high relative abundance of *Lactobacillus-Enterococcus*.

This study demonstrated that the DMBs prebiotic effect depended on the microalgae species used. However, all microalgae biomasses presented a higher prebiotic index than FOS after 48 h fermentation, one of the most established prebiotic compounds. This could be evidenced in the PCA map, confidence ellipses, and HCA. The utilization of SPI biomass resulted in the highest prebiotic index after 24 h of fermentation, which can be related to the most significant relative abundance of *Lactobacillus-Enterococcus* and *Bifidobacterium*, and the lowest *Eubacterium rectale* group and *Clostridium coccoides*, *Clostridium histolyticum* and *Prevotellaceae*, *Bacteroidaceae* and some *Porphyromonadaceae* relative abundance in this medium. The prebiotic effect of SPI may be related to the presence of oligosaccharides (nystose and raffinose) in its composition and the highest phenolic compound content (particularly of chlorogenic acid, epicatechin, and myricetin). Oligosaccharides can be used by gut bacteria in their metabolism, increasing their viability and survival [35]. *Bifidobacterium* prefers D3 oligosaccharides for their growth, such as raffinose, compared to FOS and other oligosaccharides [51]. Furthermore, nystose may increase the number of beneficial microorganisms, mainly *Bifidobacterium* and *Lactobacillus* [47].

Although the prebiotic effect of non-digestible carbohydrates is well recognized, dietary phenolic compounds seem to be involved with the modulation of the intestinal microbiota and the production of metabolites important for host health [10,52]. Several phenolic compounds affect the adhesion, growth, survival, and functionality of beneficial microorganisms through bidirectional interactions with the intestinal microbiota, positively impacting the host health. Furthermore, some phenolic compounds can inhibit the growth of pathogenic intestinal

Table 5
Phenolic compound contents in medium with digested microalgae biomass, fructooligosaccharides (FOS) and control at time zero and after 24 and 48 h of an in vitro colonic fermentation.

Phenolic compounds (mg/L)	Samples	Time of incubation		
		0 h	24 h	48 h
Gallic acid	CHL	0.45 ± 0.01^{Ac}	0.05 ± 0.01^{Cc}	0.30 ± 0.02^{Bb}
	DES	0.36 ± 0.03^{Ad}	0.02 ± 0.00^{Bc}	0.02 ± 0.00^{Bd}
	CHLO	0.70 ± 0.03^{Ab}	0.73 ± 0.02^{Ab}	0.56 ± 0.02^{Ba}
	SPI	0.85 ± 0.03^{Aa}	0.91 ± 0.03^{Aa}	0.20 ± 0.01^{Bc}
Naringenin	CHL	0.56 ± 0.02^{Aa}	0.41 ± 0.02^{Ba}	0.30 ± 0.03^{Ca}
	DES	0.70 ± 0.05^{Ba}	0.39 ± 0.04^{Ba}	0.28 ± 0.02^{Ca}
	CHLO	<LOD	<LOD	<LOD
	SPI	<LOD	<LOD	<LOD
Catechin	CHL	0.69 ± 0.03^{Bb}	2.35 ± 0.05^{Aa}	0.45 ± 0.04^{Cb}
	DES	0.32 ± 0.01^{Bc}	0.47 ± 0.03^{Ad}	0.20 ± 0.00^{Cc}
	CHLO	0.38 ± 0.02^{Cc}	0.63 ± 0.04^{Ac}	0.48 ± 0.02^{Bb}
	SPI	0.91 ± 0.03^{Ca}	1.59 ± 0.02^{Ab}	1.37 ± 0.04^{Ba}
Epicatechin	CHL	0.39 ± 0.02^{Bb}	0.33 ± 0.01^{Bc}	0.57 ± 0.03^{Ab}
	DES	0.15 ± 0.01^{Bc}	0.35 ± 0.02^{Ac}	0.13 ± 0.03^{Bc}
	CHLO	0.65 ± 0.03^{Ca}	0.96 ± 0.03^{Ba}	1.08 ± 0.05^{Aa}
	SPI	0.22 ± 0.02^{Cc}	0.44 ± 0.01^{Bb}	0.57 ± 0.04^{Ab}
Epigallocatechin gallate	CHL	0.42 ± 0.02^{Ab}	0.17 ± 0.01^{Bc}	0.13 ± 0.02^{Bb}
	DES	0.47 ± 0.03^{Ab}	0.50 ± 0.02^{Aa}	<LOD
	CHLO	0.58 ± 0.04^{Aa}	0.29 ± 0.03^{Bb}	<LOD
	SPI	0.12 ± 0.03^{Ac}	0.12 ± 0.02^{Ac}	0.07 ± 0.01^{Aa}
Epicatechin gallate	CHL	0.46 ± 0.02^{Ba}	0.41 ± 0.01^{Bab}	0.63 ± 0.03^{Ab}
	DES	0.35 ± 0.03^{Bb}	0.31 ± 0.02^{Bc}	0.46 ± 0.05^{Ac}
	CHLO	0.45 ± 0.03^{Ba}	0.47 ± 0.04^{Ba}	0.61 ± 0.03^{Ab}
	SPI	0.15 ± 0.01^{Cc}	0.39 ± 0.04^{Bab}	2.27 ± 0.04^{Aa}
Procyanidin B1	CHL	0.16 ± 0.01^{Cb}	0.37 ± 0.02^{Ba}	0.95 ± 0.03^{Aa}
	DES	0.35 ± 0.03^{Aa}	0.10 ± 0.01^{Bc}	0.35 ± 0.01^{Ab}
	CHLO	0.11 ± 0.02^{Bb}	0.12 ± 0.02^{Bbc}	0.90 ± 0.02^{Aa}
	SPI	0.11 ± 0.03^{Bb}	0.15 ± 0.01^{Bb}	0.25 ± 0.01^{Ab}
Procyanidin B2	CHL	0.35 ± 0.01^{Ac}	0.35 ± 0.03^{Ac}	0.15 ± 0.02^{Bd}
	DES	0.21 ± 0.03^{Bd}	0.20 ± 0.04^{Bd}	0.70 ± 0.02^{Ac}
	CHLO	1.27 ± 0.06^{Bb}	1.54 ± 0.05^{Aa}	1.08 ± 0.04^{Cb}
	SPI	1.99 ± 0.05^{Ba}	1.22 ± 0.03^{Cb}	2.38 ± 0.05^{Aa}
Procyanidin A2	CHL	0.37 ± 0.03^{Bc}	4.23 ± 0.05^{Aa}	<LOD
	DES	0.42 ± 0.02^{Ab}	0.37 ± 0.01^{Bb}	<LOD
	CHLO	0.44 ± 0.01^{Cb}	0.48 ± 0.02^{ABb}	0.50 ± 0.02^{Ab}
	SPI	0.58 ± 0.04^{Ca}	4.18 ± 0.04^{Aa}	1.36 ± 0.02^{Ba}
Hesperidin	CHL	<LOD	<LOD	<LOD
	DES	<LOD	<LOD	<LOD
	CHLO	0.48 ± 0.02^{Bb}	1.00 ± 0.02^{Ab}	<LOD
	SPI	1.40 ± 0.04^{Aa}	1.32 ± 0.05^{Aa}	0.95 ± 0.02^{Ba}
Quercetin 3-glucoside	CHL	0.14 ± 0.01^{Aa}	0.14 ± 0.01^{Ab}	0.16 ± 0.02^{Ab}
	DES	0.13 ± 0.02^{Aa}	0.12 ± 0.01^{Ab}	0.11 ± 0.02^{Ab}
	CHLO	0.15 ± 0.01^{Ca}	0.18 ± 0.02^{ABa}	0.22 ± 0.01^{Aa}
	SPI	<LOD	<LOD	<LOD
Rutin	CHL	0.05 ± 0.01^{Ab}	0.05 ± 0.00^{Ab}	0.06 ± 0.01^{Ab}
	DES	0.06 ± 0.01^{Ab}	0.06 ± 0.01^{Ab}	0.06 ± 0.02^{Ab}
	CHLO	0.04 ± 0.00^{Ab}	0.07 ± 0.02^{Ab}	0.05 ± 0.01^{Ab}
	SPI	0.15 ± 0.02^{Ca}	0.17 ± 0.02^{ABa}	0.24 ± 0.03^{Aa}
Kaempferol 3-glucoside	CHL	0.06 ± 0.01^{Aa}	0.04 ± 0.02^{ABa}	0.02 ± 0.00^{Ca}
	DES	0.05 ± 0.01^{Aa}	0.03 ± 0.00^{Ba}	0.02 ± 0.00^{Ba}
	CHLO	0.06 ± 0.02^{Aa}	0.05 ± 0.01^{ABa}	0.03 ± 0.01^{Ba}
	SPI	0.03 ± 0.01^{Aab}	0.05 ± 0.01^{Aa}	0.04 ± 0.01^{Aa}
Myricetin	CHL	<LOD	<LOD	<LOD
	DES	<LOD	<LOD	<LOD
	CHLO	<LOD	<LOD	<LOD
	SPI	0.42 ± 0.04^{Ca}	1.72 ± 0.05^{Ba}	2.30 ± 0.05^{Aa}

Data reported as mean ± standard deviation, n = 3 repetitions. CHL: *Chlorella vulgaris*; DES: *Desmodesmus maximus*; CHLO: *Chlorococcum* cf. *hypnosporum*; SPI: *Spirulina platensis*. A–C: Different superscript capital letters in the same row for the same cultivation media denotes differences ($p < 0.05$), based on Tukey's test. a–d: different superscript small letters in the same column at a same time interval and measured sugar denotes difference ($p < 0.05$) among cultivation media, based on Tukey's test.
LOD: limit of detection. LOD of gallic acid 0.02 mg/L. LOD of other compounds cited in Table 2 footnote.

bacteria without affecting the growth of probiotic bacteria [53]. Recently, the importance of the colonic microbiota in biotransformation and, consequently, in phenolic compounds' bioactivity has been reported [43]. In this study, the medium with SPI had the highest contents of some phenolic compounds (gallic acid, hesperidin, rutin, and myricetin) after 24 h of fermentation, which could be related to the biotransformation promoted by the higher relative abundance of the beneficial microbiota. Polymeric phenolics, such as galloylated compounds, with gallic acid in their structure, mostly reach the colon and are degraded by the gut microbiota into flavonoids and phenolic acids [54]. In addition, *Lactobacillus* and *Bifidobacterium* can hydrolyze glycosidic bound in the structure of the phenolic compounds through β-glucosidase, releasing flavonoids from their *O*-glycosylated form [49].

During fermentation in the SPI medium, the concentration of succinic acid and acetic acid was increased. This medium showed the highest acetic acid and propionic acid concentrations after 24 h of fermentation. Succinic acid is considered a critical propionate precursor [47]. The most increased acetic acid production may be associated with inhibition of enteropathogens, as well as to the higher relative abundance of *Bifidobacterium* and *Lactobacillus–Enterococcus* [55]. In addition, *Bifidobacterium* produces a high concentration of acetic acid in media with raffinose [48]. Acetic acid is considered an energy source for peripheral and liver issues, necessary in lipogenesis and gluconeogenesis [56]. Additionally, SPI also showed higher growth and productivity compared to the other microalgae species. This is of paramount

importance, considering that the production costs are related to biomass productivity, cultivation method, scale, and nutrient costs. Furthermore, the growth and biomass productivity differences are associated with differences in the light penetration for each species and different capabilities of assimilating the medium nutrients [14].

The utilization of DES biomass resulted in the highest prebiotic index after 48 h of fermentation, which was related to the most significant relative abundance of *Lactobacillus-Enterococcus*. In this medium, increases in the concentration of butyric acid and propionic acid were observed during fermentation. These results could be associated with the highest concentrations of insoluble fibers and oligosaccharides (kestose) in DES biomass. Kestose is mainly consumed by *Lactobacillus* species [57]. In this way, kestose and insoluble fibers probably were not absorbed in the small intestine mimicking step used in this study, further fermented by the gut microbiota (mainly *Lactobacillus*) in the colonic mimicking conditions with the production of short-chain organic acids [35]. Microbial synthesis of short-chain organic acids are essential for intestinal integrity, luminal pH regulation, mucus synthesis, provision of energy for epithelial cells and intestinal mucosal immune function, and direct modulation of host metabolism [58]. Butyric acid is considered an important energy source for colonocytes, responsible for functions linked to the gut microbiota and physiology, maintaining cell growth and differentiation. It is also associated with a reduction in the incidence of colon cancer and inflammation improvements. At the same time, propionic acid is an important energy source for colon cells, involved in gluconeogenesis and inhibition of fat and cholesterol synthesis [56].

The utilization of CHL biomass resulted in the highest *Bifidobacterium* relative abundance (similar to SPI), which could be associated with the glucose and fructose concentration in the CHL medium and their utilization during fermentation. Furthermore, it could be related to the concentration of specific phenolic compounds (e.g., catechin, procyanidin A2, procyanidin B2) over the fecal fermentation. *Bifidobacterium* spp. is a saccharolytic microorganism, and their abundance in a medium is dependent on their ability to metabolize carbohydrates [59]. *Bifidobacterium* can metabolize low molecular carbohydrates, while *Lactobacillus* can metabolize low and high molecular carbohydrates due to the presence of specific enzymes [11]. Therefore, the presence of monosaccharides is essential and can accelerate the growth of *Bifidobacterium* [58], which should explain in part the highest BIF abundance in the CHL fermentation medium. *Bifidobacterium* can produce acetate, lactate, formate, and ethanol but usually do not produce butyrate [58]. This reflects the lowest concentration of butyric acid observed in this medium. Furthermore, phenolic compounds, such as catechin, epicatechin gallate, and procyanidins could alter gut microecology by enhancing the growth of beneficial bacteria, including *Bifidobacterium* and *Enterococcus* [60,53].

The present study results demonstrated that the biomass of novel microalgae species, mainly DES, may result in a higher prebiotic effect than SPI and CHL, which are the most recognized and used microalgae species. DES could result in a higher abundance of *Lactobacillus–Enterococcus* and decreased abundance of *Clostridium histolyticum* compared to CHL, with a consequent high concentration of short-chain organic acids (butyric and propionic acid). When evaluated after 24 h of fermentation, CHLO could increase the abundance of *Lactobacillus–Enterococcus* and decreases the abundance of *Clostridium histolyticum* and *Eubacterium rectale-Clostridium coccoides*. In this way, novel microalgae species may be future important prebiotic ingredients.

The results of this study show potential prebiotic effects of digested microalgae biomass on the human colonic microbiota in vitro tests, as higher production of short-chain organic acids, decreased pH values, and modulatory effects on gut microbiota through the increase in the relative abundance of beneficial bacteria and suppressing of potentially pathogenic microorganism were observed. Microalgae species with high carbohydrate content (mainly oligosaccharides) and phenolic compounds showed better performance, suggesting a possible synergistic activity among these compounds to reach the reported prebiotic effects. It is important to highlight that the microalgae biomasses were subjected to simulated gastrointestinal digestion, as the resistance to gastric acidity and enzymes are a requisite for prebiotic compounds. In this way, freshwater microalgae biomass may be considered an emergent prebiotic ingredient. Future studies should evaluate these effects in vivo tests, as many factors may influence the prebiotic properties, such as diet composition, initial host microbiota, and individual host response.

5. Conclusion

This study provides evidence on the prebiotic effect of different freshwater microalgae species biomass, with a higher prebiotic index than FOS (8.51–10.10 vs. 4.67 after 48 h fermentation), an established prebiotic compound. Modulatory effects on the intestinal microbiota were observed due to the increased relative abundance of beneficial microorganisms (*Lactobacillus–Enterococcus* and *Bifidobacterium*) and inhibition of undesirable bacteria (*Eubacterium rectale* group and *Clostridium coccoides*, *Prevotellaceae*, *Bacteroidaceae*, and *Clostridium histolyticum*). In addition, the presence of insoluble dietary fiber, oligosaccharides, and phenolic compounds in microalgae biomasses was associated with their modulatory effects on intestinal microbiota, with consequent metabolites production (short-chain organic acids).

S. platensis biomass increased the relative abundance of *Lactobacillus-Enterococcus* and *Bifidobacterium*, and decreased *Eubacterium rectale-Clostridium coccoides*, *Clostridium histolyticum*, and *Prevotellaceae-Bacteroidaceae*, which was associated with the presence of nystose, raffinose, and phenolics and resulted in an increase in the concentration of acetic and propionic acids. *D. maximus* biomass showed the greatest *Lactobacillus-Enterococcus* relative abundance and increased the butyric and propionic acids, which was associated with the presence of higher insoluble fibers and kestose contents. *C. vulgaris* biomass showed the highest *Bifidobacterium* relative abundance, which was associated with the highest glucose and fructose concentrations and some phenolics.

The present study results are important to expand the knowledge and application of microalgae biomass for food purposes and contribute to revealing additional beneficial species. The biomass of novel microalgae species, mainly DES, may result in a higher prebiotic effect than SPI and CHL, which are the most recognized and used microalgae species. DES is not commonly used in studies approaching the functionalities of microalgae.

CRediT authorship contribution statement

Viviane Priscila Barros de Medeiros: Conceptualization, Methodology, Writing - Original draft, Writing - Review & editing, Investigation, Formal analysis, Visualization. **Evandro Leite de Souza**: Conceptualization, Review & editing. **Thatyane Mariano Rodrigues de Albuquerque**: Methodology, Investigation. **Cristiane Francisca da Costa Sassi**: Resources, Funding acquisition. **Marcos dos Santos Lima**: Methodology. **Katia Sivieri**: Review & editing. **Tatiana Colombo Pimentel**: Methodology, Writing – Original, Review & editing. **Marciane Magnani**: Definition, Conceptualization, Writing - Review & editing, Supervision, Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors confirm that they have no conflicts of interest concerning the work described in this manuscript.

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Statement of informed consent

Volunteers signed the Informed Consent before the donation of fecal samples.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2021.102547.

References

[1] S. Abu-Ghosh, Z. Dubinsky, V. Verdelho, D. Iluz, Unconventional high-value products from microalgae: a review, Bioresour. Technol. 329 (2021), 124895, https://doi.org/10.1016/j.biortech.2021.124895.

[2] W. Levasseur, P. Perré, V. Pozzobon, A review of high value-added molecules production by microalgae in light of the classification, Biotechnol. Adv. 41 (2020), 107545, https://doi.org/10.1016/j.biotechadv.2020.107545.

[3] Food and Drug Administration (FDA), Original Submission, in: Spirulina platensis GRAS self affirmation 7-1-2011, 2011, pp. 1–126.

[4] C.A. Laamanen, S.M. Desjardins, G.N.A. Senhorinho, J.A. Scott, Harvesting microalgae for health beneficial dietary supplements, Algal Res. 54 (2021), 102189, https://doi.org/10.1016/j.algal.2021.102189.

[5] C. Enzing, M. Ploeg, M. Barbosa, L. Sijtsma, Microalgae-based products for the food and feed sector: an outlook forEurope, 2014, https://doi.org/10.2791/3339.

[6] K.K.B. Sassi, J.A.D. Silva, C.D. Calixto, R. Sassi, C.F.D.C. Sassi, Metabolites of interest for food technology produced by microalgae from the Northeast Brazil, Rev. Ciênc. Agron. 50 (2019) 54–65, https://doi.org/10.5935/1806-6690.20190007.

[7] R.M. González-Balderas, S.B. Velásquez-Orta, I. Valdez-Vazquez, M.O. Ledesma, Ultrason. Sonochem. 62 (2020), 104852, https://doi.org/10.1016/j.ultsonch.2019.104852.

[8] L.S. Rizza, M.E.S. Smachetti, M. Nascimento, G.L. Salerno, L. Curatti, Bioprospecting for native microalgae as an alternative source of sugars for the production of bioethanol, Algal Res. 22 (2017) 140–147, https://doi.org/10.1016/j.algal.2016.12.021.

[9] G.R. Gibson, R. Hutkins, M.E. Sanders, S.L. Prescott, R.A. Reimer, S.J. Salminen, K. Scott, C. Stanton, K.S. Swanson, P.D. Cani, K. Verbeke, G. Reid, Expert consensus document: the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics 14 (2017) 491–502, https://doi.org/10.1038/nrgastro.2017.75.

[10] M. Cunningham, M.A. Azcarate-Peril, A. Barnard, V. Benoit, R. Grimaldi, D. Guyonnet, H.D. Holscher, K. Hunter, S. Manurung, D. Obis, M.I. Petrova, R. E. Steinert, K.S. Swanson, D. van Sinderen, J. Vulevic, G.R. Gibson, Shaping the future of probiotics and prebiotics, Trends Microbiol. xx (2021) 1–19, https://doi.org/10.1016/j.tim.2021.01.003.

[11] A. Peredo-lovillo, H.E. Romero-luna, M. Jiménez-fernández, Health promoting microbial metabolites produced by gut microbiota after prebiotics metabolism 136, 2020, https://doi.org/10.1016/j.foodres.2020.109473.

[12] M.E. Sanders, D.J. Merenstein, G. Reid, G.R. Gibson, R.A. Rastall, Probiotics and prebiotics in intestinal health and disease: from biology to the clinic 16 (2019) 605–616, https://doi.org/10.1038/s41575-019-0173-3.

[13] B.E.S. Leal, M.R. Prado, A. Grzybowski, M. Tiboni, H.S. Koop, L.B. Scremin, A. C. Sakuma, A.A. Takamatsu, A.F. dos Santos, V.F. Cavalcanti, J.D. Fontana, Potential prebiotic oligosaccharides from aqueous thermopressurized phosphoric acid hydrolysates of microalgae used in treatment of gaseous steakhouse waste, Algal Res. 24 (2017) 138–147, https://doi.org/10.1016/j.algal.2017.03.020.

[14] V.P. Barros de Medeiros, W.K.A. da Costa, R.T. da Silva, T.C. Pimentel, M. Magnani, Microalgae as source of functional ingredients in new-generation foods: challenges, technological effects, biological activity, and regulatory issues, Crit. Rev. Food Sci. Nutr. (2021) 10408398, https://doi.org/10.1080/10408398.2021.1879729.

[15] J.B. Jin, J.W. Cha, I.S. Shin, J.Y. Jeon, K.H. Cha, C.H. Pan, Supplementation with Chlorella vulgaris, Chlorella protothecoides, and Schizochytrium sp. increases propionate-producing bacteria in in vitro human gut fermentation, J. Sci. Food Agric. 100 (2020) 2938–2945, https://doi.org/10.1002/jsfa.10321.

[16] A. Nakashima, K. Sasaki, D. Sasaki, K. Yasuda, K. Suzuki, A. Kondo, The alga Euglena gracilis stimulates Faecalibacterium in the gut and contributes to increased defecation, Sci. Rep. 11 (1) (2021) 1–8, https://doi.org/10.1038/s41598-020-80306-0.

[17] T. Bhattad, A. Koradiya, G. Prakash, Prebiotic activity of paramylon isolated from heterotrophically grown Euglena gracilis, Heliyon (2021), e07884, https://doi.org/10.1016/j.heliyon.2021.e07884 (1-6).

[18] V.P.B. de Medeiros, T.C. Pimentel, R.C.R. Varandas, S.A. dos Santos, G.T. de Souza Pedrosa, C.F. da Costa Sassi, M.M. da Conceição, M. Magnani, Exploiting the use of agro-industrial residues from fruit and vegetables as alternative microalgae culture medium, Food Res. Int. 137 (2020), https://doi.org/10.1016/j.foodres.2020.109722.

