Interreg Austria – Czechia
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UTILIZATION OF MICROALGAE IN BAKERY PRODUCTS

Study of the Current State of Knowledge and Market Research

This study was conducted as part of the **Bio2AgroFood** project – Utilization of Biotechnology in the Agricultural and Food Sector within the **Interreg Austria – Czech Republic** program.

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1. SUMMARY

This study comprehensively explores the integration of microalgae into bakery products, focusing on nutritional, technological, legislative, commercial and research aspects. Microalgae, such as *Spirulina*, *Chlorella*, and *Dunaliella*, have gained traction in the food industry due to their rich protein content, essential fatty acids, vitamins, and bioactive compounds. Their incorporation into baked goods can enhance nutritional value, texture, and shelf stability, making them attractive for the functional food market.

The study identifies key challenges, including taste, color changes, and sensory acceptability, which influence consumer perception. To address these, methods such as ethanol treatment, fermentation, and encapsulation have been explored to improve sensory characteristics while maintaining nutritional integrity. Furthermore, this study reviews patents and ongoing research projects, highlighting innovations in microalgae-based bakery applications and market trends.

Finally, the study offers recommendations for future research and development, focusing on optimizing formulation techniques, assessing bioavailability, and evaluating prebiotic effects. As microalgae continue to gain recognition as a sustainable and functional food source, their role in bakery products is expected to expand, contributing to the advancement of health-conscious and environmentally friendly food solutions.

2. INTRODUCTION

Microalgae have emerged as a valuable ingredient in the food industry due to their high nutritional content, sustainability, and versatility. Rich in proteins, essential fatty acids, vitamins, antioxidants, and bioactive compounds, microalgae offer potential benefits for functional food applications. In the bakery sector, their incorporation has been explored to enhance the nutritional profile, texture, and shelf life of various products. Given the increasing demand for plant-based, protein-rich, and functional foods, microalgae represent a promising alternative to conventional ingredients.

The integration of microalgae into bakery products presents both opportunities and challenges. While their high protein and omega-3 fatty acid content supports enhanced nutritional properties, concerns such as color changes, taste alterations, and textural modifications require further research and technological advancements. This study aims to analyze the current state of knowledge and market trends regarding microalgae in bakery products, highlighting scientific advancements, patents, and commercial applications....xxx…

2.1 OBJECTIVE OF THE STUDY

The aim of this study is to analyze the current state of knowledge and market trends in the use of microalgae in bakery products. Microalgae represent an innovative ingredient with potentially significant benefits for the food industry, particularly due to their high content of proteins, essential fatty acids, vitamins, and antioxidants. The study will focus on scientific findings, patent research, and existing market products to identify the main reasons for adding microalgae to baked goods, the nutritional and functional benefits they provide, and their technological contributions to bakery manufacturing.

The research will also explore the specific properties of microalgae relevant to baking, such as their influence on the sensory properties of products (taste, color, texture) and their potential application in gluten-free baked goods. An important aspect will be evaluating their potential prebiotic effects, meaning their ability to support gut microbiota health, which is highly relevant for functional foods. Special attention will be given to the most commonly used microalgae species in the food sector, such as *Spirulina platensis*, *Chlorella vulgaris*, and *Dunaliella salina*, as well as other species and their role in modern food trends, including organic and plant-based nutrition.

Based on these findings, the study will provide a systematic overview of current research and development, analyze European research projects, relevant patents, and market segments that incorporate microalgae in bakery applications. Finally, recommendations will be formulated for further applied research and possible directions for developing new bakery products with microalgae. This study will thus serve as a comprehensive foundation for strategic decision-making on the use of microalgae in this segment of the food industry.

2.2 METHODS

This study focuses on a systematic analysis of available scientific knowledge, patent databases, and market trends related to the use of microalgae in bakery products. To gather relevant information, it utilizes scientific publications, patent databases (e.g., Espacenet, WIPO, Google Patents), market reports, and analyses focused on functional foods and innovations in the bakery industry. Emphasis is placed on identifying the key bioactive components of microalgae, their nutritional benefits, and technological properties, which can improve the quality of bakery products.

The first step is a literature review focusing on scientific articles and review studies published in peer-reviewed journals and relevant professional sources. This section maps the current state of research, particularly the reasons for adding microalgae to baked goods and the benefits they provide in terms of nutrition, sensory properties, and dough functionality. Special attention is given to gluten-free bakery products and the potential prebiotic properties of microalgae.

Additionally, the study conducts a patent analysis, providing an overview of innovations in the field of bakery products enriched with microalgae. It identifies key patents related to their use in food production and evaluates their significance for potential commercial applications. Simultaneously, it examines the market to determine which microalgae-based products are already available, which companies are active in this area, and what the main trends are in the commercial utilization of microalgae in the bakery sector.

The collected information is systematically processed and synthesized into a structured overview, allowing for the formulation of conclusions about the current state of knowledge and the identification of areas with potential for further research and innovation. The study concludes with recommendations for the application of microalgae in the bakery industry and future research opportunities, which may contribute to the further development of this sector.

3. USE OF MICROALGAE IN BAKERY PRODUCTS

3.1 HISTORY AND TREND IN USE OF MICROALGAE IN BAKERY PRODUCTS

The use of microalgae in food has a long history, dating back over 2000 years when the Chinese consumed Nostoc during famines and the Aztecs harvested Spirulina from Lake Texcoco. Modern scientific interest began post-WWII due to global food shortages, with institutions like the Carnegie Institution and Stanford University studying its feasibility. In the 1960s, Dr. Hiroshi Nakamura and Christopher Hills pioneered Spirulina cultivation, recognizing its high protein content and potential against malnutrition.

Production has evolved significantly: in the 1960s-70s, research focused on food security, but large-scale cultivation remained challenging. By the 1980s-90s, improved techniques led to increased production and a growing market for health supplements. The 2000s saw microalgae incorporated into food products like beverages and snacks. In the 2010s-20s, sustainability and plant-based diets boosted their prominence, with innovations in biorefinery approaches expanding applications to biofuels and bioplastics.

Microalgae are primarily used in three categories: nutraceuticals, such as Spirulina and Chlorella dietary supplements; natural pigments, like chlorophylls, carotenoids, and phycobiliproteins used in food colorants; and functional foods, where microalgae enhance nutritional profiles.

In the food industry, algae are primarily used as raw materials (note: the term “raw material” is not officially recognized in legislation) or as dietary supplements. Microalgae are an important source of proteins, essential amino acids, lipids—including unsaturated fatty acids (primarily EPA and DHA)—bioactive polysaccharides, vitamins, and carotenoid pigments with antioxidant properties.

The most commonly used species are Spirulina, Chlorella, Dunaliella, and Haematococcus.

3.1.1 Spirulina

The annual production of Spirulina is approximately 15,000 tons. This microalga is a significant source of proteins (up to 70% of dry weight biomass) and also a source of the blue pigment phycocyanin.

Phycocyanin is not considered a colorant under European legislation according to Regulation (EC) No. 1333/2008 on food additives, and therefore, it does not have an E-number designation. The aqueous extract of phycocyanin is derived from Spirulina, which itself is a food product. Since it does not undergo selective extraction and retains some properties of the original food, it is classified as a "coloring foodstuff," similar to beet juice.

For comparison, beta-carotene extracted from the alga *Dunaliella* is classified as a food additive and colorant and has an E-number (E160a - carotenes). The estimated annual production of phycocyanin is around 200 tons.

The following Spirulina species are considered traditional foods and are not subject to Novel Food regulations:

Arthrospira platensis
Limnospira fusiformis
Limnospira indica
Limnospira maxima

Spirulina major

More on the issue of Spirulina and coloring foods:

(Bratinova, 2015)
(https://publications.jrc.ec.europa.eu/repository/bitstream/JRC96974/final%20report_colouring%20food.pdf)

Guidance notes on the classification of food extracts with colouring properties (EU) - https://english.nvwa.nl/binaries/nvwa-en/documenten/consumers/food/safety/documents/guidance-notes-on-the-classification-of-food-extracts-with-colouring-properties-eu/leidraad-europese-commissie-criteria-extract-kleurstof-of-kleurend-ingredi%C3%ABnt-29-november-2013.pdf

Colouring foods, also known as colouring foodstuffs, are food ingredients used by the food industry primarily to deliver colour to food and beverage products. They are obtained from foods such as fruits, vegetables, flowers, spices, algae and/or other edible source materials. The difference with food colour additives is that there is no selective extraction.