[19] C. Zarrouk, Contribution a l'etude d'une Cyanobacterie: Influence de Divers Facteurs Physiques et Chimiques sur la Croissanceet la Photosynthese de Spirulina maxima (Setchell et Gardner) Geitler, University of Paris, France, 1966. Ph. D. Thesis.

[20] R.R. Guillard, C.J. Lorenzen, Yellow-green algae with chlorophyllide C 1, 2, J. Phycol. 8 (1972) 10–14, https://doi.org/10.1111/j.1529-8817.1972.tb03995.x.

[21] C.D. Calixto, J.K. da Silva Santana, E.B. de Lira, P.G.P. Sassi, R. Rosenhaim, C.F. da Costa Sassi, M.M. da Conceição, R. Sassi, Biochemical compositions and fatty acid profiles in four species of microalgae cultivated on household sewage and agro-industrial residues, Bioresour. Technol. 221 (2016) 438–446, https://doi.org/10.1016/j.biortech.2016.09.066.

[22] Association of Official Analytical Chemists (AOAC), Official Methods of Analysis of the AOAC International, 20th ed., 2016. Rockville, MD, USA. ISBN: 97-8-093558487-5.

[23] M. dos Santos Lima, P.C. Nunes, B. de Lourdes de Araújo Silva, C.V. da Silva Padilha, T.H.F. do Bonfim, T.L.M. Stamford, M.A. da Silva Vasconcelos, J. de Souza Aquino, Determining 1-kestose, nystose and raffinose oligosaccharides in grape juices and wines using HPLC: method validation and characterization of products from Northeast Brazil, J. Food Sci. Technol. 56 (2019) 4575–4584, https://doi.org/10.1007/s13197-019-03936-x.

[24] T. Dong, S. Van Wychen, N. Nagle, P.T. Pienkos, L.M.L. Laurens, Impact of biochemical composition on susceptibility of algal biomass to acid-catalyzed pretreatment for sugar and lipid recovery, Algal Res. 18 (2016) 69–77, https://doi.org/10.1016/j.algal.2016.06.004.

[25] E.M. Coelho, C.V. da Silva Padilha, G.A. Miskinis, A.G.B. de Sá, G.E. Pereira, L.C. de Azevêdo, M. dos Santos Lima, Simultaneous analysis of sugars and organic acids in wine and grape juices by HPLC: method validation and characterization of products from Northeast Brazil, J. Food Compos. Anal. 66 (2018) 160–167, https://doi.org/10.1016/j.jfca.2017.12.017.

[26] P.T. Scaglioni, L. Quadros, M. de Paula, V.B. Furlong, P.C. Abreu, E. Badiale-Furlong, Inhibition of enzymatic and oxidative processes by phenolic extracts from Spirulina sp. and Nannochloropsis sp. Food Technol.Biotechnol. 56 (2018) 344–353, https://doi.org/10.17113/ftb.56.03.18.5495.

[27] C.V. da S. Padilha, G.A. Miskinis, M.E.A.O. de Souza, G.E. Pereira, D. de Oliveira, M.T. Bordignon-Luiz, M. dos S. Lima, Rapid determination of flavonoids and phenolic acids in grape juices and wines by RP-HPLC/DAD: method validation and characterization of commercial products of the new Brazilian varieties of grape, Food Chem. 228 (2017) 106–115, https://doi.org/10.1016/j.foodchem.2017.01.137.

[28] M. Minekus, M. Alminger, P. Alvito, S. Ballance, T. Bohn, C. Bourlieu, F. Carrière, R. Boutrou, M. Corredig, D. Dupont, C. Dufour, L. Egger, M. Golding, S. Karakaya, B. Kirkhus, S. Le Feunteun, U. Lesmes, A. Macfarzanka, A. MacKie, S. Marze, D. J. McClements, O. Ménard, I. Recio, C.N. Santos, R.P. Singh, G.E. Vegarud, M.S. J. Wickham, W. Weitschies, A. Brodkorb, A standardised static in vitro digestion method suitable for food-an international consensus, Food Funct. 5 (2014) 1113–1124, https://doi.org/10.1039/c3fo60702j.

[29] K.B. Guergoletto, A. Costabile, G. Flores, S. Garcia, G.R. Gibson, In vitro fermentation of juçara pulp (Euterpe edulis) by human colonic microbiota, Food Chem. 196 (2016) 251–258, https://doi.org/10.1016/j.foodchem.2015.09.048.

[30] D. Rodrigues, G. Walton, S. Sousa, T.A.P. Rocha-Santos, A.C. Duarte, A.C. Freitas, A.M.P. Gomes, In vitro fermentation and prebiotic potential of selected extracts from seaweeds and mushrooms, LWT Food Sci. Technol. 73 (2016) 131–139, https://doi.org/10.1016/j.lwt.2016.06.004.

[31] P.S. Langendijk, F. Schut, G.J. Jansen, G.C. Raangs, G.R. Kamphuis, M. H. Wikinson, G.W. Welling, Quantitative fluorescence in situ hybridization of Bifidobacterium spp. with genus-specific 16S rRNA-targeted probes and its application in fecal samples, Appl. Environ. Microbiol. 61 (1995) 3069.

[32] H.J. Harmsen, P. Elfferich, F. Schut, G.W. Welling, A 16S rRNA-targeted probe for detection of lactobacilli and enterococci in faecal samples by fluorescent in situ hybridization, Microbial Ecol. Health Dis. 11 (1999) 3–12, https://doi.org/10.1080/089106099435862.

[33] W. Manz, R. Amann, W. Ludwig, M. Vancanneyt, K.H. Schleifer, Application of a suite of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum cytophaga-flavobacter-bacteroides in the natural environment 142 (1996) 1097–1106, https://doi.org/10.1099/13500872-142-5-1097.

[34] A.H. Franks, H.J. Harmsen, G.C. Raangs, G.J. Jansen, F. Schut, G.W. Welling, Variations of bacterial populations in human feces measured by fluorescent in situ hybridization with group-specific 16S rRNA-targeted oligonucleotide probes, Appl. Environ. Microbiol. 64 (1998) 3336.

[35] F.N.D.D. Menezes, F.H.C. de Melo, A.R.S. Vieira, T.C. Almeida, M.S. Lima, J. S. Aquino, A. Gomez-Zavaglia, M. Magnani, E.L. de Souza, Acerola (Malpighia glabra L.) and guava (Psidium guayaba L.) industrial processing by-products stimulate probiotic lactobacillus and bifidobacterium growth and induce beneficial changes in colonic microbiota, J. Appl. Microbiol. 130 (2021) 1323–1336, https://doi.org/10.1111/jam.14824.

[36] L. Conterno, F. Martinelli, M. Tamburini, F. Fava, A. Mancini, M. Sordo, M. Pindo, S. Martens, D. Masuero, U. Vrhovsek, C. Dal Lago, G. Ferrario, M. Morandini, K. Tuohy, Measuring the impact of olive pomace enriched biscuits on the gut microbiota and its metabolic activity in mildly hypercholesterolaemic subjects, Eur. J. Nutr. 58 (2019) 63–81, https://doi.org/10.1007/s00394-017-1572-2.

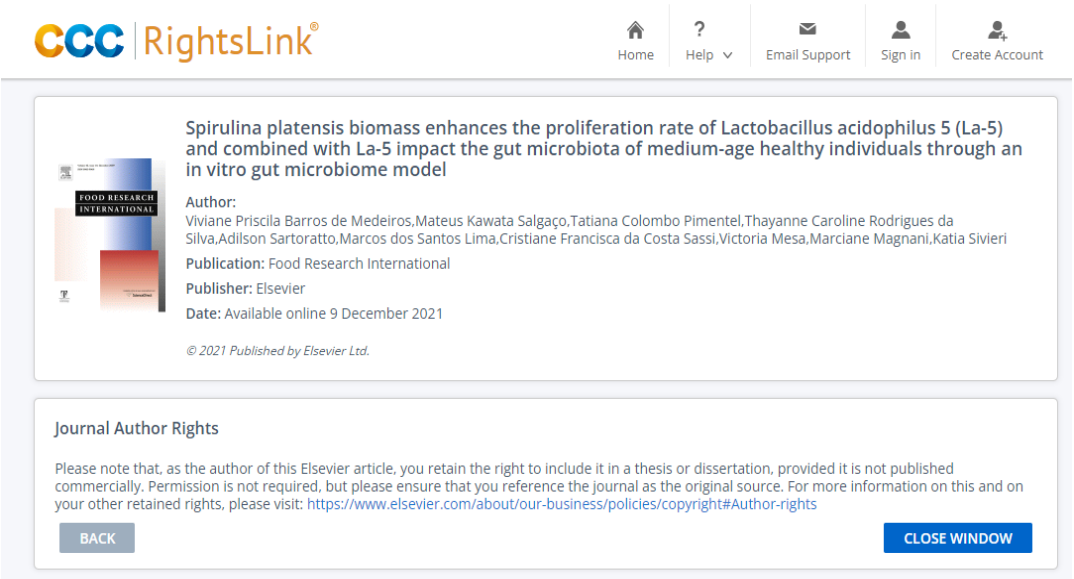
[37] T.M.R. de Albuquerque, M. Magnani, M. dos S. Lima, L.R.C. Castellano, E.L. de Souza, Effects of digested flours from four different sweet potato (Ipomoea batatas L.) root varieties on the composition and metabolic activity of human colonic

microbiota in vitro, J. Food Sci. (2021), https://doi.org/10.1111/1750-3841.15852.
[38] F.N.D.D. Menezes, É.T. da Cruz Almeida, A.R. da Silva Vieira, J. de Souza Aquino, M. dos Santos Lima, M. Magnani, E.L. de Souza, Impact of cashew (Anacardium occidentale L.) by-product on composition and metabolic activity of human colonic microbiota in vitro indicates prebiotic properties, Curr. Microbiol. 78 (2021) 2264–2274, https://doi.org/10.1007/s00284-021-02502-z.
[39] C.L.C. Meira, C.G. Novaes, F.C. Novais, V.D.S. de Jesus, D.M. de Oliveira, R. M. Aguiar, Application of principal component analysis for the evaluation of the chemical constituents of Mimosa tenuiflora methanolic extract by DLLME/GC–MS, Microchem. J. 152 (2020), 104284, https://doi.org/10.1016/j.microc.2019.104284.
[40] M. Sharma, A. Wasan, R.K. Sharma, Recent developments in probiotics: an emphasis on bifidobacterium, Food Biosci. 41 (2021), 100993, https://doi.org/10.1016/j.fbio.2021.100993.
[41] Y. Nami, R.V. Bakhshayesh, H.M. Jalaly, H. Lotfi, S. Eslami, M.A. Hejazi, Probiotic properties of enterococcus isolated from artisanal dairy products, Front. Microbiol. 10 (2019) 1–13, https://doi.org/10.3389/fmicb.2019.00300.
[42] A. Iljazovic, L. Amend, E.J.C. Galvez, R. de Oliveira, T. Strowig 311 (2021), 151472, https://doi.org/10.1016/j.ijmm.2021.151472.
[43] A. Iljazovic, U. Roy, E.J.C. Gálvez, T.R. Lesker, B. Zhao, A. Gronow, L. Amend, S. E. Will, J.D. Hofmann, M.C. Pils, K. Schmidt-Hohagen, M. Neumann-Schaal, T. Strowig, Perturbation of the gut microbiome by Prevotella spp. enhances host susceptibility to mucosal inflammation, Mucosal Immunol. 14 (2021) 113–124, https://doi.org/10.1038/s41385-020-0296-4.
[44] Q. Song, Y. Wang, L. Huang, M. Shen, Y. Yu, Q. Yu, Y. Chen, J. Xie, Review of the relationships among polysaccharides, gut microbiota, and human health, Food Res. Int. 140 (2021), 109858, https://doi.org/10.1016/j.foodres.2020.109858.
[45] C.B. Comerlato, A.C. Ritter, K.N. Miyamoto, A. Brandelli, Proteomic study of Enterococcus durans LAB18S growing on prebiotic oligosaccharides, Food Microbiol. 89 (2020), 103430, https://doi.org/10.1016/j.fm.2020.103430.
[46] H. Hanchi, W. Mottawea, K. Sebei, R. Hammami, The genus Enterococcus: between probiotic potential and safety concerns-an update, Front. Microbiol. 9 (2018) 1–16, https://doi.org/10.3389/fmicb.2018.01791.
[47] T.M.R. de Albuquerque, C.W.P. Borges, M.T. Cavalcanti, M. dos S. Lima, M. Magnani, E.L. de Souza, Potential prebiotic properties of flours from different varieties of sweet potato (Ipomoea batatas L.) roots cultivated in Northeastern Brazil, Food Biosci. 36 (2020), 100614, https://doi.org/10.1016/j.fbio.2020.100614.
[48] S.G. Sáyago-Ayerdi, K. Venema, M. Tabernero, B. Sarriá, L.L. Bravo, R. Mateos, Bioconversion by gut microbiota of predigested mango (Mangifera indica L) 'Ataulfo' peel polyphenols assessed in a dynamic (TIM-2) in vitro model of the human colon, Food Res. Int. 139 (2021), https://doi.org/10.1016/j.foodres.2020.109963.
[49] F.H.C. de Melo, F.N.D.D. Menezes, J.M.B. de Sousa, M. dos Santos Lima, G. da Silva Campelo, E.L. de Borges, M.Magnani Souza, Prebiotic activity of monofloral honeys produced by stingless bees in the semi-arid region of Brazilian Northeastern toward Lactobacillus acidophilus LA-05 and Bifidobacterium lactis BB-12, Food Res. Int. 128 (2020), 108809, https://doi.org/10.1016/j.foodres.2019.108809.
[50] G.Jing Chen, Q.Yue Hong, N. Ji, W.Neng Wu, L.Zhi Ma, Influences of different drying methods on the structural characteristics and prebiotic activity of polysaccharides from bamboo shoot (Chimonobambusa quadrangularis) residues, Int. J. Biol. Macromol. 155 (2020) 674–684, https://doi.org/10.1016/j.ijbiomac.2020.03.223.
[51] R. Ose, K. Hirano, S. Maeno, J. Nakagawa, S. Salminen, T. Tochio, A. Endo, The ability of human intestinal anaerobes to metabolize different oligosaccharides: novel means for microbiota modulation? Anaerobe 51 (2018) 110–119, https://doi.org/10.1016/j.anaerobe.2018.04.018.
[52] S. Zheng, H. Zhang, R. Liu, C. Huang, H. Li, Do short chain fatty acids and phenolic metabolites of the gut have synergistic anti-inflammatory effects ? – new insights from a TNF- α -induced Caco-2 cell model, Food Res. Int. (2020), 109833, https://doi.org/10.1016/j.foodres.2020.109833.
[53] M. Makarewicz, I. Drożdż, T. Tarko, A. Duda-Chodak, The interactions between polyphenols and microorganisms, especially gut microbiota 10 (2021) 1–70, https://doi.org/10.3390/antiox10020188.
[54] D. Karas, J. Ulrichov 105 (2017), https://doi.org/10.1016/j.fct.2017.04.021.
[55] R. Kumar Suryawanshi, N. Kango, Production of mannooligosaccharides from various mannans and evaluation of their prebiotic potential, Food Chem. 334 (2021), 127428, https://doi.org/10.1016/j.foodchem.2020.127428.
[56] T. Di, G. Chen, Y. Sun, S. Ou, X. Zeng, H. Ye, In vitro digestion by saliva, simulated gastric and small intestinal juices and fermentation by human fecal microbiota of sulfated polysaccharides from Gracilaria rubra, J. Funct. Foods 40 (2018) 18–27, https://doi.org/10.1016/j.jff.2017.10.040.
[57] K. Ide, M. Shinohara, S. Yamagishi, A. Endo, K. Nishifuji, T. Tochio, Kestose supplementation exerts bifidogenic effect within fecal microbiota and increases fecal butyrate concentration in dogs, J. Vet. Med. Sci. 82 (2020) 1–8, https://doi.org/10.1292/jvms.19-0071.
[58] E.E. Blaak, E.E. Canfora, S. Theis, G. Frost, A.K. Groen, G. Mithieux, A. Nauta, K. Scott, B. Stahl, J. van Harsselaar, R. van Tol, E.E. Vaughan, K. Verbeke, Short chain fatty acids in human gut and metabolic health 11 (2020) 411–455, https://doi.org/10.3920/BM2020.0057.
[59] M. Egan, D.Van Sinderen, Bifidobacteria, in, Elsevier Inc., 2018, https://doi.org/10.1016/B978-0-12-805060-6/00008-9.
[60] Y. Qiao, J. Sun, S. Xia, X. Tang, Y. Shi, G. Le, Effects of resveratrol on gut microbiota and fat storage in a mouse model with high-fat-induced obesity, Food Funct. 5 (2014) 1241–1249, https://doi.org/10.1039/c3fo60630a.

APÊNDICE B – ARTIGO II

Spirulina platensis biomass enhances the proliferation rate of _Lactobacillus acidophilus_ 5 (La-5) and combined with La-5 impact the gut microbiota of medium-age healthy individuals through an in vitro gut microbiome model.

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Spirulina platensis biomass enhances the proliferation rate of *Lactobacillus acidophilus 5* (La-5) and combined with La-5 impact the gut microbiota of medium-age healthy individuals through an *in vitro* gut microbiome model

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ABSTRACT

This study first evaluated the stimulatory effect of *S. platensis* biomass on the growth of *L. acidophilus* and the metabolic activity during fermentation (37 °C, 72 h) in a culture medium. The results demonstrated a higher impact of *S. platensis* biomass than fructooligosaccharide (FOS), an established prebiotic. Higher *L. acidophilus* proliferation rates and metabolic activity were observed (lower pH values and higher concentrations of acetic, lactic, and propionic acids) in the presence of *S. platensis*. Then, we evaluated the effects of the *S. platensis* biomass (1.5 g, twice a day, 5 days) in association with *L. acidophilus* (10^6 CFU/g) on the gut microbiota composition of medium-age healthy individuals through the Simulator of Human Intestinal Microbial Ecosystem (SHIME®) and measurement of metabolites. *L. acidophilus* (La5) and *L. acidophilus* + *S. platensis* (Spi-La5) could positively modulate the intestinal microbiota. The administration of La5 resulted in increases in *Bacteroides*, *Megasphaera*, *Lactobacillus*, and *Parabacteroides* genus abundance, with a consequent decrease in ammonium ions. The administration of Spi-La5 increased the abundance of the genus *Erysipelatoclostridium*, *Roseburia*, *Enterococcus*, *Bifidobacterium*, *Coriobacteriaceae* UCG-003, *Enterobacter*, and *Paraclostridium*. The results demonstrate that the intestinal microbiota was differently modified by administrating La5 and Spi-La5 and indicate the latter as an alternative for microbiota positive modulation in healthy individuals.

1. Introduction

The gut microbiota of healthy individuals is predominantly composed of strictly anaerobic microorganisms' members of the phyla *Firmicutes, Bacteroidetes, Actinobacteria*, and *Proteobacteria*. These microorganisms are responsible for performing functions fundamental to the maintenance of host health through the synthesis of metabolites that promote the regulation of various physiological processes, including immune homeostasis and the exclusion of potentially pathogenic microorganisms (Coman & Vodnar, 2020; Kim & Benayoun, 2020; Kriss, Hazleton, Nusbacher, Martin, & Lozupone, 2018).

However, with advancing age, changes in the composition of the intestinal microbiota can occur because of the action of external factors (use of antibiotics, eating habits, physical and psychological stress) and internal factors (host genetics and immunosenescence), resulting in an imbalance of the intestinal microbiota (Coman & Vodnar, 2020). Therefore, studies involving the human gut microbiota are commonly performed using children (Roswall et al., 2021), young adults (Lee et al., 2021), and the elderly (Zhang et al., 2021). They reported increases in *Bacteriodetes* and decreases in *Firmicutes* with age, mainly due to the

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changes in *Clostridium* groups. Furthermore, decreases in the abundance of health-related microorganisms, such as Bifidobacteria, have been reported in the elderly compared to younger adults (Claesson et al., 2011; Maynard & Weinkove, 2018). In contrast, the human microbiota of medium-aged individuals has been scarcely evaluated.

In recent years the administration of probiotics, particularly *Lactobacillus acidophilus*, has been suggested as a promising strategy for modulating the composition and metabolic activity of the intestinal microbiota with positive repercussions on the host's immune regulation (Zhao, Liu, Kwok, Cai, & Zhang, 2020). The mechanisms of action involving *L. acidophilus* on intestinal microbiota and immunity include increased relative abundance of beneficial bacteria and protection against intestinal infection (Vemuri et al., 2018).

Modulation of the intestinal microbiota can also be achieved through the consumption of prebiotics. Previous studies have already reported the positive impact of different microalgae species on probiotic strains or gut microbiota, such as *Chlorella vulgaris* (Hyrslova et al., 2021), *Chlorella protothecoides*, *Schizochytrium* sp. (Jin et al., 2020), and *Euglena gracilis* (Nakashima et al., 2021). Furthermore, in our previous study, we demonstrated that different microalgae species (*Desmodesmus maximus, Chlorella vulgaris, Chlorococcum* sp. cf *hypnosporum,* and *Spirulina platensis*) could exert a potentially prebiotic effect on the intestinal microbiota in an *in vitro* static colonic fermentation system. However, *S. platensis* biomass had a more pronounced effect, as increases in the relative abundance of *Bifidobacterium* and *Lactobacillus-Enterococcus* and decreases in *Clostridium histolyticum, Eubacterium rectale-Clostridium coccoides,* and *Prevotellaceae-Bacteroidaceae* were observed (de Medeiros et al., 2021).

S. platensis utilization is also recommended due to its classification as Generally Recognized as Safe (GRAS- No. 127) by the Food and Drug Administration (FDA). In addition, it is a natural source of insoluble fibers, proteins, polyunsaturated fatty acids, phenolic compounds, and bioactive pigments (Lafarga, Fernández-Sevilla, González-López, & Acién-Fernández, 2020), which may present prebiotic potential and enhance the metabolic activity of *L. acidophilus*. In vitro research involving its incorporation into fermented foods (yogurt, ayran, kefir) has shown that *S. platensis* biomass promotes a significant increase in the growth, viability, and metabolic activity of *L. acidophilus* (Atik, Gürbüz, Bölük, & Palabıyık, 2021; Celekli, Alslibi, & üseyin Bozkurt, 2019; Patel et al., 2019). Furthermore, a prior study showed that the whole biomass of *S. platensis* could alter the composition of the colonic microbial community in mice after 24 days of treatment, suggesting it as a source of prebiotics (Hu et al., 2019). However, there are no reports on the effects of *S. platensis* biomass combined with probiotics in human gut microbiota using simulated digestion in a dynamic colonic model.

Therefore, this study aimed to evaluate the stimulatory effect of the microalgae *S. platensis* on the growth of *L. acidophilus-La 5* and the modulatory effects of *S. platensis* in association with *L. acidophilus* (10^6 CFU/g) on gut microbiota composition of medium-age healthy individuals using the Simulator of Human Intestinal Microbial Ecosystem (SHIME®) and measurement of metabolites.

2. Material and methods

2.1. Cultivation and production of biomass

The microalgae *S. platensis* belonging to the culture collection of the Laboratory of Reef Environments and Biotechnology with Microalgae (Federal University of Paraíba, João Pessoa, Paraíba, Brazil) was cultivated in Zarrouk medium following the conditions described by de Medeiros et al. (2020). The culture development was accompanied every 24 h through cell counts in chamber Sedgewick Rafter and *in vivo* fluorescence measurements using Turner-Design 10005R fluorometer to reach the stationary phase (13 days). After that period, the experiments were interrupted. The cells were harvested by filtration in a 60 μm mesh strainer. The wet biomass was frozen at −30 °C, lyophilized, packaged in polyethylene bags, and stored at −18 °C. The *S. platensis* biomass showed 50.11 g/100 g of protein and 18.38 g/100 g of lipids. Furthermore, it had 1.5 g/100 g of soluble dietary fiber, 27 g/100 g of insoluble dietary fiber, 3.71 g/100 g of nystose, 6.14 g/100 g of raffinose, 4 g/100 g of glucose, 12 g/100 g of rhamnose, and 4 g/100 g of fructose (de Medeiros et al., 2021).

2.2. In vitro gastrointestinal digestion of microalgae biomass

The whole biomass of *S. platensis* was submitted to *in vitro* digestion, simulating the oral, gastric, and intestinal phases following INFOGEST's protocol (Minekus et al., 2014). Previous studies recommended biomass digestion before being subjected to colonic fermentation to evaluate its prebiotic properties (Jin et al., 2020). After the digestion, the solutions containing the final digestion phase were dialyzed for 24 h against 0.01 M NaCl at 5 ± 0.5 °C (Guergoletto, Costabile, Flores, Garcia, & Gibson, 2016). The digested *S. platensis* biomass (DB SPI) was frozen (−18 °C), freeze-dried, and stored (−20 ± 0.2 °C) in hermetically sealed polyethylene bags. The dialysis membrane, enzymes (α-amylase, lipase, pepsin, pancreatin), bile salts, and all other reagents were purchased from Sigma-Aldrich.

2.3. Effect of digested S. platensis biomass on the growth of L. acidophilus La-5

Lyophilized culture of *L. acidophilus-La 5* obtained from Chr. Hansen S.A. (Valinhos, São Paulo, Brasil) was previously cultivated in de Man, Rogosa and Sharpe (MRS) broth (Hi-Media, Mumbai, India) at 37 °C under anaerobic conditions (Anaerobic System Anaerogen, Oxoid) for 24 h. The cells were collected by centrifugation (4500g, 15 min, 4 °C), washed twice, resuspended in sterile saline solution (NaCl 0.85 g/ 100 mL), and homogenized for the 30 s using a vortex to obtain the initial inoculum. Cell suspensions with optical density (OD) at 660 nm (OD_{660}) of 0.8 provided viable counts of approximately 6 log CFU/mL. Subsequently, 1% (v/v) of the cell suspension was inoculated individually in MRS medium (10 mL) containing the digested *S. platensis* biomass or fructooligosaccharides (FOS) (positive control) at the final concentration of 0.5 mg/mL. The negative control was elaborated without the addition of FOS or digested *S. platensis* biomass.

Immediately after homogenization and after 24, 48, and 72 h of incubation, an aliquot of 100 μL of each assay was serially diluted in sterile saline solution and plated on MRS agar to perform cell count using the micro drop technique (Herigstad, Hamilton, & Heersink, 2001). The viable cells were counted after anaerobic incubation at 37 °C, and the results were expressed as log CFU/mL. The proliferation rate was calculated according to the equation described by Li et al. (2019), which considers the growth in medium (absence of the substance evaluated) and the medium added of the substance under evaluation. The number of colonies in the MRS medium without the digested biomass is considered 100%, and the proliferation rate was calculated using Equation 1.

$$\text{Proliferation rate} = \frac{\text{The number of colonies in MRS medium with digested biomass}}{\text{The number of colonies in MRS medium}} \times 100\%$$

2.4. Evaluation of the metabolic activity of L. acidophilus-La 5

The metabolites were evaluated during fermentation (time zero - just after homogenization), after 24, 48, and 72 h of incubation. The measurement of pH values (method 981.12) was performed in a digital potentiometer (Quimis, Diadema, São Paulo, Brazil) (AOAC, 2016). Furthermore, the content of sugars (glucose and fructose), organic acids (lactic acid, citric acid, succinic acid, malic acid, acetic acid, and propionic acid), and phenolic compounds were evaluated.

At each time interval, aliquots of MRS media containing FOS, DB SPI, and negative control were collected, centrifuged (4500*g*, 5 min, 4 °C), and the supernatants were filtered using a 0.45-μm membrane Millex-HA filter (Millipore Co., Bedford, MA, USA). Then, 10 μL was injected into the High-Performance Liquid Chromatography (HPLC) using a chromatography (model 1260 Infinity LC, Agilent Technologies, USA) coupled with a diode array detector (DAD) (Agilent G1315D) and refractive index detector (RID) (Agilent G1362A).

HPLC-DAD-RID simultaneously determined the quantification and profile of sugars and organic acids according to the conditions described by Coelho et al. (2018). An Agilent Hi-Plex H column (7.7 × 300 mm, 8 μm) and 4 M H_2SO_4 in ultrapure water as mobile phase (flow rate 0.5 mL/min) were used. The phenolic compounds were analyzed by chromatography according to the conditions referred by da Silva Padilha et al. (2017). The chromatographic separation of the phenolic compounds was performed using a Zorbax Eclipse Plus RP-C18 column (100 × 4.6 mm, 3.5 μm) and the Zorbax C18 pre-column (12.6 × 4.6 mm, 5 μm). The column temperature was adjusted to 35 °C, and the mobile phases were composed of acidified water (pH 2) with 0.1 mM/L phosphoric acids (phase A) and acidified methanol with 0.5% phosphoric acid (phase B). The flow rate was maintained at 0.8 mL/min. Data acquisitions from DAD were processed using OpenLAB CDS ChemStation Edition software (Agilent Technologies).

The quantification was carried out by using calibration curves of the external standard according to the validated methods (Coelho et al., 2018; da Silva Padilha et al., 2017). All analyzed compounds presented calibration curves with $R^2 \geq 0.998$. In addition, the peak spectral purity was verified using the Threshold tool to ensure the identification accuracy compared with the external standard (Sigma Aldrich, St. Louis, USA).

2.5. Simulated digestion in the dynamic colonic model

The SHIME® is computer-controlled, and it consists of 5 closed compartments representing the stomach, small intestine, ascending colon, transverse colon, and descending colon (Molly, Woestyne, Smet, & Verstraete, 1994). In this experiment, SHIME® was adapted for the triplication of the ascending colon, where the transverse and descending colon have been substituted, according to da Cruz Rodrigues et al. (2020). A schematic representation of SHIME® is provided in Fig. 1.

The volumetric capacity, pH, temperature (37 °C), and retention time (24 h) were controlled (Possemiers, Verthe, Uyttendaele, & Verstraete, 2004). Anaerobiosis of the system was achieved by adding nitrogen and the pH value corrected in each vessel using hydrochloric acid or sodium hydroxide accordingly, ranging from 5.6 to 5.9 (Molly et al., 1994; Possemiers et al., 2004). The compartments were colonized with feces from five healthy medium-aged volunteers. Inclusion criteria were ages 40–50 years who had not used antibiotics, probiotics, or prebiotics 6 months before the study. They were homogenized, and a portion was stored in a sterile plastic tube (10 g) and held at −80 °C until analysis.

A portion of 40 g of feces was homogenized and diluted in 200 mL of phosphate buffer (pH 6.5). The buffer was composed of 7.08 g/L of monosodium phosphate (Synth, SP, Brazil), 5.98 g/L of disodium phosphate (Synth, SP, Brazil), and 1 g/L of sodium thioglycollate (Merck, Brazil) (Possemiers, Marzorati, Verstraete, & Van de Wiele, 2010). This mixture was then homogenized in a stomacher and centrifuged at 3000*g* for 15 min. The supernatant was collected and 10 mL added to each of the last 3 compartments together with 500 mL of sterile feed medium (Van den Abbeele et al., 2019). The feed medium is carbohydrate-based [4.0 g/L starch (Maizena, São Paulo, BR), 4.0 g/L mucin (Sigma, St. Louis, USA), 3.0 g/L yeast extract (Difco, Becton Dickinson Co., Sparks, MD, USA), 1.0 g/L arabinogalactan (Sigma, St. Louis, USA), 1.0 g/L xylan (Sigma, St. Louis, USA), 1.0 g/L peptone (Himedia, Mumbai, India), 0.5 g/L cysteines (Sigma, St. Louis, USA), and 0.4 g/L glucose (Synth, Diadema, BR]. A feed medium should have complex carbohydrate and protein sources with the addition of mucins and a mineral and vitamin mix to maintain the survival of the microbiota over a longer timeframe (Van de Wiele, Van den Abbeele, Ossieur, Possemiers, & Marzorati, 2015). The concentration of the compounds used in the present study has been used in previous studies with SHIME® (Bianchi et al., 2018; Roselino et al., 2020; Salgaço et al., 2021). Stomach conditions were simulated in reactor 1 by adding HCl to regulate pH, along with the carbohydrate-based medium. The passage through the duodenum was simulated by 60 mL of artificial pancreatic juice (12.5 g/L of $NaHCO_3$, 6 g/L of Oxgall, and 1.9 g/L of pancreatin, Sigma-Aldrich, St. Louis, USA) with an essential role in environmental adaptation and inoculum growth, with the formation of a stable and

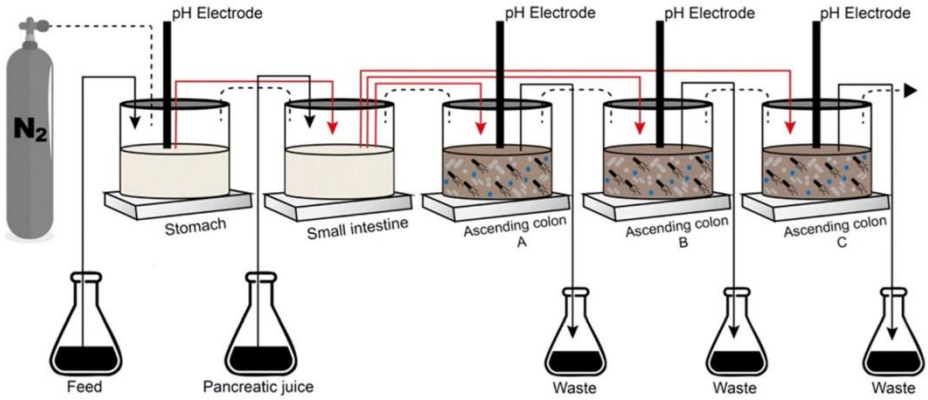


Fig. 1. Schematic representation of the SHIME® adapted from Machado Ribeiro et al. (2021).

representative community (Molly et al., 1994).

2.6. Experimental protocol in the SHIME reactor

The experimental period in the SHIME® reactor lasted 4 weeks. First, the feed medium (240 mL) was inserted into the system and left to stabilize for 14 days (Bianchi et al., 2018). After these two weeks, for one week, daily, the 210 mL of feed medium and 60 mL of pancreatic juice were inserted into the system (control treatment). Afterward, the treatment was administered two times a day for 5 days. Treatments: *L. acidophilus* (La5, 10^6 CFU/g) or 1.5 g of biomass of *S. platensis* with *L. acidophilus* - *La 5* (Spi-La5, 10^6 CFU/g). The experiment was performed in biological triplicate. The *S. platensis* concentration was selected based on guidelines for safe daily consumption (FDA, 2011; Huang, Liao, Pu, & Cui, 2018). Furthermore, we considered the FAO recommended dosage for applying *S. platensis* in food products (1.5 g/100 g), such as meal replacement and mixes, sports beverages, energy soft drinks, fruit juices, low-fat soy milk, among others (FDA, 2011). Finally, a previous study observed that similar *S. platensis* concentrations (1.5 and 3.0 g/kg) promoted an increase in the proportion of *Firmicutes* and *Bacteroidetes* after the 21 days of administration to healthy mice (Hu et al., 2019). Thus, from the perspective of adding more health benefits to the host, we believe that the combination of *Spirulina* biomass (1.5 g, twice a day) and *L. acid*ophilus (10^6 CFU/g) could be considered a suitable evaluation strategy.

2.7. Metabolic Activity: Ammonium (NH^+4) analysis and short-chain fatty acids (SCFAs)

After the simulated digestion, the samples from the colon compartment (n = 3) were collected and stored at −20 °C. First, the ammonium ions (NH^{+4}) were quantified according to Bianchi et al. (2014) using a specific ion meter (Model 710A, Orion) coupled to a selective ammonia ion electrode (Model 95–12, Orion). Next, short-chain fatty acids (SCFA) were analyzed according to the protocol adopted by Duque, Monteiro, Adorno, Sakamoto, and Sivieri (2016), with modifications. First, the samples (n = 3, second week of the colon reactors) were centrifuged (14,000g, 5 min) followed by the dilution of 100 μL of the supernatant in 1900 μL of ultrapure water. Next, NaCl (1g) and crotonic acid (100 μL) were added, as well as isobutanol (70 μL) and 2 M H_2SO_4 (200 μL).

Analytical curves were constructed from stock solutions of the acids of interest (acetic, propionic, and butyric). The SCFAs analysis was conducted using an HP-5975 model gas chromatograph (Agilent Technologies, Santa Clara-CA, USA) equipped with a split/splitless injector, a mass selective detector, and an HP-7683B automated sampler. The SCFAs were separated through a ZB-WAX column (60 m × 0.25 mm × 0.25 μm) (Phenomenex, Torrance-CA, USA). The GC was performed using the column set at 35 °C, 2 °C/min, 38 °C; 10 °C/min, 75°C; 35 °C/min, 120 °C (1 min); 10 °C/min, 170 °C (2 min); 40 °C/min, 170 °C (2 min), and the temperature of the injector and the detector was maintained at 220 °C and 250 °C, respectively. Helium was used as the carrier gas; the flow rate was set at 1 mL/min (Hoving, Heijink, van Harmelen, van Dijk, & Giera, 2018).

2.8. Microbiological analysis using 16S rRNA gene sequencing

The microbiological analysis employing 16S rRNA gene sequencing (n = 2) was performed by Next-generation Sequencing (Neoprospecta Microbiome Technologies, Brazil) with specific primers amplified the V3–V4 region of the 16S rRNA, 341F and 806R of 1 ng of DNA (Caporaso et al., 2011; Wang & Qian, 2009). The analysis of the sequences and the identification of the taxonomic units were based on the methodology of Giongo, Davis-Richardson, Crabb, and Triplett (2010) and Hong, Bunge, Jeon, and Epstein (2006). The PCR was carried out in triplicate using a Platinum Taq (Invitrogen USA) under the following conditions: initial denaturation at 94 °C for 3 min; 20 cycles of denaturation at 94 °C for 45 s, annealing at 50 °C for 30 s, extension at 65 °C for 90 s and final extension at 65 °C for 10 min. The PCR products were purified using AMPure beads (Beckman coulter) (Christoff, Sereia, Boberg, Moraes, & Oliveira, 2017) and quantified by fluorometry using Qubit® 2.0 Fluorometer (Invitrogen, USA) (Fagen et al., 2012).

2.9. Statistical analysis

The analyses were conducted in triplicate in three independent experiments, and the results were expressed as means ± standard deviation. First, data were submitted to a Student's *t*-test or analysis of variance (ANOVA) to determine significant differences at a level of 5% significance, followed by the Tukey's multiple comparisons test, using computational software GraphPad Prism software, version 8.0 (La Jolla, USA).

For the microbiological analysis using 16S rRNA gene sequencing data, the sequencing libraries were prepared according to Neoprospecta® Microbiome Technologies, and the sequencing was carried out using the MiSeq platform (Illumina). First, bioinformatics analyzes were performed using the QIIME program (Quantitative Insights Into Microbial Ecology) according to procedures described by Caporaso et al. (2011). Next, taxonomic descriptions were generated based on the NCBI taxonomy database (http://www.ncbi.nlm.nih.gov) for the RDP database entries using the set of scripts listed in the TaxCollector pipeline Giongo et al. (2010). Then, the strings were filtered and classified according to similarity. A minimum of 80% similarity was considered for domain and phylum identification, 90% for order and class identification, 90% for family, 95% for the genus, and 99% similarity for species identification (Savy et al., 2018).

The 16S rRNA gene sequencing analyses were conducted in RStudio, version 3.2.4 (R Core Team, 2018), utilizing the phyloseq package (McMurdie & Holmes, 2013) to import sample data and calculate alpha and beta diversity. Alpha diversity was calculated using Chao1 index, Shannon index et al. with QIIME (V1.7.0), and displayed with R software (V2.15.3). Beta diversity was measured using both weighted and unweighted unifrac distance metrics by QIIME software (V1.7.0). The significance of categorical variables was determined using the non-parametric Wilcoxon test for two category comparisons or the Kruskal-Wallis test when comparing three or more categories. Principal coordinate plots relied on the PERMANOVA test to estimate p values (Fierer et al., 2010). The p values were adjusted for multiple comparisons using the FDR algorithm (Benjamini, Drai, Elmer, Kafkafi, & Golani, 2001). To compare the relative abundance of the different taxa between groups, we performed Linear Discriminant Analysis (LDA) effect implemented in LEfSe (Dei-Cas, Giliberto, Luce, Dopazo, & Penas-Steinhardt, 2020). Correlation analysis between microbial groups and the metabolism products was performed using the Spearman correlation test using the cor.mtest function from the corrplot stats package (Wei & Simko, 2021).

Table 1
Viability cell counts (log CFU/mL) of the *Lactobacillus acidophilus* LA-5 in medium with digested *Spirulina platensis* biomass, fructooligosaccharides (FOS), and control during 72 h of fermentation.

Samples	Time of incubation			
	0 h	24 h	48 h	72 h
CONTROL	6.4 ± 0.02^{Da}	7.58 ± 0.02^{Bc}	7.77 ± 0.03^{Ac}	7.04 ± 0.04^{Cc}
FOS	6.1 ± 0.01^{Ca}	8.74 ± 0.03^{Ab}	8.80 ± 0.01^{Ab}	8.05 ± 0.04^{Bb}
DB SPI	6.2 ± 0.02^{Ca}	9.00 ± 0.01^{Aa}	8.97 ± 0.01^{Aa}	8.28 ± 0.01^{Ba}

DB SPI: Digested biomass of *Spirulina platensis*. A– D: different superscript capital letters in the same row for the same cultivation medium and at different time intervals

a – c: Different superscript small letters in the same column at the same time interval denotes difference among cultivation media.

3. Results

3.1. Effect of DB SPI on the growth of L. acidophilus-La 5

Table 1 presents viable cell counts (log CFU/mL) of *L. acidophilus- La 5* in a medium containing DB SPI, FOS, and control during fermentation. At the beginning of cultivation, all tested media showed similar viable cell counts ($p > 0.05$), as expected. However, during cultivation, there was an increase in the viable cell counts ($p < 0.05$), especially in the media containing DB SPI that promoted higher ($p < 0.05$) counts when compared to the medium containing FOS or control. At the end of the incubation period, the counts of *L. acidophilus- La 5* were > 8 logs CFU/mL in media with DB SPI and FOS. On the other hand, the control showed lower counts (7.04 log CFU/ mL).

The proliferation of *L. acidophilus- La 5* cultured in a medium containing DB SPI and FOS is shown in Fig. 2. Medium containing DB SPI promoted a greater proliferation rate ($p < 0.05$) than FOS medium at all times evaluated. The highest proliferation of *L. acidophilus La 5* was observed in the first 24 h of incubation, followed by decreases after 48 h and 72 h in media containing DB SPI and FOS, respectively.

3.2. Metabolic activity of L. acidophilus- La 5 in media with DB SPI and FOS

The pH values, sugar (glucose and fructose), and organic acid contents in media with DB SPI, FOS, and control during the cultivation are shown in Table 2. The pH values of all media decreased during the culture period and were lower ($p < 0.05$) after 72 h compared to the initial pH. At the end of incubation, the DB SPI medium exhibited the lowest pH value ($p < 0.05$), while the medium with FOS showed similar values compared to the control ($p > 0.05$). As expected, the highest ($p < 0.05$) glucose concentrations were detected in the medium containing DB SPI compared to the medium with FOS. In contrast, the highest fructose content was found in the medium with FOS. Contents of fructose and glucose decreased ($p < 0.05$) during the cultivation period in media containing FOS and DB SPI. The decreases in glucose and fructose contents at the end of the cultivation period (time 72 h) were higher ($p < 0.05$) in the medium containing FOS and DB SPI, respectively.

The organic acid produced in the highest ($p < 0.05$) amounts regardless of the cultivation medium was lactic acid. The lactic acid content increased ($p < 0.05$) in all media when the length of the cultivation time increased. The media with DB SPI exhibited higher lactic acid content than FOS ($p < 0.05$) at all incubation times evaluated. The most significant increase ($p < 0.05$) of lactic acid was detected after 72 h of cultivation in medium with DB SPI, followed by medium with FOS.

Contents of citric acid and succinic acid decreased ($p < 0.05$) over time in all evaluated media. Medium with DB SPI exhibited the highest ($p < 0.05$) citric acid contents at the beginning of the fermentation and after 24 h when compared to the medium with FOS in the same period. At the end of incubation, citric acid contents between the media with DB SPI and FOS were similar ($p > 0.05$). The lowest range of citric acid was found in the control medium ($p < 0.05$). Similarly, the higher ($p < 0.05$)

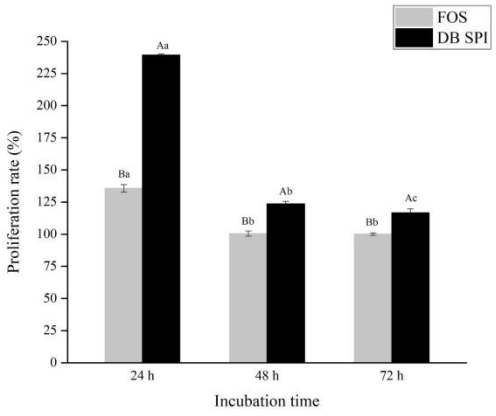


Fig. 2. Proliferation rate of *L. acidophilus*. FOS = treatment with *L. acidophilus*-La5 and FOS, Spi-La5 = treatment with *L. acidophilus*- La5 and spirulina.

Table 2
pH values, sugar, and organic acids amounts (g/L) in media with digested *Spirulina platensis* biomass, fructooligosaccharides (FOS), and control during 72 h of fermentation.