Colours, as defined in Regulation (EC) No 1333/2008 on food additives, are “substances which add or restore colour in a food, and include natural constituents of foods and natural sources which are normally not consumed as foods as such and not normally used as characteristic ingredients of food. Preparations obtained from foods and other edible natural source materials obtained by physical and/or chemical extraction resulting in a selective extraction of the pigments relative to the nutritive or aromatic constituents are colours within the meaning of this Regulation”

As an example, the pigment Anthocyanins E163 is a purple natural colour additive extracted from elderberry, whereas elderberry juice concentrate, which does not undergo selective pigment extraction and can be consumed as such, is an example of a colouring food.

https://www.cbi.eu/market-information/natural-food-additives/natural-food-colours/market-entry

3.1.2 Chlorella

The industrial-scale production of Chlorella began in the early 1960s in Japan and Taiwan. Today, Chlorella is cultivated using autotrophic, heterotrophic, and mixotrophic methods. The estimated annual production of Chlorella is around 5,000 tons, with half of the global production occurring in China.

The original genus Chlorella has been taxonomically split into multiple genera, but the general term “Chlorella” is still commonly used for all of them.

The following Chlorella species are considered traditional foods and are not subject to Novel Food regulations:

Chlorella vulgaris
Chlorella sorokiniana
Auxenochlorella protothecoides
Jaagichlorella luteoviridis

Parachlorella kessleri
Auxenochlorella pyrenoidosa

Amino Acid Contents in Chlorella Biomass from MBÚ Production

		Ch. sorokiniana G-120	Ch. vulgaris R-117
aspartic acid	g/100 g	2,06	4,98
serine	g/100 g	1,04	2,43
glutamic acid	g/100 g	7,83	5,70
glycine	g/100 g	1,30	3,14
histidine	g/100 g	0,55	1,14
arginine	g/100 g	5,72	3,28
threonine	g/100 g	1,36	2,61
alanine	g/100 g	3,01	4,23
proline	g/100 g	1,35	2,50
cystine	g/100 g	0,22	0,59
tyrosine	g/100 g	0,65	2,10
valine	g/100 g	1,38	3,30
methioninee	g/100 g	0,62	1,22
lysine	g/100 g	2,65	3,11
isoleucine	g/100 g	1,01	2,07
leucine	g/100 g	1,83	4,35
fenylalanine	g/100 g	1,03	2,78

3.1.3 Dunaliella

The mass production of Dunaliella began in the 1980s with the goal of obtaining natural beta-carotene, which can make up up to 10% of the microalgal cells. The annual production of Dunaliella is approximately 2,000 tons, with the largest suppliers located in Australia, Israel, and China.

Beta-carotene from Dunaliella is an approved food additive and colorant (E160a) in the food industry.

3.1.4 Haematococcus

This microalga is the largest natural producer of astaxanthin, a carotenoid pigment. In commercial production, astaxanthin concentrations reach 4–5%, and the annual production is estimated at 1,000–2,000 tons, with the largest producers located in Israel and China.

Astaxanthin oleoresin, obtained through CO_2 or ethyl acetate extraction, is an approved Novel Food. However, the biomass of Haematococcus lacustris (syn. Haematococcus pluvialis) is considered a traditional food, but only for use in dietary supplements (see Directive 2002/46/EC).

3.1.5 Other microalgae used in food industry

<u>Aphanizomenon flos-aquae</u> (Not novel in food supplements)

Aphanizomenon flos-aquae (AFA) is a freshwater cyanobacterium valued in the food sector for its high nutritional and bioactive content. It contains 60–70% protein, making it a rich dietary protein source, alongside essential vitamins, minerals, and trace elements. Additionally, AFA is abundant in bioactive compounds like C-phycocyanin and β-phenylethylamine, which offer nutraceutical benefits. While AFA has beneficial properties, it's important to note that certain strains can produce toxins.

Therefore, proper sourcing and testing are crucial to ensure safety when incorporating AFA into food products.

<u>Euglena gracilis</u> (Whole: Novel Food, dried biomass - authorized novel food (Union List))

Euglena gracilis is a microalga recognized for its exceptional nutritional profile, making it valuable in food applications. It contains all 20 essential amino acids, ensuring a complete protein source. Additionally, it is rich in β-glucan, particularly paramylon, which has been linked to immune system enhancement and the removal of undesirable substances like fats and cholesterol.