Parameter	Samples	Time of incubation			
		0 h	24 h	48 h	72 h
pH value	CONTROL	5.48 ± 0.03^{Aa}	3.47 ± 0.02^{Ba}	3.29 ± 0.03^{Ca}	3.27 ± 0.03^{Ca}
	FOS	5.64 ± 0.05^{Aa}	3.41 ± 0.03^{Ba}	3.25 ± 0.05^{Ca}	3.21 ± 0.01^{Cab}
	DB SPI	5.58 ± 0.03^{Aa}	3.28 ± 0.03^{Bb}	3.22 ± 0.02^{BCa}	3.16 ± 0.03^{Dc}
Glucose	CONTROL	1.18 ± 0.03^{Ac}	0.80 ± 0.03^{Bc}	0.50 ± 0.01^{Cc}	0.36 ± 0.02^{Db}
	FOS	1.82 ± 0.04^{Bb}	1.25 ± 0.02^{Bb}	0.82 ± 0.04^{Cb}	0.38 ± 0.03^{Db}
	DB SPI	3.28 ± 0.05^{Aa}	2.70 ± 0.01^{Ba}	1.60 ± 0.01^{Ca}	0.42 ± 0.04 ^{Da}
Fructose	CONTROL	< LOD	< LOD	< LOD	< LOD
	FOS	6.66 ± 0.05^{Aa}	4.35 ± 0.04^{Ba}	3.88 ± 0.04^{Ca}	2.20 ± 0.03 ^{Da}
	DB SPI	0.11 ± 0.01^{Ab}	0.05 ± 0.00^{Bb}	0.04 ± 0.01^{Bb}	< LOD
Lactic acid	CONTROL	5.88 ± 0.05^{Db}	9.84 ± 0.03^{Cc}	10.50 ± 0.04^{Bc}	13.16 ± 0.04^{Ac}
	FOS	5.83 ± 0.02^{Cb}	13.14 ± 0.02^{Bb}	14.09 ± 0.04^{Bb}	19.51 ± 0.04^{Ab}
	DB SPI	6.69 ± 0.01 ^{Da}	13.72 ± 0.03^{Ca}	14.83 ± 0.03^{Ba}	22.06 ± 0.02^{Aa}
Citric acid	CONTROL	1.40 ± 0.04^{Ab}	0.99 ± 0.05^{Bb}	0.70 ± 0.05^{Cb}	0.22 ± 0.03^{Db}
	FOS	1.40 ± 0.01^{Ab}	0.99 ± 0.03^{Bb}	0.81 ± 0.01^{Ca}	0.59 ± 0.02 ^{Da}
	DB SPI	1.48 ± 0.01^{Aa}	1.28 ± 0.03^{Ba}	0.75 ± 0.01^{Cab}	0.53 ± 0.01 ^{Da}
Succinic acid	CONTROL	2.37 ± 0.02^{Ab}	1.36 ± 0.02 ^{Da}	1.81 ± 0.02^{Ba}	1.44 ± 0.02^{Ca}
	FOS	2.25 ± 0.03^{Ac}	1.79 ± 0.03^{Bb}	1.57 ± 0.03^{Cb}	1.27 ± 0.03^{Db}
	DB SPI	2.62 ± 0.01^{Aa}	2.37 ± 0.01^{Ba}	1.56 ± 0.01^{Cb}	1.03 ± 0.03^{Dc}
Malic acid	CONTROL	0.15 ± 0.01^{Cb}	0.17 ± 0.02^{Cb}	0.24 ± 0.02^{Bb}	0.37 ± 0.04^{Ab}
	FOS	0.13 ± 0.00^{Ca}	0.28 ± 0.02^{Ba}	0.47 ± 0.01^{Aa}	0.48 ± 0.01^{Aa}
	DB SPI	0.08 ± 0.02^{Ca}	0.18 ± 0.01^{Bb}	0.49 ± 0.00^{Aa}	0.48 ± 0.02^{Aa}
Acetic acid	CONTROL	1.23 ± 0.03 ^{Da}	1.29 ± 0.02^{Cb}	1.59 ± 0.01^{Bb}	2.13 ± 0.02^{Ab}
	FOS	1.04 ± 0.02^{Db}	1.31 ± 0.01^{Cb}	1.58 ± 0.02^{Bb}	2.11 ± 0.01^{Ab}
	DB SPI	0.93 ± 0.02^{Dc}	1.42 ± 0.03^{Ca}	2.14 ± 0.01^{Ba}	2.26 ± 0.00^{Aa}
Propionic acid	CONTROL	0.25 ± 0.01^{Dc}	0.36 ± 0.00^{Ca}	0.40 ± 0.02^{Ab}	0.39 ± 0.01^{ABc}
	FOS	0.36 ± 0.03^{Ba}	0.39 ± 0.00^{Ba}	0.47 ± 0.01^{Aa}	0.46 ± 0.02^{Ab}
	DB SPI	0.31 ± 0.02^{Dab}	0.39 ± 0.02^{Ca}	0.51 ± 0.01^{Ba}	0.89 ± 0.04^{Aa}

DB SPI: Digested biomass of *Spirulina platensis*. LOD: limit of detection. A–D: Different superscript capital letters in the same row for the same cultivation media denotes differences.
a-c: different superscript small letters in the same column at the same time interval denotes difference ($p < 0.05$) among cultivation media.

succinic acid contents were identified in medium with DB SPI at times zero and 24 h (2.37 g/L), while the medium with FOS showed lower values ($p < 0.05$). The media with FOS exhibited similar content ($p > 0.05$) to the medium with DB SPI after 48 h and higher ($p < 0.05$) after 72 h of cultivation.

An increase ($p < 0.05$) of malic acid contents was observed after 24 h compared to the initial cultivation time. Highest ($p < 0.05$) contents of malic acid were found in media with FOS after 24 h, however, after 48 and 72 h of cultivation, the contents of malic acid in media with FOS and DB SPI did not differ ($p > 0.05$). Acetic acid content increased ($p < 0.05$) over time and was higher at the end of cultivation in all media. Higher ($p < 0.05$) content of acetic acid was identified in medium with DB SPI at times 24 h, 48 h, or 72 h, while medium with FOS and control exhibited similar values ($p > 0.05$). The propionic acid content did not differ ($p > 0.05$) between the media with FOS and DB SPI after 24 h and 48 h of incubation. Then, after 72 h of incubation, the medium with DB SPI showed a higher ($p < 0.05$) content of propionic acid when compared to FOS.

A total of 9 phenolic compounds have been identified. None of these phenolic compounds were detected in a medium with FOS or control (Table 3). Over the measured cultivation period, changes in phenolic contents were observed. The content of syringic acid and epicatechin decreased ($p < 0.05$) after 24 h and was followed by increases ($p > 0.05$) after 48 h and/or 72 h. On the other hand, chlorogenic acid, catechin, epigallocatechin gallate, epicatechin gallate, and procyanidins B1, B2, and A2 decreased ($p < 0.05$) after 24 h, with the lowest values found at the end of cultivation time (time 72 h).

3.3. Richness and diversity of the gut microbiota

A total of >4.8 million high-quality reads were obtained from microbiota samples collected during the control period, treatment with *L. acidophilus*- La, and combination of *L. acidophilus*- La with biomass of *S. platensis*. After normalizing the data, 40.948,00 sequences were produced, resulting in 368,000 sequences per sample. Chao1 and Shannon's indexes were used to describe alpha diversity (Table 4). Significant differences were found between the control group and Spi-La5 treatment ($p < 0.05$) for Shannon index showing a higher diversity in Spi-La5 treatment than in the control group. Significantly, the Simpson index that weighted more on the dominant species revealed that the treatment with Spi-La5 exhibited alteration patterns of the α-diversity.

Table 3
Phenolic compound amounts in media with digested *Spirulina platensis* biomass during 72 h of fermentation.

Phenolic compounds (mg/L)	Samples	Time of incubation			
		0 h	24 h	48 h	72 h
Syringic acid	DB SPI	0.31 ± 0.01^{B}	0.23 ± 0.01^{C}	0.37 ± 0.01^{A}	0.39 ± 0.02^{A}
Chlorogenic acid	DB SPI	0.22 ± 0.01^{A}	0.15 ± 0.02^{B}	0.08 ± 0.02^{C}	0.05 ± 0.01^{C}
Catechin	DB SPI	52.94 ± 0.06^{A}	45.66 ± 0.05^{B}	1.79 ± 0.04^{C}	1.67 ± 0.03^{C}
Epicatechin	DB SPI	3.36 ± 0.05^{A}	0.09 ± 0.02^{C}	0.11 ± 0.01^{C}	3.01 ± 0.03^{B}
Epigallocatechin gallate	DB SPI	9.65 ± 0.05^{A}	9.01 ± 0.02^{B}	7.41 ± 0.05^{C}	5.13 ± 0.02^{D}
Epicatechin gallate	DB SPI	3.40 ± 0.03^{A}	3.01 ± 0.03^{B}	0.92 ± 0.03^{C}	0.70 ± 0.02^{D}
Procyanidin B1	DB SPI	5.98 ± 0.03^{A}	0.61 ± 0.03^{B}	0.40 ± 0.02^{C}	0.37 ± 0.02^{C}
Procyanidin B2	DB SPI	3.60 ± 0.05^{A}	3.03 ± 0.03^{B}	1.54 ± 0.04^{C}	1.20 ± 0.04^{D}
Procyanidin A2	DB SPI	6.81 ± 0.01^{A}	6.39 ± 0.05^{B}	1.14 ± 0.02^{C}	0.94 ± 0.02^{D}

DB SPI: Digested biomass of *Spirulina platensis*.
A–D: Based on Tukey's test, different superscript capital letters in the same row for the same cultivation media denotes differences.

Table 4
Alpha diversity within microbiota samples expressed by indices Chao1 (species richness) and Shannon and Simpson (diversity) in SHIME® ascending colon vessel.

Treatment	Chao 1	Shannon and Simpson
Control	49.33 ± 1.24	2.54 ± 0.07
La-5	34.00 ± 6.97	2.80 ± 0.23
Spi-La5	47.66 ± 10.53	2.90 ± 0.12*

Control = control period, La-5 = treatment with *L. acidophilus*- La5, Spi-La5 = treatment with biomass of Spirulina + *L. acidophilus*- La5. * Asterisk denote statistical differences between the treatments and control period.

In addition to the α-diversity evaluation, the unweighted UniFrac analysis was applied to compare similarities among gut microbial communities (β-diversity). The bacterial composition profiles showed significant differences between the treatments ($P = 0.003$, Permanova analysis). The structural difference in the colonic microbiota community was also observed by the gradual shifts of distance matrix among treatment groups using PCoA (Fig. 3). The observation revealed significant differences in gut microbial community structure between control, La5, and Spi-La5 treatments.

3.4. Changes in the gut microbiota during the treatments with *L. acidophilus*- La 5 and La5 combined Spirulina

Fig. 4 shows the abundance of the main phylum and genus in ascending region of the Shime® model. A high abundance of *Firmicutes* and *Bacteriodetes* was found during the control period. The administration of La5 resulted in a decrease in *Firmicutes* and *Actinobacteria* abundance and increases in *Bacteriodetes* and *Proteobacteria* abundance. On the other hand, the administration of Spi-La5 resulted in increases in *Actinobacteria* and *Proteobacteria* abundance, decreases in *Bacteriodetes*, and maintenance of *Firmicutes* compared to control. Fig. 5 shows that the ratio of bacterial phyla *Firmicutes* to *Bacteroidetes* (F/B ratio) ranged from 0.56 to 1.71. F/B ratio was significantly reduced ($p < 0.001$) with La5 treatment compared to the control period and Spi-La5 treatment. Otherwise, Spi-La5 didn't show the statistical difference with the control period ($p > 0.05$).

Analysis of the abundance of bacterial taxonomic groups showed an increase ($p < 0.05$) of *Bacteroides*, *Megasphaera*, *Lactobacillus*, and *Parabacteroides* genus in La5 treatment. However, in the Spi-La5 treatment, an increase ($p < 0.05$) of the genus *Enterococcus*, *Roseburia*, *Bifidobacterium*, *Coriobacteriaceae* UCG-003, and *Paraclostridium* was observed (Fig. 4).

To identify specific bacterial genus in treatments (La5 and Spi-La5), the abundance of colonic bacteria in SHIME® ascending colon vessel was analyzed by the LEfSe method, and the significantly differentiated phylotypes by abundance were visualized using the histogram of LDA scores. The histogram reflected the LDA scores computed for the features at the OTU level with LDA values > 2.0, and they were depicted in Fig. 5.

The abundance of *Lactobacillus* and *Bacteroides* genus was higher in the La5 treatment than in control, which presented higher abundances of *Clostridia*, *Streptococcus*, *Caproiciproducens,* and *Lachnospira*. On the other hand, *Erysipelatoclostridium*, *Paraclostridium*, *Enterobacter*, and *Enterococcus* genus were found in considerably high abundances in the Spi-La5 treatment compared with the control. Therefore, when we compare La5 with the Spi-La5 treatment, we observed higher abundances of *Erysipelaclostridium*, *Paraclostridium*, *Enterobacter*, *and Enterococcus genus* Spi-La5 and *Bacteroides* and *Lactobacillus* genus for La5 treatment.

3.5. Metabolic activity

Levels of acetic and butyric acids had no change with La5 or Spi-La5 treatment ($p > 0.05$). However, a significant decrease ($p < 0.05$) in

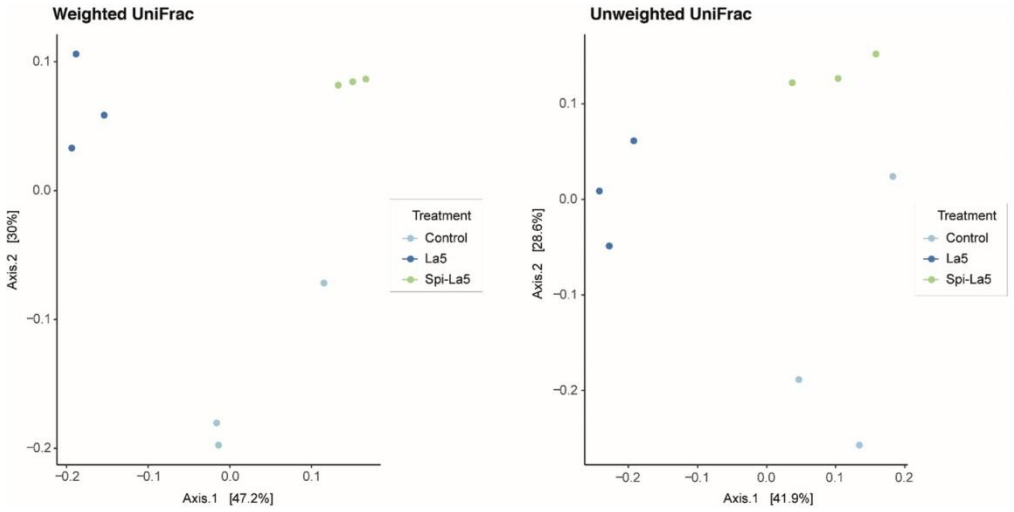


Fig. 3. PCoA based on the abundance of OTUs. Control = control period, La-5 = treatment with *L. acidophilus*- La5, Spi-La5 = treatment with spirulina + *L. acidophilus*- La5.

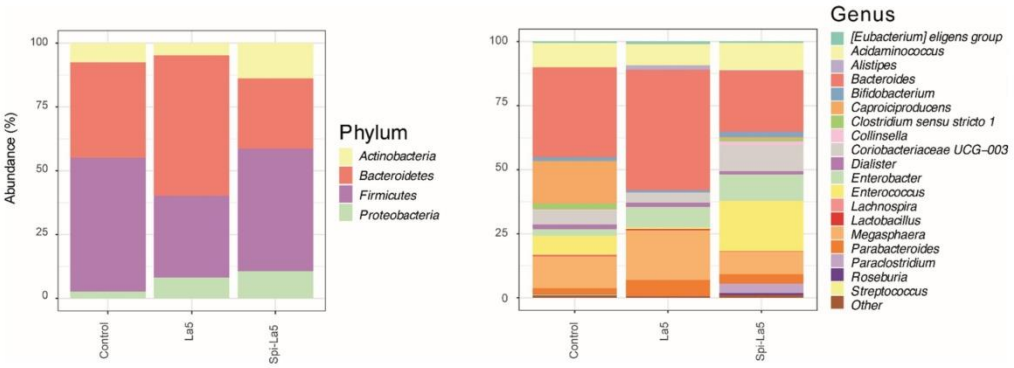


Fig. 4. Histogram of the community composition of gut microbiota at the phylum level (A) and genus level (B). Control = control period, La-5 = treatment with *L. acidophilus*- La5, Spi-La5 = treatment with spirulina + *L. acidophilus*- La5.

propionic acid was observed during the Spi-La5 treatment (Table 5). A significant decrease ($p < 0.05$) in NH_4^+ production was observed during La5 treatment compared with the Spi-La5 treatment or control period. However, Spi-La5 treatment had similar values to control ($p > 0.05$).

3.6. Correlation between metabolite production and bacterial genera during the treatments

We assessed the correlation between the relative abundance of bacterial genera, SCFAs, and NH_4^+ to identify the genera that might contribute to the production of SCFA and NH_4^+ (Fig. 6). Genus *Bacteroides* positively correlated ($p < 0.05$) with acetic acid levels, while *Lactobacillus* and *Vellonella* had a positive correlation ($p < 0.05$) with propionic acid (Fig. 7). However, the other genera didn't significantly correlate with the bacterial metabolites measured ($p > 0.05$).

4. Discussion

4.1. Growth and metabolic activity of L. acidophilus associated with FOS or DB SPI

The primary objective was to assess the possible stimulatory effect of *S. platensis in vitro* digested biomass (DB SPI) toward *L. acidophilus* using a culture medium. This analysis is commonly used to evaluate the selective stimulatory effects of potentially prebiotic compounds on *Bifidobacterium* or *Lactobacillus* species, known as beneficial groups in gut microbiota (de Melo et al., 2020; Albuquerque et al., 2020; Menezes et al., 2020). The results demonstrated a higher *in vitro* stimulatory effect of *S. platensis* biomass than FOS (control), an established prebiotic compound. Furthermore, during cultivation in media with FOS and DB SPI, consumption of sugars (glucose and fructose) and production of some organic acids (acetic acid, lactic acid, and propionic acid) was observed. At the same time, the content of other organic acids was

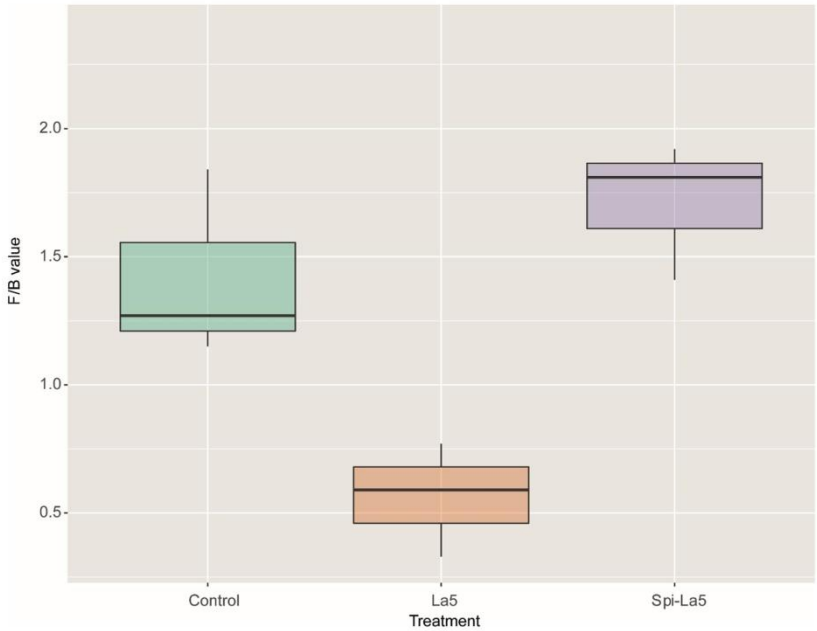


Fig. 5. The ratio of *Firmicutes* to *Bacteroidetes* (F/B) boxplot. Control = control period, La-5 = treatment with *L. acidophilus*- La5, Spi-La5 = treatment with spirulina + *L. acidophilus*- La5.

Table 5
Production of gut microbiota metabolites (mmol/L) [acetic, propionic and butyric acids and ammonium ion (NH_4^+)] in SHIME® ascending colon vessel.

Treatment	Acetic acid	Propionic acid	Butyric acid	NH_4^+
Control	$19.53^a \pm 4.16$	$6.79^a \pm 0.11$	$9.34^a \pm 2.05$	$529.33^a \pm 38.89$
La-5	$23.56^a \pm 2.18$	$8.91^a \pm 1.47$	$9.75^a \pm 0.71$	$246.22^b \pm 33.22$
Spi-La5	$18.30^a \pm 2.01$	$5.99^b \pm 0.16$	$7.89^a \pm 0.92$	$476.66^a \pm 1.44$

Control = control period, La-5 = treatment with *L. acidophilus*- La5, Spi-La5 = treatment with biomass of Spirulina + *L. acidophilus*- La5. Different letters denote statistical differences between the treatments for the same column.

reduced (citric acid and succinic acid), the pH was reduced, and higher viability of *L. acidophilus* was found.

The effects were more pronounced for the DB SPI media, which showed the highest proliferation rates of *L. acidophilus*, mainly after 24 h of fermentation. Furthermore, it showed the most increased metabolic activity. Thus, the increased cellular proliferation of *L. acidophilus* may be associated with the presence of compounds with dietary fiber, polysaccharides, oligosaccharides, and phenolic compounds in the biomass of *S. platensis* (Leal et al., 2017).

L. acidophilus can metabolize sugars and other carbohydrates (Song et al., 2021), with lactic acid production as the primary end product (Hanchi, Mottawea, Sebei, & Hammami, 2018). Acetic acid may have been produced from citrate metabolism (Menezes et al., 2020), which may explain the decrease in the citric acid content in the media. The reduction in the succinic acid content suggests a high fermentative metabolism, as succinic acid is commonly consumed in the citric acid route (de Melo et al., 2020),

The higher concentration of glucose in the DB SPI medium may have contributed to the increased viability of the probiotic culture. Glucose is rapidly metabolized by probiotic cultures with a consequent higher probiotic population in medium with glucose than fructose (FOS) (Chen, Hong, Ji, Wu, & Ma, 2020). Furthermore, in the medium with DB SPI, the phenolic compound contents were decreased for most compounds, while others were increased at the end of fermentation time (syringic acid and epicatechin). Phenolic compounds, such as epigallocatechin gallate and catechin, can stimulate the growth of probiotic *Lactobacillus* species as these microorganisms metabolize phenolic compounds (Albuquerque et al., 2020). The changes in the phenolics can also be attributed to the decreased pH values, with a consequent release of bound phenolic forms. Procyanidin B2 and epicatechin gallate may be converted to epicatechin by probiotic cultures (de Melo et al., 2020), which may explain the increase in epicatechin content at the end of fermentation time.

Evaluating the effect of potentially prebiotic compounds on probiotic cultures' growth and metabolic activity may provide important information about their selective stimulatory effects. However, the results cannot be extrapolated to a prebiotic effect on gut microbiota (Albuquerque et al., 2020). Furthermore, it is important to previously evaluate the selective utilization of a potentially prebiotic on the probiotic culture when aiming their utilization in combination. Therefore, as *S. platensis* biomass showed stimulatory properties toward *L. acidophilus* in culture medium, we evaluated the potentially prebiotic effect of the microalgae *S. platensis* and/or probiotic *L. acidophilus-La 5* on the intestinal microbiota of medium-age healthy individuals in dynamic microbiome model (SHIME®).

4.2. Changes in gut microbiota with L. acidophilus associated and S. platensis biomass

The gut microbiota profile of adults is composed predominantly of *Firmicutes* and *Bacteroidetes* phyla, while minor phyla include *Proteobacteria*, *Actinobacteria*, and *Verrucomicrobia*. Furthermore, eight genus

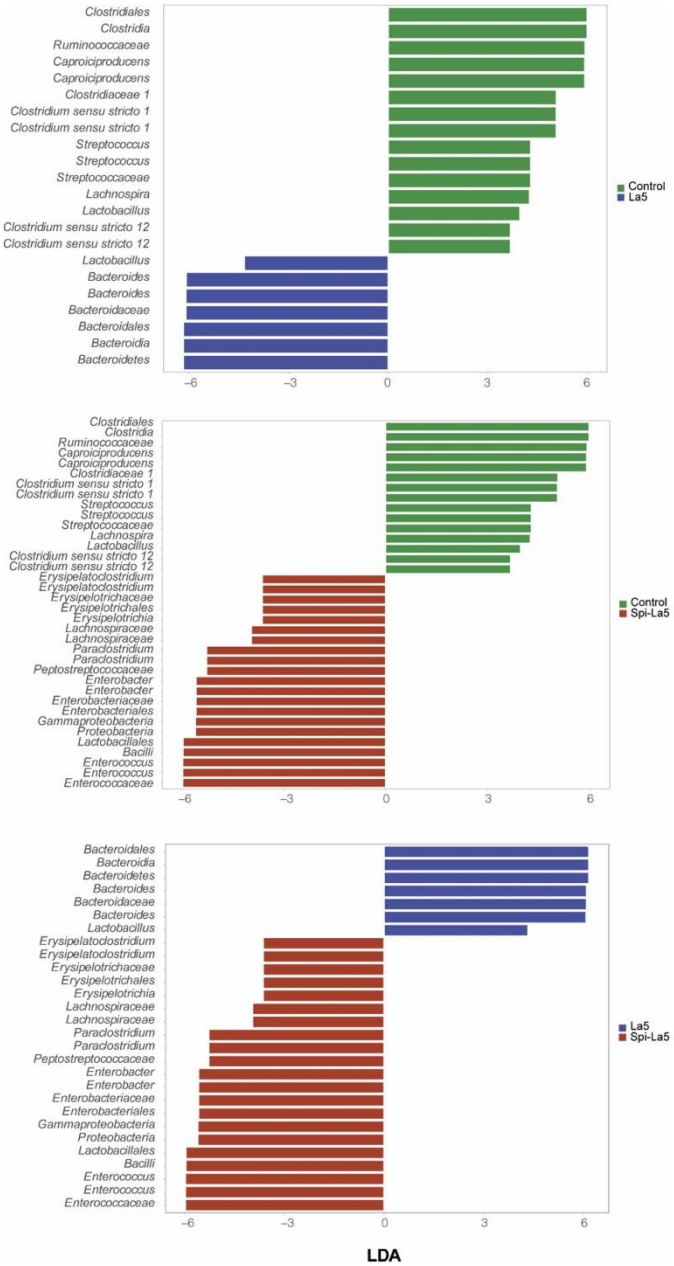


Fig. 6. The bar graph indicates the taxa that discriminate among the two groups, based on linear discriminant analysis (LDA). The statistical test was performed using the LDA effect size method. Only taxa with an alpha value of 0.05 and with absolute LDA (log10) scores > 2.0 were considered significant. Control = control period, La-5 = treatment with *L. acidophilus*- La5, Spi-La5 = treatment with spirulina + *L. acidophilus*- La5.

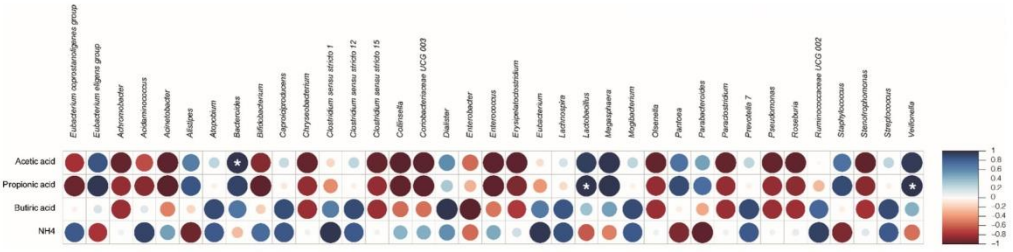


Fig. 7. Correlation between short chain fat acids production, ammonium ions, and bacterial genera. Positive correlations are represented by blue color and negative correlations by red color. Color intensities are proportional to the correlation coefficients (Spearman correlation). Significant correlations are indicated by ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

are considered more abundant, including *Eubacterium, Bacteroides, Alistipes, Faecalibacterium, Clostridium, Ruminococcus, Blautia*, and *Roseburia* (Blaak et al., 2020). In the present study, *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* were the four most abundant phyla following findings previously reported in the human gut (Yao et al., 2020). Furthermore, the Shannon and Simpson values in the present study were in the range of 2.54–2.90, which were correlated with a normal bacterial diversity (Pickard, Zeng, Caruso, & Núñez, 2017). Therefore, the microbiota of the medium-aged individuals of the present study was correlated to healthy individuals.

Analysis of the abundance of bacterial taxonomic groups showed an increase of *Bacteroides*, *Megasphaera*, *Lactobacillus*, and *Parabacteroides* genus in La-5 treatment. *Lactobacillus* and amended genera are considered probiotic cultures, and they can contribute indirectly or directly to beneficial effects in the human body. Therefore, an increase in its relative abundance in the gut is desired (Sharma, Wasan, & Sharma, 2021). *Megasphaera* has been associated with health effects due to the production of SCFA and vitamins. It was found in the intestinal microbiota of long-living and healthy older adults (Yao et al., 2020). *Bacteroides spp.* can degrade carbohydrates in the colon, and some species may protect against intestinal inflammatory diseases (Yao et al., 2020). In addition, *Parabacteroides* are known for their anti-obesity effects (Beisner, Gonzalez-Granda, Basrai, Damms-Machado, & Bischoff, 2020).

The administration of Spi-La5 resulted in increases in *Actinobacteria* and *Proteobacteria* abundance, decreases in *Bacteroidetes* abundance, and maintenance of *Firmicutes* compared to control. Including products with high dietary fiber content in the diet has been associated with reductions in the *Bacteroidetes* phylum and increases or maintenance of *Firmicutes* phylum (Beisner et al., 2020). However, the administration of Spi-La5 showed an increase in *Firmicutes:Bacteriodetes* ratio, in agreement with findings reported after oral administration of 1.5 g/ Kg of *Spirulina* biomass (28 days) in healthy rats by Hu et al. (2019).

An increase of the genus *Erysipelatoclostridium*, *Enterococcus*, *Coriobacteriaceae* UCG-003, *Enterobacter*, *Roseburia*, *Bifidobacterium*, and *Paraclostridium* was observed for the Spi-La5 group. *Bifidobacterium* and some *Enterococcus* species are members of the healthy intestinal microbiota, and they are considered potentially probiotic cultures. They may present co– and auto-aggregation ability, pH and bile salts resistance, hydrocarbons adherence, antibiotic susceptibility, and ability to produce exopolysaccharides (Nami et al., 2019). They also have bactericidal effects against *E. coli,* improve antibiotic-induced dysbiosis, and show anticancer and antitumor properties and immune system modulation (Azad, Kalam, Sarker, Li, & Yin, 2018). *Coriobacteriaceae* might regulate host glucose homeostasis and shows lipid and bile acid metabolism (Liu et al., 2018). *Erysipelatoclostridium* belongs to the *Firmicutes* phylum and is recognized as a butyrate-producing bacteria (Beisner et al., 2020). It can ferment complex carbohydrates (dietary fibers) (Pi et al., 2021). *Paraclostridium* and *Roseburia* are also butyrate-producing bacteria commonly found in media with carbohydrates and oligosaccharides. It has been associated with intestinal microecology regulation, anti-inflammatory properties, and immunity increase (Ji et al., 2021; Scott, Jean-Michel, Midtvedt, & van Hemert, 2015). The presence of complex carbohydrates and oligosaccharides in *S. platensis* may explain the abundance of some genus in the microbiota of this group (Leal et al., 2017).

The administration of La5 or SPi-La5 had no impact on the acetic acid and butyric acid contents, while the administration of Spi-La5 resulted in a lower concentration of propionic acid. Therefore, the reduction in *Bacteriodetes* after Spi-La5 treatment may be associated with the reduced propionic acid content. Propionate can be produced via three routes in the gut, namely propanediol, succinate, and acrylate pathways. Several microorganisms may be involved in these routes, including *Bacteroides, Bifidobacterium, Dialister, Akkermansia, Clostridium, Coprococcus, Megasphaera, Eubacterium*, and *Ruminococcus* (Jin et al., 2020).

The maintenance of similar SCFA content after the administration of La5 or Spi-La5 (acetic, butyric, and propionic acids) compared to control may be associated with their utilization by specific groups of microorganisms and conversion to other metabolites. The gut is composed of a complex microbiota, existing cross-feeding interactions. Cross-feeding may alter the SCFA content, as some microorganisms use the end products from the metabolism of other groups (Bianchi et al., 2018). For example, *Lactobacillus* are saccharolytic microorganisms and can produce ethanol, lactate, and succinate as end products, intermediates in the fermentation process by microbiota, and metabolized to SCFA by the cross-feeding species (Roselino et al., 2020). Furthermore, a low acetic: propionic ratio (2.64–3.05) was observed in the present study. Low acetic: propionic ratios are associated with positive impacts on human health and hypolipidemic properties due to the cholesterol biosynthesis inhibition (da Cruz Rodrigues et al., 2020).

The administration of La5 reduced the NH_4^+ ions. These ions result from urea hydrolysis and amino acid deamination by intestinal microbiota. The decrease in their contents suggests favoring carbohydrate fermentation and inhibiting amino acid and peptide fermentation (Bianchi et al., 2018). In this way, the reduction in NH_4^+ ions is desired, as they may alter the intermediate metabolism and morphology of intestinal cells, promote tumor development, and increase DNA synthesis (Bianchi et al., 2018). In general, the increase or decrease in the synthesis of ammonium ions depends on the substrate administered as well as the duration of the treatment (Casarotti, Borgonovi, de Mello, Sivieri, & Penna, 2020). Considering that ammonia is the main metabolite produced by the fermentation of proteins by intestinal bacteria, the lack of decrease in $NH4^+$ ions in the treatment Spi-La5 was probably associated with the presence of proteins in its composition that can vary up to 70 g/100 g dw (Käferböck et al., 2020) and can be metabolized by gut bacterial groups. However, the $NH4^+$ levels in Spi-La5 did not differ ($p < 0.05$) from the control treatment.

5. Conclusion

This study provides essential information about the potentially prebiotic effect of *S. platensis* biomass and in combination with *L. acidophilus* La-5. First, a higher *in vitro* prebiotic effect of *S. platensis* biomass was demonstrated compared to FOS using a culture medium. In a general view, both interventions may improve the gut microbiota of healthy medium-aged in dynamic microbiome model. However, in-depth future investigation utilizing metabolomics, metagenomics, and transcriptomics is needed to assess the specific microbial functions. Finally, prospective clinical studies should demonstrate the beneficial effects of the changes on human health. These studies are critical as it was observed in the present study differences when *L. acidophilus* La-5 was administrated solely or in combination with *S. platensis* biomass concerning the types of microorganisms that were modulated. Furthermore, although SHIME may be a critical approach to analyzing the impact of dietary compounds on the human colonic microbiota, clinical trials are necessary to corroborate its results and associate the gut microbiota modulation to possible health effects.

CRediT authorship contribution statement

Viviane Priscila Barros de Medeiros: Conceptualization, Investigation, Formal analysis, Visualization. **Mateus Kawata Salgaço:** Conceptualization, Investigation. **Tatiana Colombo Pimentel:** Investigation, Formal analysis, Visualization. **Thayanne Caroline Rodrigues da Silva:** Investigation. **Adilson Sartoratto:** Investigation. **Marcos dos Santos Lima:** Investigation. **Cristiane Francisca da Costa Sassi:** Investigation. **Victoria Mesa:** Formal analysis. **Marciane Magnani:** Conceptualization, Formal analysis, Visualization, Project administration. **Katia Sivieri:** Formal analysis, Visualization, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Albuquerque, T. M. R., Borges, C. W. P., Cavalcanti, M. T., Lima, M. dos S., Magnani, M., & Souza, E. L. (2020). Potential prebiotic properties of flours from different varieties of sweet potato (*Ipomoea batatas* L.) roots cultivated in Northeastern Brazil. *Food Bioscience, 36*, 100614. https://doi.org/10.1016/j.fbio.2020.100614, 1–13.

Association of Official Analytical Chemists (AOAC). (2016). *20th ed. Official Methods of Analysis of the AOAC International*. Rockville, MD, USA. ISBN: 97-8-093558487-5.

Atik, D., Gürbüz, B., Bölük, E., & Palabıyık, İ. (2021). Development of vegan kefir fortified with *Spirulina platensis*. *Food Bioscience, 42*(101050), 1–8. https://doi.org/10.1016/j.fbio.2021.101050

Azad, M., Kalam, A., Sarker, M., Li, T., & Yin, J. (2018). Probiotic species in the modulation of gut microbiota: An overview. *BioMed Research International, 2018*, 1–8. https://doi.org/10.1155/2018/9478630

Benjamini, Y., Drai, D., Elmer, G., Kafkafi, N., & Golani, I. (2001). Controlling the false discovery rate in behavior genetics research. *Behavioural Brain Research, 125*, 279–284. https://doi.org/10.1016/s0166-4328(01)00297-2

Beisner, J., Gonzalez-Granda, A., Basrai, M., Damms-Machado, A., & Bischoff, S. C. (2020). Fructose-induced intestinal microbiota shift following two types of short-term high-fructose dietary phases. *Nutrients, 12*(11), 3444. https://doi.org/10.3390/nu12113444

Bianchi, F., Larsen, N., de Mello Tieghi, T., Adorno, M. A. T., Kot, W., Saad, S. M. I., … Sivieri, K. (2018). Modulation of gut microbiota from obese individuals by in vitro fermentation of citrus pectin in combination with *Bifidobacterium longum* BB-46. *Applied Microbiology and Biotechnology, 102*(20), 8827–8840. https://doi.org/10.1007/s00253-018-9234-8

Bianchi, F., Rossi, E. A., Sakamoto, I. K., Adorno, M. A. T., Van de Wiele, T., & Sivieri, K. (2014). Beneficial effects of fermented vegetal beverages on human gastrointestinal microbial ecosystem in a simulator. *Food Research International, 64*, 43–52. https://doi.org/10.1016/j.foodres.2014.05.072

Blaak, E. E., Canfora, E. E., Theis, S., Frost, G., Groen, A. K., Mithieux, G., … Verbeke, K. (2020). Short chain fatty acids in human gut and metabolic health. *Beneficial Microbes, 11*, 411–455. https://doi.org/110.3920/BM2020.0057.

Casarotti, S. N., Borgonovi, T. F., de Mello, T. T., Sivieri, K., & Penna, A. L. B. (2020). Probiotic low-fat fermented goat milk with passion fruit by-product: In vitro effect on obese individuals' microbiota and on metabolites production. *Food Research International, 136*(109453), 1–12. https://doi.org/10.1016/j.foodres.2020.109453

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., … Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences, 108*(1), 4516–4522. https://doi.org/10.1073/pnas.1000080107

Celekli, A., Alslibi, Z. A., & üseyin Bozkurt, H. (2019). Influence of incorporated Spirulina platensis on the growth of microflora and physicochemical properties of ayran as a functional food. *Algal Research, 44*, 101710. https://doi.org/10.1016/j.algal.2019.101710

Chen, G., Hong, Q., Ji, N., Wu, W., & Ma, L.i. (2020). Influences of different drying methods on the structural characteristics and prebiotic activity of polysaccharides from bamboo shoot (*Chimonobambusa quadrangularis*) residues. *International Journal of Biological Macromolecules, 155*, 674–684. https://doi.org/10.1016/j.ijbiomac.2020.03.223

Christoff, A. P., Sereia, A. F. R., Boberg, D. R., Moraes, R. L. V., & Oliveira, L. F. V. (2017). Bacterial identification through accurate library preparation and high-throughput sequencing. *Neoprospecta Microbiome Technologies, 25*.

Claesson, M. J., Cusack, S., O'Sullivan, O., Greene-Diniz, R., de Weerd, H., Flannery, E., … O'Toole, P. W. (2011). Composition, variability, and temporal stability of the intestinal microbiota of the elderly. *Proceedings of the National Academy of Sciences, 108*(Supplement 1), 4586–4591. https://doi.org/10.1073/pnas.1000097107

Coelho, E. M., da Silva Padilha, C. V., Miskinis, G. A., de Sá, A. G. B., Pereira, G. E., de Azevêdo, L. C., & dos Santos Lima, M. (2018). Simultaneous analysis of sugars and organic acids in wine and grape juices by HPLC: Method validation and characterization of products from northeast Brazil. *Journal of Food Composition and Analysis, 66*, 160–167. https://doi.org/10.1016/j.jfca.2017.12.01

Coman, V., & Vodnar, D. C. (2020). Gut microbiota and old age: Modulating factors and interventions for healthy longevity. *Experimental Gerontology, 141*(111095), 1–18. https://doi.org/10.1016/j.exger.2020.111095

da Cruz Rodrigues, V. C., Duque, A. L. R. F., de Carvalho Fino, L., Simabuco, F. M., Sartoratto, A., Cabral, L., … Antunes, A. E. C. (2020). Modulation of the intestinal microbiota and the metabolites produced by the administration of ice cream and a dietary supplement containing the same probiotics. *British Journal of Nutrition, 124*, 57–68. https://doi.org/10.1017/S0007114520000896

da Silva Padilha, C. V., Miskinis, G. A., de Souza, M. E. A. O., Pereira, G. E., de Oliveira, D., Bordignon-Luiz, M. T., & dos Santos Lima, M. (2017). Rapid determination of flavonoids and phenolic acids in grape juices and wines by RP-HPLC/DAD: Method validation and characterization of commercial products of the new Brazilian varieties of grape. *Food Chemistry, 228*, 106–115. https://doi.org/10.1016/j.foodchem.2017.01.137

de Medeiros, V. P. B., Pimentel, T. C., Varandas, R. C. R., Dos Santos, S. A., de Souza Pedrosa, G. T., da Costa Sassi, C. F., … Magnani, M. (2020). Exploiting the use of agro-industrial residues from fruit and vegetables as alternative microalgae culture medium. *Food Research International, 137*, 1–11. https://doi.org/10.1016/j.foodres.2020.109722

de Medeiros, V. P. B., de Souza, E. L., de Albuquerque, T. M. R., da Costa Sassi, C. F., dos Santos Lima, M., Sivieri, K., … Magnani, M. (2021). Freshwater microalgae biomasses exert a prebiotic effect on human colonic microbiota. *Algal Research, 60*, Article 102547. https://doi.org/10.1016/j.algal.2021.102547

de Melo, F. H. C., Menezes, F. N. D. D., de Sousa, J. M. B., dos Santos Lima, M., Borges, G. D. S. C., de Souza, E. L., & Magnani, M. (2020). Prebiotic activity of monofloral honeys produced by stingless bees in the semi-arid region of Brazilian Northeastern toward Lactobacillus acidophilus LA-05 and Bifidobacterium lactis BB-12. *Food Research International, 128*, Article 108809. https://doi.org/10.1016/j.foodres.2019.108809

Dei-Cas, I., Giliberto, F., Luce, L., Dopazo, H., & Penas-Steinhardt, A. (2020). Metagenomic analysis of gut microbiota in non-treated plaque psoriasis patients stratified by disease severity: Development of a new Psoriasis-Microbiome Index. *Scientific Report, 10*(12754), 1–11. https://doi.org/10.1038/s41598-020-69537-3

Duque, A. L. R. F., Monteiro, M., Adorno, M. A. T., Sakamoto, I. K., & Sivieri, K. (2016). An exploratory study on the influence of orange juice on gut microbiota using a dynamic colonic model. *Food Research International, 84*, 160–169. https://doi.org/10.1016/j.foodres.2016.03.028

Fagen, J. R., Giongo, A., Brown, C. T., Davis-Richardson, A. G., Gano, A. K., & Triplett, E. W. (2012). Characterization of the relative abundance of the citrus pathogen Ca. *Liberibacter asiaticus* in the microbiome of its insect vector, *Diaphorina citri*, using high throughput 16s rRNA sequencing. *Open Microbiology Journal, 6*, 29–33. https://doi.org/10.2174/1874285801206010029

FDA (2011). Food and Drug Administration. 2011. *Spirulina platensis* GRAS self affirmation 7-1-2011. Original Submission, 477, 1–126.