E. gracilis was approved as a novel food (whole biomass) in 2020, but with exclusive right to Kemin Foods until the end of 2025 (only this company can place E. gracilis on european market, because toxicity tests are patented, so results cannot be used by others, unless they apply for approval with other toxicity tests). https://eur-lex.europa.eu/eli/reg_impl/2020/1820/oj

Betaglucan from E. gracilis has been also approved (since 2024). Similarly as in whole biomass Kemin Foods has exclusive right to place E. gracilis betaglucan on EU market until end of April 2029. https://eur-lex.europa.eu/eli/reg_impl/2024/1046/oj

In both cases, the reason is that the applicant (Kemin Foods) based both approved applications on proprietary studies (structure, stability, toxicology, etc.), which are protected under a patent. The EU approval is granted based on these studies, which is why it is exclusively awarded to Kemin Foods for five years from the decision date.

Any other applicant may submit a request for approval, but they cannot rely on or reference these studies in their application.

<u>Graesiella emersonii</u> (Not novel in food)

Synonymum Chlorella emersonii.

Graesiella emersonii is a unicellular green alga recognized for its applications in the food industry due to its nutritional profile and bioactive compounds. In the European Union, it is classified as a non-novel food, indicating its history of consumption prior to May 15, 1997, which allows its use in food products without the need for novel food authorization.

While specific companies placing G. emersonii on the market are not detailed in the available sources, its approval as a non-novel food facilitates its incorporation into various food products. In France, for instance, G. emersonii has been consumed and is approved as a food supplement.

G. emersonii is valued for its potential in sustainable biodiesel production during wastewater treatment, highlighting its versatility beyond the food sector.

Compared to other Chlorella species, G. emersonii has been noted for its lipid production capabilities. Studies have shown that under nitrogen limitation conditions, G. emersonii can achieve lipid contents of up to 63%, which is higher than some other Chlorella strains. (Kang et al., 2022; Wang et al., 2023)

Ievina, Baiba, and Francesco Romagnoli. 2020. "Potential of Chlorella Species as Feedstock for Bioenergy Production: A Review." Environmental and Climate Technologies 24(2): 203–220. https://doi.org/10.2478/rtuect-2020-0067.

<u>Odontella aurita</u> (Whole: authorized novel food (Union list))

A marine diatom, has gained attention in the food industry primarily due to its high content of eicosapentaenoic acid (EPA), an omega-3 fatty acid known for its cardiovascular benefits. The biomass of O. aurita contains approximately 25–26% EPA of its total fatty acid composition, making it a valuable source for health-promoting ingredients. ((An et al., 2023)

In 2002, the Agence Française de Sécurité Sanitaire des Aliments (AFSSA) approved the consumption of O. aurita, citing its substantial equivalence to other edible seaweeds already approved under EC Regulation 258/97. Following this, O. aurita was officially designated as a Novel Food in the European Union. According to EU regulations, the entire biomass of O. aurita can be utilized in specific food products, subject to maximum content limitations.

The French company Innovalg has successfully cultivated O. aurita on a large scale in raceway ponds and commercialized it as a dietary supplement. The supplement is available in the form of capsules containing dried cells. While human nutrition tests are pending, studies have shown that O. aurita has the potential to mitigate the risk of metabolic syndrome in mice fed a high-fat diet.

Advantages of Odontella aurita:

- Nutritional Benefits: Rich in EPA, which supports cardiovascular health.
- Bioactive Compounds: Contains fucoxanthin, a marine xanthophyll with antioxidant, anti-cancer, and anti-obesity properties.
- Versatility: Potential applications in food, feed, and cosmetics due to its beneficial components, including fiber, phytosterols, protein, and minerals.

It's important to note that while O. aurita is authorized as a Novel Food in the EU, its use is subject to specific conditions. For instance, the maximum levels permitted in certain food categories are as follows:

- Broth preparations: 1%
- Crackers: 1.5%
- Fish soups: 1%
- Flavored pasta: 1.5%
- Frozen breaded fish: 1.5%
- Marine terrines: 0.5%

The designation of the novel food on the labeling of foodstuffs containing it must be 'Odontella aurita microalgae'.

Products with Odontela on market

Solmon by Odontella

A French startup named Odontella has developed "Solmon," a vegan alternative to smoked salmon. This product is crafted using Odontella aurita, the only microalgae certified for human consumption. Solmon mimics the flavor and texture of traditional smoked salmon and is rich in omega-3 fatty acids.

salmonbusiness.com

Odontella aurita Powder by ALGANEX

ALGANEX offers Odontella aurita in powder form, highlighting its high protein content and omega-3 fatty acids, particularly EPA. The powder also contains the carotenoid pigment fucoxanthin and is suitable for applications in food, dietary supplements, animal feed, and cosmetics.

alganex.com

Odontella aurita Powder by Alfa Chemistry

Alfa Chemistry provides Odontella aurita powder, emphasizing its richness in chrysolaminarin—a β-1,3-glucan with strong antioxidant and anti-inflammatory properties. This powder is intended for use in boosting immunity and regulating lipid metabolism.

marinechem.alfa-chemistry.com

Scenedesmus vacuolatus (Not novel in food)

Syn. Chlorella vacuolatus.