Fierer, N., Lauber, C. L., Zhou, N., McDonald, D., Costello, E., & Knight, K. R. (2010). Forensic identification using skin bacterial communities. *Proceedings of the National Academy of Sciences, 107*, 6477–6481. https://doi.org/10.1073/pnas.1000162107

Giongo, A., Davis-Richardson, A., Crabb, D. B., & Triplett. (2010). TaxCollector: Modifying current 16S rRNA databases for the rapid classification at six taxonomic levels. *Diversity, 2*(7), 1015–1025. https://doi.org/10.3390/d2071015

Guergoletto, K. B., Costabile, A., Flores, G., Garcia, S., & Gibson, G. R. (2016). In vitro fermentation of juçara pulp (*Euterpe edulis*) by human colonic microbiota. *Food Chemistry, 196*, 251–258. https://doi.org/10.1016/j.foodchem.2015.09.048

Hanchi, H., Mottawea, W., Sebei, K., & Hammami, R. (2018). The genus *Enterococcus*: Between probiotic potential and safety concerns–an update. *Frontiers in Microbiology, 1791*, 1–16. https://doi.org/10.3389/fmicb.2018.01791

Herigstad, B., Hamilton, M., & Heersink, J. (2001). How to optimize the drop plate method for enumerating bacteria. *Journal of Microbiological Methods, 44*(2), 121–129. https://doi.org/10.1016/S0167-7012(00)00241-4

Hong, S. H., Bunge, J., Jeon, S. O., & Epstein, S. S. (2006). Predicting microbial species richness. *Proceedings of the National Academy of Sciences of the United States of America, 103*(1), 117–122. https://doi.org/10.1073/pnas.0507245102

Hoving, L. R., Heijink, M., van Harmelen, V., van Dijk, K. W., & Giera, M. (2018). GC-MS analysis of short-chain fatty acids in feces, cecum content, and blood samples. *Clinical Metabolomics, 1730*, 247–256. https://doi.org/10.1007/978-1-4939-7592-1_17

Hu, J., Li, Y., Pakpour, S., Wang, S., Pan, Z., Liu, J., … Zhang, R. X. (2019). Dose effects of orally administered *Spirulina* suspension on colonic microbiota in healthy mice. *Frontiers in Cellular and Infection Microbiology, 9*, 1–13. https://doi.org/10.3389/fcimb.2019.00243

Huang, H., Liao, D., Pu, R., & Cui, Y. (2018). Quantifying the effects of spirulina supplementation on plasma lipid and glucose concentrations, body weight, and blood pressure. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy, 11*(729), 729–742. https://doi.org/10.2147/DMSO.S185672

Hyrslova, I., Krausova, G., Smolova, J., Stankova, B., Branyik, T., Malinska, H., … Curda, L. (2021). Prebiotic and immunomodulatory properties of the microalga chlorella vulgaris and its synergistic triglyceride-lowering effect with bifidobacteria. *Fermentation, 7*, 125. https://doi.org/10.3390/fermentation7030125

Ji, X. G., Chang, K. L., Chen, M., Zhu, L. L., Osman, A., Yin, H., & Zhao, L. M. (2021). In vitro fermentation of chitooligosaccharides and their effects on human fecal microbial community structure and metabolites. *LWT- Food Science and Technology, 144*(111224), 1–11. https://doi.org/10.1016/j.lwt.2021.111224

Jin, J. B., Cha, J. W., Shin, I. S., Jeon, J. Y., Cha, K. H., & Pan, C. H. (2020). Supplementation with *Chlorella vulgaris*, *Chlorella protothecoides*, and Schizochytrium sp. increases propionate-producing bacteria in vitro human gut fermentation. *Journal of the Science of Food and Agriculture, 100*, 2938–2945. https://doi.org/10.1002/jsfa.10321

Käferböck, A., Smetana, S., de Vos, R., Schwarz, C., Toepfl, S., & Parniakov, O. (2020). Sustainable extraction of valuable components from Spirulina assisted by pulsed electric fields technology. *Algal Research, 48*, 1–7. https://doi.org/10.1016/j.algal.2020.101914

Kim, M., & Benayoun, B. A. (2020). The microbiome: An emerging key player in aging and longevit. *Translational Medicine of Aging, 4*, 103–116. https://doi.org/10.1016/j.tma.2020.07.004

Kriss, M., Hazleton, K. Z., Nusbacher, N. M., Martin, C. G., & Lozupone, C. A. (2018). Low diversity gut microbiota dysbiosis: Drivers, functional implications and recovery. *Current Opinion in Microbiology, 44*, 34–40. https://doi.org/10.1016/j.mib.2018.07.003

Lafarga, T., Fernández-Sevilla, J. M., González-López, C., & Acién-Fernández, F. G. (2020). Spirulina for the food and functional food industries. *Food Research International, 137*(109356), 1–10. https://doi.org/10.1016/j.foodres.2020.109356

Leal, B. E. S., Prado, M. R., Grzybowski, A., Tiboni, M., Koop, H. S., Scremin, L. B., … Fontana, J. D. (2017). Potential prebiotic oligosaccharides from aqueous thermopressurized phosphoric acid hydrolysates of microalgae used in treatment of gaseous steakhouse waste. *Algal Research, 24*, 138–147. https://doi.org/10.1016/j.algal.2017.03.020

Lee, S. Y., Kang, H. J., Kang, J. H., Cho, M. G., Jang, H. W., Kim, B. K., & Hur, S. J. (2021). Differences in the gut microbiota between young and elderly persons in Korea. *Nutrition Research, 87*, 31–40. https://doi.org/10.1016/j.nutres.2020.12.013

Li, E., Yang, H., Zou, Y., Wang, H., Hu, T., Li, Q., & Liao, S. (2019). In-vitro digestion by simulated gastrointestinal juices of *Lactobacillus rhamnosus* cultured with mulberry oligosaccharides and subsequent fermentation with human fecal inocula. *LWT- Food Science and Technology, 101*(133), 61–68. https://doi.org/10.1016/j.lwt.2018.11.029

Liu, H., Zhang, H., Wang, X., Yu, X., Hu, C., & Zhang, X. (2018). The family Coriobacteriaceae is a potential contributor to the beneficial effects of Roux-en-Y gastric bypass on type 2 diabetes. *Surgery for Obesity and Related Diseases, 14*, 584–593. https://doi.org/10.1016/j.soard.2018.01.012

Maynard, C., & Weinkove, D. (2018). The Gut microbiota and ageing. *Biochemistry and Cell Biology of Ageing: Part I Biomedical Science, 351–371*. https://doi.org/10.1007/978-981-13-2835-0_12

McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PloS One, 8*, Article e61217. https://doi.org/10.1371/journal.pone.0061217

Menezes, F. N. D. D., de Melo, F. H. C., Vieira, A. R. S., Almeida, T. C., Lima, M. S., Aquino, J. S., … de Souza, E. L. (2020). Acerola (*Malpighia glabra* L.) and guava (*Psidium guayaba* L.) industrial processing by-products stimulate probiotic Lactobacillus and Bifidobacterium growth and induce beneficial changes in colonic microbiota. *Journal of Applied Microbiology, 130*, 1323–1336. https://doi.org/10.1111/jam.14824

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., … Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food-an international consensus. *Food and Function, 5*(6), 1113–1124. https://doi.org/10.1039/c3fo60702j

Molly, K., Woestyne, M. V., Smet, I. D., & Verstraete, W. (1994). Validation of the simulator of the human intestinal microbial ecosystem (SHIME) reactor using microorganism-associated activities. *Microbial Ecology in Health and Disease, 7*(4), 191–200. https://doi.org/10.3109/08910609409141354

Nami, Y., Bakhshayesh, R. V., Jalaly, H. M., Lotfi, H., Eslami, S., & Hejazi, M. A. (2019). Probiotic properties of *Enterococcus* isolated from artisanal dairy products. *Frontiers in Microbiology, 10*(300), 1–13. https://doi.org/10.3389/fmicb.2019.00300

Nakashima, A., Sasaki, K., Sasaki, D., Yasuda, K., Suzuki, K., & Kondo, A. (2021). The alga Euglena gracilis stimulates Faecalibacterium in the gut and contributes to increased defecation. *Scientific Reports, 11*, 1–8. https://doi.org/10.1038/s41598-020-80306-0

Patel, P., Jethani, H., Radha, C., Vijayendra, S. V. N., Mudliar, S. N., Sarada, R., & Chauhan, V. S. (2019). Development of a carotenoid enriched probiotic yogurt from fresh biomass of Spirulina and its characterization. *Journal of Food Science and Technology, 56*(8), 3721–3731. https://doi.org/10.1007/s13197-019-03844-0

Pi, Y., Hu, J., Bai, Y., Wang, Z., Wu, Y., Ye, H., … Wang, J. (2021). Effects of dietary fibers with different physicochemical properties on fermentation kinetics and microbial composition by fecal inoculum from lactating sows in vitro. *Journal of the Science of Food and Agriculture, 101*(3), 907–917. https://doi.org/10.1002/jsfa.10698

Pickard, J. M., Zeng, M. Y., Caruso, R., & Núñez, G. (2017). Gut Microbiota: Role in pathogen colonization, immune responses and inflammatory disease. *Immunological Reviews, 279*(1), 70–89. https://doi.org/10.1111/imr.12567

Possemiers, S., Marzorati, M., Verstraete, W., & Van de Wiele, T. (2010). Bacteria and chocolate: A successful combination for probiotic delivery. *International Journal of Food Microbiology, 141*(1–2), 97–103. https://doi.org/10.1016/j.ijfoodmicro.2010.03.008

Possemiers, S., Verthe, K., Uyttendaele, S., & Verstraete, W. (2004). PCR-DGGE based quantification of stability of the microbial community in a simulator of the human intestinal microbial ecosystem. *FEMS Microbiology Ecology, 49*(3), 495–507. https://doi.org/10.1016/j.femsec.2004.05.002

Machado Ribeiro, T. R., Salgaço, M. K., Adorno, M., da Silva, M. A., Piazza, R., Sivieri, K., & Moreira, C. G. (2021). Human microbiota modulation via QseC sensor kinase mediated in the Escherichia coli O104:H4 outbreak strain infection in microbiome model. *BMC Microbiology, 21*(1), 163. https://doi.org/10.1186/s12866-021-02220-3

Roswall, J., Olsson, L. M., Kovatcheva-Datchary, P., Nilsson, S., Tremaroli, V., Simon, M. C., … Bäckhed, F. (2021). Developmental trajectory of the healthy human gut microbiota during the first 5 years of life. *Cell Host and Microbe, 29*(5), 765–776. e3. https://doi.org/10.1016/j.chom.2021.02.021

Roselino, M. N., Sakamoto, I. K., Tallarico Adorno, M. A., Márcia Canaan, J. M., de Valdez, G. F., Rossi, E. A., … Umbelino Cavallini, D. C. (2020). Effect of fermented sausages with probiotic Enterococcus faecium CRL 183 on gut microbiota using dynamic colonic model. *LWT- Food Science and Technology, 132*, 1–8. https://doi.org/10.1016/j.lwt.2020.109876

R Core Team. (2018). *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. https://www.R-project.org.

Salgaço, M. K., Perina, N. P., Tomé, T. M., Mosquera, E. M. B., Lazarini, T., Sartoratto, A., & Sivieri, K. (2021). Probiotic infant cereal improves children's gut microbiota: Insights using the Simulator of Human Intestinal Microbial Ecosystem (SHIME®). *Food Research International, 143*, Article 110292. https://doi.org/10.1016/j.foodres.2021.110292

Savy, N., Brossier, D., Brunel-Guitton, C., Ducharme-Crevier, L., Du Pont-Thibodeau, G., & Jouvet, P. (2018). Acute pediatric hyperammonemia: Current diagnosis and management strategies. *Hepatic Medicine: Evidence and Research, 10*, 105–115. https://doi.org/10.2147/hmer.s140711

Scott, K. P., Jean-Michel, A., Midtvedt, T., & van Hemert, S. (2015). Manipulating the gut microbiota to maintain health and treat disease. *Microbial Ecology in Health and Disease, 26*(1), 1–10. https://doi.org/10.3402/mehd.v26.25877

Sharma, M., Wasan, A., & Sharma, R. K. (2021). Recent developments in probiotics: An emphasis on *Bifidobacterium*. *Food Bioscience, 41*, 1–10. https://doi.org/10.1016/j.fbio.2021.100993

Song, Q., Wang, Y., Huang, L., Shen, M., Yu, Y., Yu, Q., … Xie, J. (2021). Review of the relationships among polysaccharides, gut microbiota, and human health. *Food Research International, 140*, 1–10. https://doi.org/10.1016/j.foodres.2020.109858

Van den Abbeele, P., Duysburgh, C., Vazquez, E., Chow, J., Buck, R., & Marzorati, M. (2019). 2′ -Fucosyllactose alters the composition and activity of gut microbiota from formula-fed infants receiving complementary feeding in a validated intestinal model. *Journal of Functional Foods, 61*(103484), 1–8. https://doi.org/10.1016/j.jff.2019.103484

Van de Wiele, T., Van den Abbeele, P., Ossieur, W., Possemiers, S., & Marzorati, M. (2015). The simulator of the human intestinal microbial ecosystem (SHIME®). *The Impact of Food Bioactives on Health, 305–317*. https://doi.org/10.1007/978-3-319-16104-4_27

Venuri, R., Shinde, T., Gundamaraju, R., Gondalia, S. V., Karpe, A. V., Beale, D. J., … Eri, R. (2018). *Lactobacillus acidophilus* DDS-1 modulates the gut microbiota and improves metabolic profiles in aging mice. *Nutrients, 10*(9), 1–19. https://doi.org/10.3390/nu10091255

Wang, Y., & Qian, P. Y. (2009). Conservative fragments in bacterial 16S rRNA genes and primer design for 16S ribosomal DNA amplicons in metagenomic studies. *PLoS ONE, 4*(10), 1–9. https://doi.org/10.1371/journal.pone.0007401

Wei, T., & Simko, V. (2021). *R package "corrplot": Visualization of a correlation matrix*. https://github.com/taiyun/corrplot.

Yao, Y., Yan, L., Chen, H., Wu, N., Wang, W., & Wang, D. (2020). Cyclocarya paliurus polysaccharides alleviate type 2 diabetic symptoms by modulating gut microbiota and short-chain fatty acids. *Phytomedicine, 77*(153268), 1–14. https://doi.org/10.1016/j.phymed.2020.153268

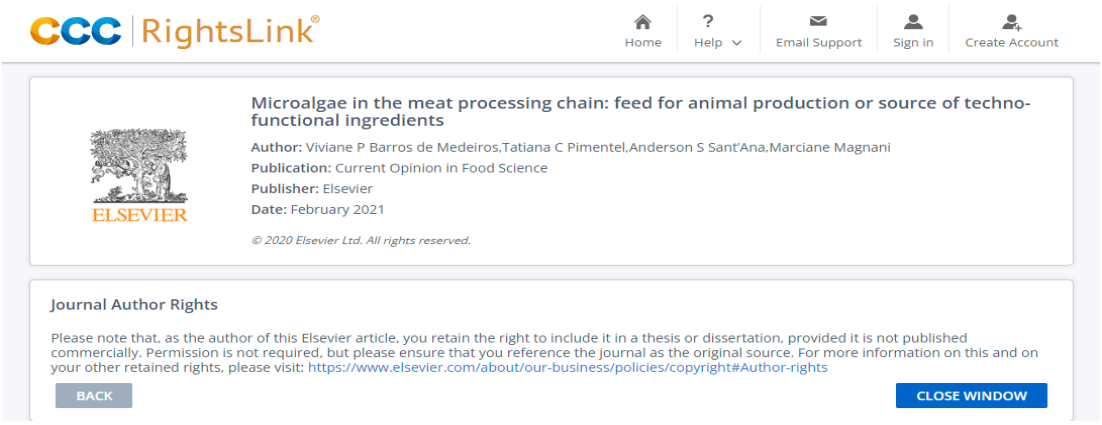
Zhao, W., Liu, Y., Kwok, L. Y., Cai, T., & Zhang, W. (2020). The immune regulatory role of *Lactobacillus acidophilus*: An updated meta-analysis of randomized controlled trials. *Food Bioscience, 36*(100656), 1–6. https://doi.org/10.1016/j.fbio.2020.100656

Zhang, S., Zeng, B., Chen, Y., Yang, M., Kong, F., Wei, L., … Li, Y. (2021). Gut microbiota in healthy and unhealthy long-living people. *Gene, 779*(145510), 1–9. https://doi.org/10.1016/j.gene.2021.145510

APÊNDICE C – ARTIGO III

Microalgae in the meat processing chain: feed for animal production or source of techno-functional ingredients.

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Microalgae in the meat processing chain: feed for animal production or source of techno-functional ingredients

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Microalgae have been recognized as natural, sustainable, and economically feasible resources of non-conventional feed ingredients able to promote benefits to animal growth and improvements in the meat quality. At the same time, microalgae biomass or their isolated compounds can be directly incorporated as techno-functional ingredients in a range of meat-processed products. This review provides the latest updates on the use of microalgae biomass as a source of nutrients in animal feed describing the main effects on the nutritional, technological, and sensory quality of the meat. Besides, relevant aspects of the direct incorporation of microalgae biomass or isolated compounds in meat-derived products are discussed, concerning changes in the nutritional composition, sensory characteristics, and technological properties. Finally, challenges and perspectives are presented.

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Introduction

Because of the increase in the world population, it has been estimated that by 2050 there will be an increase in the demand for animal origin products, particularly due to the growing consumption of proteins [1]. If the global trend of meat consumption continues, the searching for animal proteins will double, which may lead to an over-exploitation and consequently limitation of conventional sources widely used in livestock and aquatic animal feed such as corn, rice, soybeans, fish meal, among others [2,3].

Microalgae are promising candidates to overcome this challenge because of their high-quality nutritional composition [4]. Microalgae constitute a heterogeneous group of unicellular or multicellular, prokaryotic or eukaryotic photosynthetic organisms, which inhabit marine and freshwater environments [5]. Cultivation of microalgae for biomass production presents several advantages when compared to agriculture because it can be performed in small spaces and over the year without being affected by seasonality. In addition, it does not dispute the land with agricultural activities or require the use of pesticides as in conventional agriculture, and it has a higher yield compared to traditional crops [4,6•].

During growth, microalgae accumulate compounds of interest for feed or foods such as polysaccharides, protein, essential amino acids, lipids and polyunsaturated fatty acids (PUFAs), minerals, vitamins, phenolics and other antioxidants pigments [5,7]. Once the microalgae biomass is included in animal feed these compounds comply with the energy-protein needs for animal growth with satisfactory performance. In parallel to the positive impacts on the nutritional, technological and sensory quality of meat, microalgae biomass improves the feed digestibility and enhance the animal immunological response [8,9••,10]. Among the wide range of microalgae genera exploited in the feed for animal production, *Tetraselmis* sp., *Chlorella* sp., *Spirulina* sp. (*Arthrospira platensis*), *Nannochloropsis* sp., *Schizochytrium* sp., *Phaeodactylum* sp., *Scenedesmus* sp., and *Tisochrysis lutea* stand out [11].

Otherwise, microalgae have a variety of techno-functional ingredients in their composition that can be used to replace synthetic additives in meat products. The incorporation of whole microalgae biomass or isolated compounds has positive impact in a range meat-processed products including sausages [12••], burgers [13], pork liver pâté and turkey patties [14••], beef patties [15], and chicken rotti [16]. Although various microalgae have potential as natural sources of added-value food ingredients, researchers have exploited primarily *Chlorella* sp. and *Spirulina* sp.

This contribution aims to present a brief overview on the use of microalgae in the meat processing chain, covering relevant aspects of the use of whole biomass or isolated compounds in animal feed and its effects on the meat

quality, as well as the impact of their incorporation as ingredients in meat-processed products.

Microalgae in animal feed: relevant aspects and impacts in the meat quality

Microalgae has been inserted in animal feed mainly through incorporation of the whole microalgae biomass as dried powder, high moisture extruded biomass or microalgae extracts [17,18••,19]. Furthermore, docosahexaenoic acid (DHA) isolated from microalgae have been used [8,9••,20]. Table 1 shows the main effects of incorporation of microalgae biomass or derived fatty acids in the feed of ruminants and non-ruminants.

Marine microalgae are high-producers of n-3-PUFAs, and the accumulation of PUFAs varies among the microalgae phyla, being the highest proportion observed in *Chlorophyta* and *Cryptophyta* (up to 60% of dried whole biomass corresponds to fatty acids) [21]. Fan *et al.* [18••] investigated the effects of the incorporation of 3% (w/w) *Schizochytrium* sp. whole biomass in a high-energy diet (corn, wheat bran, and soybean meal) on the lipid metabolism of lambs under intensive feeding for 60 days. It was observed increases in the DHA and eicosapentanenoic (EPA) contents in the animal muscle and liver tissues, as well as modulation of the metabolic alterations caused by the high-energy diet through expression of genes involved with the lipid metabolism. Therefore, a reduction of body fat, cholesterol, and oleic acid in the muscle and liver was observed. The authors concluded that the supplementation of the high-energy diet with microalgae biomass increased the n-3PUFA and improved the metabolic disorders associated to lipid metabolism. Allen *et al.* [22] also reported increases in PUFA and proportions of n-3/n-6 in shrimps after consumption of *Schizochytrium* sp. whole biomass.

Microalgae species are also used as protein resources for replacing conventional protein or supplements in animal feed. In poultry diets, the dietary substitution (50% (w/w)) of soy protein by *Arthrospira platensis* (*Spirulina*) biomass (dried power) did not impact in the carcass performance parameters (live weight, carcass weight, breast filet yield, protein, intramuscular fat and moisture), but increased post-mortem pH and the water-holding capacity during storage and cooking. Moreover, it led to more tender and softer meat and improved quality aspects including decrease of metallic flavor and hardness. However, it impacted in the color of the products, with meats with redder, darker, and more yellow color, possibly due to the high content of carotenoids present in the microalgae biomass [23]. In other study, the microalgae biomass utilization (*Arthrospira platensis* (*Spirulina*)) was associated to a higher umami and chicken flavor in the meats [24]. Otherwise, the dietary supplementation of broilers with *Chlorella* sp. (1.0% (w/w)) did not change the pH, water holding capacity, and color of the poultry meat in comparison to basal diets (soybean and corn meal) but improved the growth, immune response and apparently metabolizable nitrogen in the blood [25]. These evidences alert for specific features of microalgae species; therefore, it is important to evaluate the impact of the specie used.

In pigs, the substitution of soy protein (50–75%) by *A. platensis* (*Spirulina*) biomass (dried power) increased PUFAs content (α-linolenic acid and γ-linolenic acid) and improved the sensory aroma of the meat. However, an increased astringent aftertaste was observed [26]. In fish, the replacement of fish meal by raw or enzyme-treated alga (*Spirulina platensis*, 10, 20 or 40% 9 (w/w)) was studied. It was suggested the utilization of up to 20% of raw alga or enzyme-treated alga, with no impact on the growth performance (weight gain, feed conversion rate) and health status (intestinal fold height, height of microvillus, and muscular wall thickness) of the animals [19]. Moreover, the replacement of fishmeal protein (15, 30 and 45%) by *T. lutea* and *T. suecica* biomass had no impact on the growth parameters and health status of fish and led to a higher nutritional value meat [3].

The supplementation of the diet of animals with microalgae biomass has also been studied. Dietary supplementation of basal weaning pigs diet with 0.5 and 1.0% (w/w) *Schizochytrium* JB5 biomass led to an improvement of the immune system (increased lymphocyte counts) and increased the digestibility of dry matter and nitrogen content (up to 44%) compared to animals that received only the control diet using soybean meal. The microalgae supplementation did not negatively influence the growth or increased cholesterol or triglycerides levels [10]. Gong and Huang [27] reported that up to 10% of *Scenedesmus* sp. biomass could be incorporated in fishmeal feeds of Atlantic salmon, while higher concentrations (20%) impacted negatively on weight gain, specific growth rate, thermal growth coefficient, feed conversion rate, condition factor, protein efficiency ratio, and digestibility (dry matter and protein). Roohani *et al.* [28] reported that 8% (w/w) was the most recommended concentration of *Spirulina platensis* to be used (compared to 0, 2, 4 and 6% (w/w)) in Caspian brown trout feed, resulting in higher nutritional value (higher contents of lipid and protein), improved fatty acid profile (higher PUFA and total n-3), and increased color parameters in the meats. Liang *et al.* [29] reported that 3% (w/w) was the most recommended concentration of *Spirulina* sp. to be used (compared to 0, 1, and 2 (w/w)) in sheep feed, resulting in reduced body fat, serum cholesterol, triacylglycerol and oxidative stress, and enhanced antioxidant capacity in the meats. Furthermore, an improvement in immunity-related parameters, including increased IgG concentration in serum and red and white blood cell counts was observed. These evidences alert for the importance of the biomass concentration used.

Table 1

Main effects of incorporation of microalgae biomass or derived fatty acids in the feed of ruminants and non-ruminants

Animals	Microalgae	Amount used (w/w)	Main effects	References
Sheep			Increase of DHA and EPA contents in the animal muscle and liver tissues	
	Schizochytrium sp. (biomass)	3%	Modulation of the metabolic alterations caused by the high-energy diet through expression of genes involved with the lipid metabolism (reduction of body fat, cholesterol and oleic acid in the muscle and liver).	[18••]
	Schizochytrium sp. (DHA)	2%	Modification of the fatty acid composition in intramuscular, subcutaneous and kidney knob and channel fat. Intramuscular fat was the most affected deposit. Intramuscular fat had higher levels of DHA and total n3 fatty acids Greater proportion of DHA and total n3 in *M. Infraspinatus* (IM) compared with *M. Longissimus* and *M. Psoas major*	[8]
	Spirulina sp. (biomass)	0, 1, 2 and 3%	3% supplementation reduced body fat, serum cholesterol, triacylglycerol and oxidative stress and enhanced antioxidant capacity Improvement in immunity-related parameters, including increased IgG concentration in serum and red and white blood cell counts.	[29]
Fish			The utilization of enzyme-treated alga did not affect the weight gain and feed conversion rate, while 40% of raw alga reduced these parameters. The apparent digestibility coefficients of protein and lipid were not affected up to 20% addition but decreased at higher concentrations. A reduction in intestinal fold height, height of microvillus, and muscular wall thickness was also observed for the highest concentration. Therefore, it was suggested the utilization of up to 20% of raw alga or enzyme-treated alga, with no impact on the growth performance and health status of the animals	
	S. platensis (biomass – raw and enzyme-treated)	0, 20 and 40%		[19]
	Scenedesmus sp. (extruded biomass)	0, 10, and 20%	Higher concentrations resulted in lower weight gain, specific growth rate, thermal growth coefficient, feed conversion rate, condition factor, protein efficiency ratio Lower digestibility of dry matter, protein, and energy Hepatosomatic and viscerosomatic indices were not impacted Increase the total n-3 and PUFA content in salmon at 10% concentration	[27]
	T. lutea and *T. suecica* (biomass)	15, 30 or 45% replacement of fishmeal protein	No effect on growth performance and feed conversion efficiency A slight decline in dry matter, protein and energy digestibility, which was compensated by increased feed intake. Higher nutritive value. No changes in biometry traits and slaughter yield while the nutritional properties of the edible muscle tissue were little affected in terms of n-3 PUFA. Greenish pigmentation of the skin, with a slight tendency towards redness and diminished lightness and hue.	[3]
	Nannochloropsis sp. (biomass)	2.5, 5, 7.5 and 10%	No effect on weight gain, specific growth rate, protein efficiency ratio, viscerosomatic index and hepatosomatic index Higher essential amino acids content Increases in pepsin, trypsin, and lipase activities, total superoxide dismutase and glutathione peroxidase activities and total antioxidant capacity of serum and liver	[39]
	S. platensis (biomass)	0, 2, 4, 6, and 8 %	6 and 8% addition diets had a significantly higher weight gain rate, specific growth rate, protein efficiency, lipid efficiency. Lower feed conversion ratio 8% addition resulted in higher protein and lipid content, higher SFAs, C20:3n-6, C18:3n-3, PUFAs and total n-3 fatty acid and increases color parameters.	[28]
Shrimp	*Schizochytrium* sp., (biomass)	50, 62 and 75 g/kg	No effect on growth and feeding efficiencies Decreased moisture and ash contents Increased amounts of LC-PUFA and proportions of n-3/n-6	[22]

Table 1 (*Continued*)

Animals	Microalgae	Amount used (w/w)	Main effects	References
Pigs				
	A. platensis (biomass)	50, 75 and 100 %	Maintenance of physical-chemical parameters of meat and increase of PUFAs in meat. More intense aroma, but higher astringent aftertaste. Diet did not influence growth performances of pigs or the technological quality of the meat	[26]
	Schizochytrium sp. (DHA)	100% of feed	The n-3 PUFA content in the longissimus dorsi muscle, subcutaneous backfat and liver increased The amount of malondialdehyde increased	[9**]
	Schizochytrium sp. (biomass)	0.5–1.0%	Higher concentrations resulted in no effect on growth performance, total cholesterol, white blood cell, and triglycerides. Higher lymphocytes were observed. The apparent total tract digestibility and ileal nutrient digestibility of protein and dry matter were improved	[10]
Rabbit				
	N. oceanica (biomass)	4.45%	No effect on growth performance, feed:gain ratio, average daily gain, cooking loss, carcass yield, perirenal fat weight, crude protein of leg meat, and digestibility Higher abundance of proteins with antioxidative activity (TXNRD1, HPX, involved in cellular iron homeostasis and heme detoxification) and LDHA, yields pyruvate.	[40]
Poultry				
	A. platensis (biomass)	50%	Maintaining animal performance and improving meat quality. Meat quality improved by 50% substitution of the soy protein by *Spirulina* (higher pH values, lower storage and cooking loses, lower metallic taste) Dark reddish-yellowish meat color. Tender and softer meats. Lower metallic taste. Higher umami taste and chicken flavor.	[23,24]
	Arthrospira platensis (biomass)	0.25, 0.5, 0.75% and 1%	Increase in the serum enzyme activity of superoxide dismutase and glutathione peroxidase, apparent total tract digestibility of dry matter and nitrogen, cecal *Lactobacillus* Decrease in excreta ammonia gas emission No significant effects on relative organ weight and breast meat quality of broilers	[41]
	Chlorella vulgaris, Spirulina platensis and Amphora coffeaformis (biomass)	1 g/kg	*A. coffeaformis* and *C. vulgaris* increased final body weight and body weight gain *A. coffeaformis* and *Spirulina platensis* increased the level of essential fatty and amino acids and decreased the microbial growth in breast muscle All studied microalgae reduced malondialdehyde and protein carbonyl levels, cooking loss, and aerobic plate count and increased the SOD activity in breast muscle The current study indicated that studied microalgae, notably *A. coffeaformis*, can be used to enhance performance and meat quality in broilers chickens.	[30]
Steers				
	Aurantiochytrium limacinum (DHA)	100 g/animal daily	No change in growth or carcass characteristic. Improved G:F ratio, increased days on feed, and increased CLA, n-3 fatty acids, and the n-3:n-6 ratio in beef. Animals secreted less insulin after a glucose infusion, resulting in less glucose clearance from the blood.	[20]

DHA: docosahexaenoic acid; EPA: eicosapentaenoico acid; IgG: G immunoglobulin; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids; LCPUFAs: long chain polyunsaturated fatty acids;TXNRD1: glutaredoxin domain-containing protein; HPX: hemopexin; LDHA: L-lactate dehydrogenase; SOD: superoxide dismutase; G:F: gain:feed; CLA: conjugated linoleic acid.

The microalgae species also has an important impact when used as supplement. El-Bahr *et al.* [30] supplemented the poultry diet (1 g/kg) with *Chlorella vulgaris* (CV), *S. platensis* (SP), and *Amphora coffeaformis* (AC) and observed that the studied microalgae, notably AC, could be used to enhance performance (higher final body weight and body weight gain) and meat quality (increased essential fatty and amino acids, lower malondialdehyde and protein carbonyl, lower cooking loss, lower microbial counts).

Residual microalgae biomass resulting from biofuel extraction could also be a viable source of crude protein for ruminant's feed. According to Morril *et al.* [31] the incorporation of 9% (w/w) *Chlorella* sp. residual biomass (derived from oil extraction) in cattle finishing diets did not affect the juiciness, flavor, and tenderness of the meat. However, there were changes in the composition of fatty acids (increased C14:0, C16:0, C:16:1n-7, and C20:2n-6 concentrations and decreased C18:1n-9 concentration).

The incorporation of DHA from *Schizochytrium* sp. in the diet of pigs results in an improvement of the nutritional value and fatty acid composition of the meat, with increase of long chain n-3 PUFA. However, an increase in the malondialdehyde (indicator of fatty acid peroxidation) was observed, with consequent development of abnormal flavor (similar to organ meat or fish) in the products [9••]. Furthermore, the use of DHA from *Aurantiochytrium limacinum* (100 g/animal day) did not change the growth and carcass characteristics in steers, while promoted increase of conjugated linoleic acid, n-3 fatty acids, and the n-3/n-6 fatty acids ratio in beef. However, after the 21 days of aging, the meat became unstable to the lipid oxidation process [20]. Therefore, the supplementation of the diet with microalgae DHA improves the fatty acid profile of the meat, but the susceptibility to oxidation should be evaluated, as it can compromise the technological, shelf life, and sensory properties of the products.

The fatty acid profile and contents accumulated vary with the animal tissue compartment [9••]. Lambs receiving basal diet (*ad libitum*) supplemented with 2% (20 g/Kg) of DHA from *Schizochytrium* sp. showed different composition and quantities of fatty acids in fat deposits (intramuscular, subcutaneous, kidney knob channel fat) and muscle (longissimus, infraspinatus, psoas major) [8]. Overall, dietary management of lambs with *Schizochytrium* DHA causes positive impacts in the nutritional quality of the muscle leading to improvement of the PUFAs/saturated fatty acids (SFAs) and n6/ n3 ratios, increased levels of DHA and total n-3 fatty acids. Similarly, pigs fed n-3 PUFA rich diets (soybean-palm diet, dehulled and extruded linseed diet; 75% dehulled and extruded linseed +25% of *Schizochytrium* DHA and 50% linseed +50% *Schizochytrium* DHA) showed distinct fatty acid composition in adipose tissues and muscles. Pigs fed with higher levels of microalgae DHA in the diet showed higher contents of n-3 PUFAs (ECA, EPA and DHA) in the neck, ham, and internal ham subcutaneous adipose tissue. In contrast, as the level of microalgae in the diet increased monounsaturated fatty acid contents decreased in all fat tissues [9••].

In view of the presented results, the use of whole biomass or isolated fatty acid from species of microalgae in animal feed represents an important route for the production of meat with improved nutritional quality and healthier lipid profile. In addition, the inclusion of microalgae in animal feed fulfill the environmental sustainability, since these microorganisms have rapid growth and their cultivation does not require the use of arable land, thus reaching the multifaceted concept of meat quality.

Microalgae as a source of techno-functional ingredients for incorporation in meat products

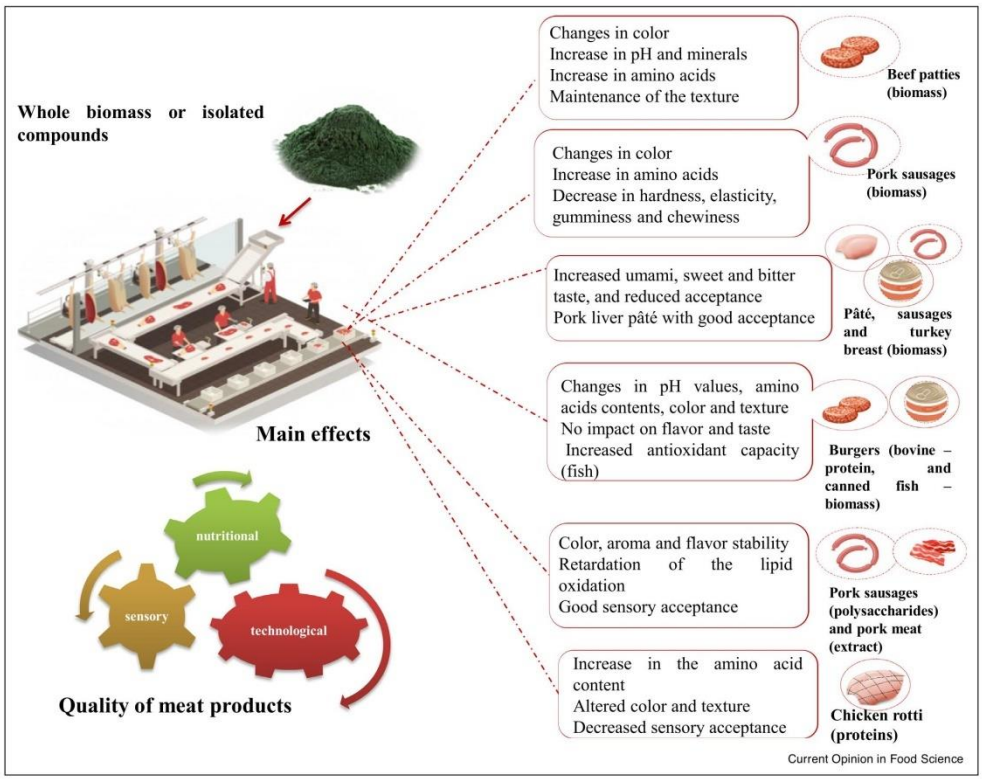
Faced to the consumer awareness of the influence of the diet on health, the meat industry has undergone continuous technological adaptations to develop healthier and/or functional meat products. The re-formulation of meat products through reduction, substitution or removal of fat and salt, as well as supplementation and/or insertion of bioactive compounds add value to meat-based products.

Microalgae are recognized resources of polysaccharides, proteins, peptides and essential amino acids, PUFA, phycobiliproteins, chlorophylls, carotenoids, vitamins, minerals and dietary fiber [32•,33•]. The techno-functional properties of these compounds guide the use of microalgae biomass or isolated compounds to improve the nutritional and technological quality of meats products, without compromising the sensory attributes. Figure 1 and Table 2 summarize the main effects of the incorporation of microalgae (biomass or isolated compounds) in distinct meat products. Most of the studies that assessed the direct incorporation of microalgae biomass or derived compounds in meat products have tested *Spirulina* sp. and *Chlorella* sp., as they are considered safe for human consumption (GRAS status by the U.S. Food and Drug Administration and validated by the World Health Organization), being well-accepted by consumers as health-promoting foods.

The replacement of soy protein as an emulsifying agent by 1% (w/w) *Chlorella* sp. and 1% (w/w) *Spirulina* sp. biomass in beef patties did not affect the chemical composition (moisture, lipids and protein), cooking loss, hardness, adhesiveness, elasticity, cohesiveness, gumminess, chewiness, and taste profile (umami, tasteless, sweet, and bitter), and increased the amino acid contents (total, essential, and non-essential). However, it promoted changes in color by decreasing lightness (L*), redness (a*), and yellowness (b*). Beef patties formulated with *Chlorella* had increased pH and decreased ashes [15]. In fresh pork sausages, the replacement of soy protein by 1% (w/w) *Spirulina* sp. or 1% (w/w) *Chlorella* sp. biomass did not affect the proximal composition, water holding capacity, adhesiveness, and cohesiveness. However, the color parameters were altered (lower L*, a* and b* values), and decreases in hardness, gumminess, elasticity, and chewiness were reported. Overall, microalgae biomass increased the total amino acid content and the ratio of essential/non-essential in the pork sausages [12••]. Therefore, the microalgae biomass could be candidates for the manufacture of new meat products, improving the amino acid profile. The effect on the texture parameters may be related to the meat product evaluated.

The improvement in the amino acid profile can impact the sensory characteristics of meat products. Zamuz *et al.* [14••] evaluated the effects of the *Chlorella* sp. and *Spirulina* sp. biomass addition on the sensory properties of pork liver pâté, turkey patties, cooked turkey breast, fresh pork sausages, and marinated turkey breast (1% (w/w), except

Figure 1



Technological effects of the utilization of microalgae biomass or isolated compounds in meat products.

in pork liver pâté which was 5% (w/w)). The microalgae addition increased the amino acid content, which was associated to increases in the umami, sweet, and bitter tastes. Consequently, a reduction in the sensory acceptance of the products was observed. Among the evaluated meat products added with microalgae biomass, only pork liver pâté had good acceptance.

Biomass of *Chlorella minutissima*, *Isochrysis galbana*, and *Picochlorum* sp. was used (0.5, 1.0, 1.5% (w/w)) in canned fish burgers and assessed for nutritional value, texture, and sensory attributes. The microalgae biomass did not change the odor but led to darker burgers (decreased a* and b* values) in a concentration-dependent way. Fish burgers added 0.5% of *C. minutissima*, 1% of *I. galbana*, and 1% of *Picochlorum* sp. presented better texture and sensory attributes than the other formulations. Burgers showed enhanced swelling ability and improved water and oil holding capacity, which may be due to the dietary fibers present in the microalgae composition. The increased antioxidant activity of formulated hamburgers was attributed to the presence of pigments (chlorophylls, carotenoids, and phycocyanin) [34].

The utilization of the whole microalgae biomass may confer undesired color, aroma, and flavor characteristics to meat formulations; thus depending upon the product, the use of isolated compounds may be more interesting. In this view, isolated proteins of *Chlorella* sp. and *Spirulina* sp. 1% (w/w) were added in turkey burgers formulations to replace soy protein. The additions resulted in reduction in the color parameters (L*, a* and b*), pH values, protein content,

Table 2

Main effects of incorporation of microalgae biomass or isolated compounds in food products

Food product	Microalgae	Form and amount used (w/w)	Main effects	References
Beef patties	*Chlorella* sp.	Biomass	No effect on the chemical composition (moisture, lipid, and protein), cooking loss, hardness, adhesiveness, elasticity, cohesiveness, gumminess, chewiness, and taste profile (umami, tasteless, sweet, and bitter)	
		1% (w/w)	Increased amino acid content (total, essential, and non-essential)	[15]
	Spirulina sp.	Replacement for soy protein (emulsifying agent)	Changes in color by decreasing lightness (L*), redness (a*) and yellowness (b*) *Chlorella* increased pH and decreased ash content	
Fresh pork sausages	*Chlorella* sp.	Biomass	No effect on proximal composition, water holding capacity, adhesiveness, and cohesiveness	
		1% (w/w)	Color parameters were altered (lower L*, a* and b* values), and decreases in hardness, elasticity, gumminess, and chewiness	[12••]
	Spirulina sp.	Replacement for soy protein (emulsifying agent)	Increases in the total amino acid content and the ratio of essential/non-essential	
Pork liver pâté, turkey patties, cooked turkey breast, fresh pork sausages, and marinated turkey breast	*Chlorella* sp.	Biomass	Increase in the amino acid content, and umami, sweet, and bitter tastes Reduction in the sensory acceptance	
	Spirulina sp.	1% (w/w), except pork liver pâté (5% w/w)	Only pork liver pâté had good acceptance	[14••]
Canned fish burgers	*Chlorella minutissima* *Isochrysis galbana*	Biomass	No changes in odor Darker burgers (decreased a* and b* values) in a concentration-dependent way	
	Picochlorum sp.	0.5, 1.0, 1.5% (w/w)	Enhanced swelling ability, improved water and oil holding capacity, and increased antioxidant activity Fish burgers added 0.5% of *C. minutissima*, 1% of *I. galbana* and 1% of *Picochlorum* sp. presented better texture and sensory attributes than the other formulations	[34]
Turkey burgers	*Chlorella* sp.,	Isolated protein	Reduction in the color parameters (L*, a* and b*), pH values, protein content, adhesiveness, and elasticity	
	Spirulina sp.	1% (w/w)	No impact on the flavor and taste characteristics, moisture, lipids and ash contents, water holding capacity, gumminess, hardness, cohesiveness, and chewiness Products with *Spirulina* sp. presented the highest total amino acid content and ratio between essential and non-essential amino acids	[13]
Turkey breasts	*Chlorella* sp.,	Isolated protein	Higher pH values and ratio between essential and non-essential amino acids Lower moisture content No impact on lipid and protein contents, and adhesiveness	
	Spirulina sp.	1% (w/w)	Altered color parameters (decreases in L*, a* and b* values) and texture (lower hardness, elasticity, cohesiveness, gumminess, and chewiness) Amino acids profile was improved with *Spirulina*	[35]
Chicken rotti	*Chlorella* sp.,	Isolated protein	Increase in the total amino acid contents and improvement in the essential/non-essential amino acids ratio Increase in the pH values Altered the color (lower L*, a* and b* values) and decreased hardness, elasticity, cohesiveness, gumminess, and chewiness No effect on the chemical composition (moisture, proteins, and ash), and adhesiveness Negative impact on the sensory acceptance	
	Spirulina sp.	1% (w/w)		[16]

Table 2 (*Continued*)

Food product	Microalgae	Form and amount used (w/w)	Main effects	References
Chinese-style pork sausage	*Spirulina platensis*	Polysaccharide 0.1, 0.25, and 0.5% (w/w)	0.5% addition caused greater stability in the red color of sausages Higher antioxidant activity No effect on the microbiological pattern Maintained aroma and flavor during refrigerated storage, decreased lipid peroxidation, and was sensorially accepted.	[36]
Raw ground pork meat	*Haematoccocus pluvialis*	Extract 0.15, 0.3 or 0.45 g/kg	0.3 and 0.45 g/kg of the extract delayed the lipid oxidation and improved the color stability of the meat, making it sensorially accepted by the tasters No antimicrobial or antioxidative activity against protein (myoglobin)	[37]
Rainbow trout	*S. platensis C. vulgaris*	Alcoholic extract 0.1% (w/w)	Increased antioxidant activity, slowed the lipid oxidation process, maintained sensory attributes, and increased the life span	[38]

adhesiveness, and elasticity of the products, without impact on the flavor and taste characteristics. The products with *Spirulina* sp. presented the highest total amino acid content and ratio between essential and non-essential amino acids [13]. Altered color parameters (decreases L*, a* and b* values) and texture (lower hardness, elasticity, cohesiveness, gumminess, and chewiness) were also observed in turkey breasts after replacement of soy by *Spirulina* sp. and *Chlorella* proteins [35]. *Spirulina* sp. and *Chlorella* sp. 1% (w/w) isolated proteins were also incorporated for replacement of soy protein in chicken rotti. The microalgae protein of both microalgae promoted an increase in the total amino acid contents and improved the essential/non-essential amino acids ratio. However, the incorporation affected the color and texture parameters decreasing hardness, elasticity, gumminess, and chewiness, as well as negatively impacted the sensory acceptance [16]. Therefore, the impact of protein from microalgae addition is dependent on the meat product.

Microalgae are source of pigments and other bioactive compounds with antioxidant activity. In this context, Luo *et al.* [36] examined the effect of the incorporation of at 0.1%, 0.25%, and 0.5% (w/w) of the polysaccharides from *S. platensis* (SPP) on lipid peroxidation, microbiological and sensory properties of Chinese-style pork sausage during 24 days of stored at 4°C. The results obtained report that the addition of 0.5% PSP caused greater stability in the red color of sausages, although it did not change the microbiological pattern. In addition, it avoided the reduction of aroma and flavor during the period of refrigerated storage, decreased lipid peroxidation, which was attributed to the polysaccharides antioxidant activity and was sensorially accepted.

The study conducted by Pogorzelska [37] investigated the antioxidant effect and microbiological stability of raw ground pork meat added with extracts of *Haematoccocus pluvialis* rich in astaxanthin in different concentrations (0.15, 0.3 or 0.45 g/kg of meat) during cold storage. The data obtained revealed that the DPPH elimination activity was dose dependent. The application of 0.3 and 0.45 g/kg of the extract delayed the lipid oxidation and improved the color stability of the meat, making it sensorially accepted by the tasters. Despite this, the extract of *H. pluvialis* had no antimicrobial or antioxidative activity against protein (myoglobin). These results corroborate with the recent study developed by Takyar *et al.* [38] were the addition of 0.1% alcoholic extract of *S. platensis* and *C. vulgaris* in rainbow trout (*Oncorhynchus mykiss*) fillet promoted increased antioxidant activity, slowed the lipid oxidation process, maintained sensory attributes, and increased the life span of chilled fish.

Despite the advances obtained over the years, it is necessary to deepen the knowledge of the functional, physicochemical, preservative and sensory properties of whole biomass and its bioactive compounds, in order to reveal the range of possible applications to be inserted in the processing chain. Still, the development of innovative strategies for incorporating microalgae as a source of bioactive compounds in meat products is necessary to reduce any undesirable changes in the formulated products.

Conclusion and future perspectives

Several species of microalgae have been used as animal feed natural resource due to the variety of nutrients, bioactive compounds, and as a way to mitigate the environmental and economic challenges of the livestock agriculture sector, resulting in meats with superior quality. Furthermore, the advance of scientific research has revealed their potential for application in the meat and meat products processing chain. To date, a limited

number of species have been explored as techno-functional ingredients in meat products. From recently published results, it can be seen that the success obtained in the nutritional and technological parameters resulting from the addition of microalgae in animal feed and in the reformulation of meat products reinforces their applicability in their whole form or in isolated bioactive compounds and suggests as a new trend to be adopted by the food industry. However, given the existing microalgal diversity and potential, more species should be explored in order to formulate healthy meat products and fill in the existing gaps.

Credit author statement

VPBM, ASS, and MM participated in the conceptualization of the study. VPBM and MM performed bibliographic survey. VPBM, TCP, and MM wrote the original draft of the manuscript. MM, TCP, and ASS participated in the supervision, review, editing, and visualization of research.

Conflict of interest statement

Nothing declared.

Acknowledgements

The authors would like to thank 'Coordenação de Aperfeiçoamento de Pessoal de Nível Superior' (CAPES-Brazil; finance code 001) and 'Fundação de Apoio à pesquisa do Estado da Paraíba' (FAPESQ-Brazil). M. Magnani thanks to CNPq (Grants #405644/2018-3 and 303437/2017-0). A.S. Sant'Ana thanks to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Grants #302763/2014-7 and #305804/2017-0). A.S. Sant'Ana is member of the HealthyMeat network, funded by CYTED (ref. 119RT0568).

References and recommended reading

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

- • of special interest
- •• of outstanding interest

1. FAO: *Meat*. 2020:162-173.

2. Madeira MS, Cardoso C, Lopes PA, Coelho D, Afonso C, Bandarra NM, Prates JAM: **Microalgae as feed ingredients for livestock production and meat quality: a review**. *Livest Sci* 2017, **205**:111-121.

3. Cardinaletti G, Messina M, Bruno M, Tulli F, Poli BM, Giorgi G, Chini-Zittelli G, Tredici M, Tibaldi E: **Effects of graded levels of a blend of *Tisochrysis lutea* and *Tetraselmis suecica* dried biomass on growth and muscle tissue composition of European sea bass (*Dicentrarchus labrax*) fed diets low in fish meal and oil**. *Aquaculture* 2018, **485**:173-182.

4. Wild KJ, Trautmann A, Katzenmeyer M, Steingaß H, Posten C, Rodehutscord M: **Chemical composition and nutritional characteristics for ruminants of the microalgae *Chlorella vulgaris* obtained using different cultivation conditions**. *Algal Res* 2019, **38**:101385.

5. Bhuvana P, Sangeetha P, Anuradha V, Ali MS: **Spectral characterization of bioactive compounds from microalgae: *N. oculata* and *C. vulgaris***. *Biocatal Agric Biotechnol* 2019, **19**:101094.

6. • Koyande AK, Chew KW, Rambabu K, Tao Y, Chu DT, Show PL: **Microalgae: a potential alternative to health supplementation for humans**. *Food Sci Hum Wellness* 2019, **8**:16-24.

This review summarizes the utilization of microalgae as supplements in the human nutrition, describing the various microalgae sources of proteins and other bioactive components.

7. Santhakumaran P, Ayyappan SM, Ray JG: **Nutraceutical applications of twenty-five species of rapid-growing green-microalgae as indicated by their antibacterial, antioxidant and mineral content**. *Algal Res* 2020, **47**:101878.

8. Díaz MT, Pérez C, Sánchez CI, Lauzurica S, Cañeque V, González C, De La Fuente J: **Feeding microalgae increases omega 3 fatty acids of fat deposits and muscles in light lambs**. *J Food Compos Anal* 2017, **56**:115-123.

9. •• de Tonnac A, Guillevic M, Mourot J: **Fatty acid composition of several muscles and adipose tissues of pigs fed n-3 PUFA rich diets**. *Meat Sci* 2018, **140**:1-8.

This study demonstrates that the fatty acid profile and contents accumulated vary with the animal tissue compartment.

10. Kibria S, Kim IH: **Impacts of dietary microalgae (*Schizochytrium* JB5) on growth performance, blood profiles, apparent total tract digestibility, and ileal nutrient digestibility in weaning pigs**. *J Sci Food Agric* 2019, **99**:6084-6088.

11. Gong Y, Huang J: **Characterization of four untapped microalgae for the production of lipids and carotenoids**. *Algal Res* 2020, **49**:101897.

12. •• Marti-Quijal FJ, Zamuz S, Tomašević I, Gómez B, Rocchetti G, Lucini L, Remize F, Barba FJ, Lorenzo JM: **Influence of different sources of vegetable, whey and microalgae proteins on the physicochemical properties and amino acid profile of fresh pork sausages**. *LWT Food Sci Technol* 2019, **110**:316-323.

This study demonstrates the influence of protein from microalgae on the quality properties of fresh pork sausages. It demonstrated that microalgae provided nutritionally favorable profiles, thus suggesting that seaweeds could be used to enrich pork sausages, as an alternative to soy protein.

13. Marti-Quijal FJ, Zamuz S, Tomašević I, Rocchetti G, Lucini L, Marszalek K, Barba FJ, Lorenzo JM: **A chemometric approach to evaluate the impact of pulses, *Chlorella* and *Spirulina* on proximate composition, amino acid, and physicochemical properties of turkey burgers**. *J Sci Food Agric* 2019, **99**:3672-3680.

14. •• Zamuz S, Purriños L, Galvez F, Zdolec N, Muchenje V, Barba FJ, Lorenzo JM: **Influence of the addition of different origin sources of protein on meat products sensory acceptance**. *J Food Process Preserv* 2019, **43**:1-12.

This study demonstrates the impact of the utilization of microalgae biomass on the sensory properties of different types of meat products.

15. Zugcic T, Abdelkebir R, Barba FJ *et al.*: **Effects of pulses and microalgal proteins on quality traits of beef patties**. *J Food Sci Technol* 2018, **55**:4544-4553.

16. Parniakov O, Toepfl S, Barba FJ, Granato D, Zamuz S, Galvez F, Lorenzo JM: **Impact of the soy protein replacement by legumes and algae based proteins on the quality of chicken rotti**. *J Food Sci Technol* 2018, **55**:2552-2559.

17. Smetana S, Sandmann M, Rohn S, Pleissner D, Heinz V: **Autotrophic and heterotrophic microalgae and cyanobacteria cultivation for food and feed: life cycle assessment**. *Bioresour Technol* 2017, **245**:162-170.

18. •• Fan Y, Ren C, Meng F, Deng K, Zhang G, Wang F: **Effects of algae supplementation in high-energy dietary on fatty acid composition and the expression of genes involved in lipid metabolism in Hu sheep managed under intensive finishing system**. *Meat Sci* 2019, **157**:107872.

This study demonstrated that the supplementation of the high-energy diet with microalgae biomass increased the n-3PUFA and improved the metabolic disorders in sheep.

19. Van Vo B, Siddik MAB, Fotedar R, Chaklader MR, Hanif MA, Foysal MJ, Nguyen HQ: **Progressive replacement of fishmeal by raw and enzyme-treated alga, *Spirulina platensis* influences growth, intestinal micromorphology and stress response in juvenile barramundi, Lates calcarifer**. *Aquaculture* 2020, **529**:735741.

20. Carvalho JRR, Brennan KM, Ladeira MM, Schoonmaker JP: **Performance, insulin sensitivity, carcass characteristics, and fatty acid profile of beef from steers fed microalgae**. *J Anim Sci* 2018, **96**:3433-3445.

21. Jónasdóttir SH: **Fatty acid profiles and production in marine phytoplankton**. *Mar Drugs* 2019, **17**.

22. Allen KM, Habte-Tsion HM, Thompson KR, Filer K, Tidwell JH, Kumar V: **Freshwater microalgae (*Schizochytrium* sp.) as a substitute to fish oil for shrimp feed**. *Sci Rep* 2019, **9**:1-10.

23. Altmann BA, Neumann C, Velten S, Liebert F, Mörlein D: **Meat quality derived from high inclusion of a micro-alga or insect meal as an alternative protein source in poultry diets: a pilot study**. *Foods* 2018, **7**.

24. Altmann BA, Wigger R, Ciulu M, Mörlein D: **The effect of insect or microalga alternative protein feeds on broiler meat quality**. *J Sci Food Agric* 2020, **100**:4292-4302.

25. Choi H, Jung SK, Kim JS, Kim KW, Oh KB, Lee PY, Byun SJ: **Effects of dietary recombinant *Chlorella* supplementation on growth performance, meat quality, blood characteristics, excreta microflora, and nutrient digestibility in broilers**. *Poult Sci* 2017, **96**:710-716.

26. Altmann BA, Neumann C, Rothstein S, Liebert F, Mörlein D: **Do dietary soy alternatives lead to pork quality improvements or drawbacks? A look into micro-alga and insect protein in swine diets**. *Meat Sci* 2019, **153**:26-34.

27. Gong Y, Bandara T, Huntley M, Johnson ZI, Dias J, Dahle D, Sørensen M, Kiron V: **Microalgae *Scenedesmus* sp. as a potential ingredient in low fishmeal diets for Atlantic salmon (*Salmo salar* L.)**. *Aquaculture* 2019, **501**:455-464.

28. Roohani AM, Abedian Kenari A, Fallahi Kapoorchali M, Borani MS, Zoriezahra SJ, Smiley AH, Esmaeili M, Rombenso AN: **Effect of spirulina *Spirulina platensis* as a complementary ingredient to reduce dietary fish meal on the growth performance, whole-body composition, fatty acid and amino acid profiles, and pigmentation of Caspian brown trout (*Salmo trutta* caspius) juvenil**. *Aquacult Nutr* 2019, **25**:633-645.

29. Liang Y, Bao Y, Gao X, Deng K, An S, Wang Z, Huang X, Liu D, Liu Z, Wang F *et al.*: **Effects of spirulina supplementation on lipid metabolism disorder, oxidative stress caused by high-energy dietary in Hu sheep**. *Meat Sci* 2020, **164**:108094.

30. El-Bahr S, Shousha S, Shehab A, Khattab W, Ahmed-Farid O, Sabike I, El-Garhy O, Albokhadaim I, Albosadah K: **Effect of dietary microalgae on growth performance, profiles of amino and fatty acids, antioxidant status, and meat quality of broiler chickens**. *Animals* 2020, **10**:1-14.

31. Morrill JC, Sawyer JE, Smith SB, Miller RK, Johnson MD, Wickersham TA: **Post-extraction algal residue in beef steer finishing diets: II. Beef flavor, fatty acid composition, and tenderness**. *Algal Res* 2017, **25**:578-583.

32. • Gullón B, Gagaoua M, Barba FJ, Gullón P, Zhang W, Lorenzo JM: **Seaweeds as promising resource of bioactive compounds: overview of novel extraction strategies and design of tailored meat products**. *Trends Food Sci Technol* 2020, **100**:1-18.
This review summarizes the recent technologies used to obtain and isolate biocompounds from seaweeds and their use in the development of meat foods with health properties and lower concentrations in unhealthy components.

33. • Gullón P, Astray G, Gullón B, Franco D, Campagnol PCB, Lorenzo JM: **Inclusion of seaweeds as healthy approach to formulate new low-salt meat products**. *Curr Opin Food Sci* 2021, **40**:20-25.
This review summarizes the use of seaweeds as salt substitutes in meat products demonstrating the effects on sensorial and physicochemical properties, microbiological stability, and shelf-life.

34. Ben Atitallah A, Barkallah M, Hentati F, Dammak M, Ben Hlima H, Fendri I, Attia H, Michaud P, Abdelkafi S: **Physicochemical, textural, antioxidant and sensory characteristics of microalgae-fortified canned fish burgers prepared from minced flesh of common barbel (*Barbus barbus*)**. *Food Biosci* 2019, **30**:100417.

35. Marti-Quijal FJ, Zamuz S, Galvez F, Roohinejad S, Tiwari BK, Gómez B, Barba FJ, Lorenzo JM: **Replacement of soy protein with other legumes or algae in turkey breast formulation: changes in physicochemical and technological properties**. *J Food Process Preserv* 2018, **42**:1-9.

36. Luo A, Feng J, Hu B, Lv J, Chen CYO, Xie S: **Polysaccharides in *Spirulina platensis* improve antioxidant capacity of Chinese-style sausage**. *J Food Sci* 2017, **82**:2591-2597.

37. Pogorzelska E, Godziszewska J, Brodowska M, Wierzbicka A: **Antioxidant potential of *Haematococcus pluvialis* extract rich in astaxanthin on colour and oxidative stability of raw ground pork meat during refrigerated storage**. *Meat Sci* 2018, **135**:54-61.

38. Taghavi Takyar MB, Haghighat Khajavi S, Safari R: **Evaluation of antioxidant properties of *Chlorella vulgaris* and *Spirulina platensis* and their application in order to extend the shelf life of rainbow trout (*Oncorhynchus mykiss*) fillets during refrigerated storage**. *LWT Food Sci Technol* 2019, **100**:244-249.

39. Qiao H, Hu D, Ma J, Wang X, Wu H, Wang J: **Feeding effects of the microalga *Nannochloropsis* sp. on juvenile turbot (*Scophthalmus maximus* L.)**. *Algal Res* 2019, **41**.

40. Ribeiro DM, Bandarrinha J, Nanni P, Alves SP, Martins CF, Bessa RJB, Falcão-e-Cunha L, Almeida AM: **The effect of *Nannochloropsis oceanica* feed inclusion on rabbit muscle proteome**. *J Proteomics* 2020, **222**:103783.

41. Park JH, Lee SI, Kim IH: **Effect of dietary *Spirulina (Arthrospira) platensis* on the growth performance, antioxidant enzyme activity, nutrient digestibility, cecal microflora, excreta noxious gas emission, and breast meat quality of broiler chickens**. *Poult Sci* 2018, **97**:2451-2459.

ANEXOS

ANEXO A – CERTIDÃO DO COMITÊ DE ÉTICAEM PESQUISA

CENTRO DE CIÊNCIAS DA SAÚDE DA UNIVERSIDADE FEDERAL DA PARAÍBA - CCS/UFPB

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: AVALIAÇÃO DO POTENCIAL PREBIÓTICO DE MICROALGAS DULCÍCOLAS ISOLADAS DA REGIÃO NORDESTE EM SISTEMAS DE FERMENTAÇÃO IN VITRO

Pesquisador: VIVIANE PRISCILA BARROS DE MEDEIROS

Área Temática:

Versão: 1

CAAE: 42495821.8.0000.5188

Instituição Proponente: Centro De Ciências da Saúde

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 4.563.908

Apresentação do Projeto:

Trata-se de avaliar o projeto de Pesquisa em tela, da pesquisadora Viviane Priscila Barros de Medeiros, para o desenvolvimento de sua tese do Programa de Pós-graduação em Ciências da Nutrição do Centro de Ciências da Saúde da Universidade Federal da Paraíba, sob a orientação da Professora Dra. Marciane Magnani.

Objetivo da Pesquisa:

Avaliar o potencial prebiótico de biomassas de microalgas isoladas da região Nordeste do Brasil, empregando sistemas de fermentação in vitro, com utilização de inóculo fecal humano.

Avaliação dos Riscos e Benefícios:

Riscos: os participantes poderão sentir pequeno desconforto ao coletar as fezes para a pesquisa.

Benefício: esta pesquisa fornecerá informação científica relevante e atualmente escassas no campo de estudo.

Comentários e Considerações sobre a Pesquisa:

Após as analises laboratórias, o estudo envolverá 04 voluntários saudáveis que farão exames de fezes, para analises in vitro de sistema de fermentação utilizando Biomassas de microalgas isoladas da região Nordeste do Brasil.

Endereço: UNIVERSITARIO S/N
Bairro: CASTELO BRANCO **CEP:** 58.051-900
UF: PB **Município:** JOAO PESSOA
Telefone: (83)3216-7791 **Fax:** (83)3216-7791 **E-mail:** comitedeetica@ccs.ufpb.br

CENTRO DE CIÊNCIAS DA SAÚDE DA UNIVERSIDADE FEDERAL DA PARAÍBA - CCS/UFPB



Considerações sobre os Termos de apresentação obrigatória:

O projeto em tela se encontra bem instruído de acordo com as normas que regem pesquisas envolvendo seres humanos da Resolução 466/12 do Conselho Nacional de Saúde.

Recomendações:

Recomenda-se manter a metodologia proposta.

Conclusões ou Pendências e Lista de Inadequações:

Sem pendências

Considerações Finais a critério do CEP:

Certifico que o Comitê de Ética em Pesquisa do Centro de Ciências da Saúde da Universidade Federal da Paraíba – CEP/CCS aprovou a execução do referido projeto de pesquisa. Outrossim, informo que a autorização para posterior publicação fica condicionada à submissão do Relatório Final na Plataforma Brasil, via Notificação, para fins de apreciação e aprovação por este egrégio Comitê.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1691727.pdf	25/01/2021 15:56:11		Aceito
Folha de Rosto	Viviane.pdf	25/01/2021 15:40:00	VIVIANE PRISCILA BARROS DE MEDEIROS	Aceito
Cronograma	CRONOGRAMA.pdf	20/01/2021 17:31:15	VIVIANE PRISCILA BARROS DE MEDEIROS	Aceito
Outros	Coleta.pdf	20/01/2021 17:27:24	VIVIANE PRISCILA BARROS DE MEDEIROS	Aceito
Projeto Detalhado / Brochura Investigador	Projeto.pdf	20/01/2021 17:25:02	VIVIANE PRISCILA BARROS DE MEDEIROS	Aceito
Declaração de concordância	Certidao.pdf	20/01/2021 17:22:04	VIVIANE PRISCILA BARROS DE MEDEIROS	Aceito
Declaração de Instituição e	Carta.pdf	20/01/2021 17:20:10	VIVIANE PRISCILA BARROS DE	Aceito

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CENTRO DE CIÊNCIAS DA SAÚDE DA UNIVERSIDADE FEDERAL DA PARAÍBA - CCS/UFPB



Infraestrutura	Carta.pdf	20/01/2021 17:20:10	MEDEIROS	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	20/01/2021 17:12:49	VIVIANE PRISCILA BARROS DE MEDEIROS	Aceito
Declaração de Instituição e Infraestrutura	Anuencia.pdf	20/01/2021 17:10:01	VIVIANE PRISCILA BARROS DE MEDEIROS	Aceito
Orçamento	Doc.docx	20/01/2021 17:03:08	VIVIANE PRISCILA BARROS DE MEDEIROS	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

JOAO PESSOA, 27 de Fevereiro de 2021

Assinado por:
Eliane Marques Duarte de Sousa
(Coordenador(a))

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ANEXO B – TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO USADO NA PESQUISA

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Prezado (a) Senhor (a)

Esta pesquisa é sobre avaliação do efeito prebiótico de microalgas dulcícolas utilizando inóculo fecal humano e está sendo desenvolvida pela pesquisadora Ms Viviane Priscila Barros de Medeiros, aluna regular do Programa de Pós-Graduação em Ciências da Nutrição, da Universidade Federal da Paraíba (UFPB), sob a orientação do Profª. Drª. Marciane Magnani, professora do Programa de Pós-Graduação em Ciências da Nutrição do Departamento de Nutrição (UFPB).

O objetivo do estudo é avaliar o potencial prebiótico de biomassas de microalgas isoladas da região Nordeste do Brasil, empregando sistemas de fermentação *in vitro,* com utilização de inóculo fecal humano. A realização desta pesquisa fornecerá informações científicas relevantes e atualmente escassas no campo de estudo.

A pesquisa apresenta abordagem inovadora considerando os aspectos tecnológicos e econômicos envolvidos. A avaliação do uso de microalgas amplamente disponíveis na região Nordeste e facilmente cultiváveis, como fonte nutricional de fibras alimentares e de compostos bioativos e suas possíveis interações benéficas com probióticos, permitirá conhecer o potencial de microalgas dulcícolas como novos ingredientes funcionais com possíveis aplicações na indústria de alimentos e/ou suplementos. A execução desta proposta representa uma oportunidade para agregar valor às biomassas provenientes de microalgas pertencentes à biodiversidade local.

Em virtude que este TCLE encontra-se em mais de uma página, as demais serão rubricadas pelo pesquisador e sujeito da pesquisa.

Solicitamos a sua colaboração para realizar a doação de material fecal, o qual será colhido no referido dia e armazenado em frasco anaeróbio cedido pelos envolvidos napesquisa, como também sua autorização para apresentar os resultados deste estudo em eventos da área de saúde e, eventualmente, publicação em revista científica. Por ocasião da publicação dos resultados, seu nome será mantido em sigilo. Informamos que essa pesquisa

não oferece riscos, previsíveis, para a sua saúde, uma vez que os resultados obtidos servirão de base para definir os efeitos prebióticos de biomassas microalgais.

Esclarecemos que sua participação no estudo é voluntária e, portanto, o(a) senhor(a) não é obrigado(a) a fornecer as informações e/ou colaborar com as atividades solicitadas pela Pesquisadora. Caso decida não participar do estudo, ou resolver a qualquer momento desistir do mesmo, não sofrerá nenhum dano.

Os pesquisadores estarão a sua disposição para qualquer esclarecimento que considere necessário em qualquer etapa da pesquisa.

Diante do exposto, declaro que fui devidamente esclarecido(a) e dou o meu consentimento para participar da pesquisa e para publicação dos resultados. Estou ciente que receberei uma cópia desse documento.

_____

Assinatura do Participante da Pesquisa

_____

Assinatura da Testemunha

Contato do Pesquisador (a) Responsável: (83) 998928341

Caso necessite de maiores informações sobre o presente estudo, favor ligar para a pesquisadora: Viviane Priscila Barros de Medeiros.

Endereço (Setor de Pesquisa): Laboratório de Processos Microbianos em Alimentos, Departamento de Engenharia de Alimentos do Centro de Tecnologia da Universidade Federal da Paraíba (UFPB), Campus I - Cidade Universitária – CEP 58051-900 – João Pessoa – PB, Telefone: (83) 3216-7576/ (83) 998928341

Comitê de Ética em Pesquisa do Centro de Ciências da Saúde da Universidade Federal da Paraíba Campus I - Cidade Universitária - 1º Andar – CEP 58051-900 – João Pessoa/PB
(83) 3216-7791 – E-mail: **comitedeetica@ccs.ufpb.br**

Atenciosamente,

_____

Assinatura do Pesquisador Responsável

ANEXO C - Sondas e condições de hibridização empregadas na análise de FISH

Tabela 1. Sondas de oligonucleotídeos de 16S rRNA e condições de hibridização usadas em análises de FISH.

Sonda	Grupos bacterianos	Sequências (5'-3')	Pré-tratamento de hibridização	Temperatura de hibridização (^{o}C)/ lavagem	Referências
Bif 164	*Bifidobacterium* spp.	CATCCGGCATTACCACCC	Lisozima	50/50	LANGENDIJIK *et al*. (1995)
Lab 158	*Lactobacillus* spp. e *Enterococcus* spp.	GGTATTAGCAYCTGTTTCCA	Lisozima	50/50	HARMSEM*et al*. (1999)
Bac 303	A maioria das *Prevotellaceae*, *Bacteroidaceae*e algumas *Porphyromonadaceae* (grupo Bacteroides)	CCAATGTGGGGGACCTT	Nenhum	46/48	MANZ*et al*. (1996).
Erec 482	A maior parte do grupo de*Eubacterium rectale*e*Clostridium coccoides* (Clostridium cluster XIVa e XIVb)	GCTTCTTAGTCARGTACCG	Nenhum	50/50	FRANKS *et al*. (1998).
Chis150	A maioria do grupo *Clostridium histolyticum*(Clostridium clusters I e II)	TTATGCGGTATTAATCTYCCTTT	Nenhum	50-50	FRANKS *et al*. (1998)