Informace o využití této řasy nejsou známy.

(Carbone, 2017)

Tetraselmis chuii (Whole: authorized novel food (Union list))

Tetraselmis chuii is a unicellular green microalga recognized for its nutritional and functional properties, making it significant in the food industry.

In March 2014, the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) authorized dried *Tetraselmis chuii* as a novel food under Regulation (EC) No 258/97. The initial authorization permitted its use in sauces, special salts, and as a condiment. Subsequently, in 2017, an application was submitted to modify the specifications of dried *Tetraselmis chuii*, adjusting the ranges of protein, ash, carbohydrates, fiber, and fat content to improve the product's nutritional profile.

(Cokdinleyen et al., 2024; Nunes, Fernandes, Vasco, Sousa, & Raymundo, 2020)

Market Introduction and Applicants:

The company Fitoplancton Marino S.L. applied for the initial authorization and has been instrumental in bringing *Tetraselmis chuii* to the market. They have marketed the dried microalga under the trade name TetraSOD® (https://tetrasod.com/en/), promoting its use in various food products and supplements. aesan.gob.es.

Tetraselmis chuii is utilized in the food industry for its nutritional benefits. It has been incorporated into gluten-free bread formulations, enhancing bioactivity and providing an innovative green appearance. Studies have shown that adding *T. chuii* can increase the antioxidant properties of bread, offering potential health benefits.

In the feed sector, *T. chuii* has a long history of use in aquaculture, serving as an efficient feed due to its high nutritional value. It is cultured and refined in large-scale facilities to support the growth and health of various aquatic species.

Advantages:

• Nutritional Profile: *T. chuii* is rich in proteins, essential fatty acids, and antioxidants, contributing to its health-promoting properties.

• Functional Benefits: Its incorporation into food products can enhance bioactivity, such as increasing antioxidant capacity, which may offer neuroprotective effects.

• Sustainability: As a microalga, *T. chuii* can be cultivated sustainably, offering an eco-friendly alternative to traditional agricultural ingredients.

For detailed information on the novel food status of *Tetraselmis chuii*, you can refer to the European Commission's Novel Food List (https://eur-lex.europa.eu/eli/reg_impl/2017/2470/oj).

Most Important Autotrophic Microalgae in the Market (Fernández et al., 2021)

Species Name	**Average Price (per DW)**	**Annual Production (ton/year)**	**Market Value (M€)**
Spirulina	5 – 50 €/kg 36 €/kg (Human nutrition); 11 €/mg (Phycobiliproteins)	5,900 - 7,000	120 - 160
Chlorella	12 - 40 €/kg 36 €/kg (Human nutrition); 50 €/L (Aquaculture)	4,000 - 7,000	100 - 130
Dunaliella	60 – 100 €/kg 215 - 2150 €/kg (beta-carotene)	1,000 - 1,600	70 - 110
Haematococcus	150 - 340 €/kg 50 €/L (Aquaculture); 7150 €/kg (Astaxanthin)	280 - 350	80 - 100
Total	227 - 530	11,180 - 15,950	370 - 500

Note: Price of astaxanthin drastically dropped to dozens up to hundreds USD per kg (Alibaba.com, made-in-china.com)

4. LEGISLATION FRAMEWORK

The European Union (EU) food regulatory framework is designed to ensure food safety, consumer protection, and fair trade practices. It is primarily governed by Regulation (EC) No 178/2002, also known as the General Food Law Regulation, which establishes the fundamental principles and requirements of food law across EU Member States. This regulation defines food as "any substance or product intended for human consumption," encompassing raw, processed, and functional foods, as well as food supplements, food additives, and novel foods. It also introduces the precautionary principle, ensuring that food placed on the EU market is safe for human consumption. Additionally, food supplements are regulated under Directive 2002/46/EC, while Regulation (EC) No 1333/2008 governs food additives, distinguishing them from food ingredients based on their technological function (e.g., preservatives, colorants, emulsifiers).

The EU food legislation consists of various regulations and directives that cover food safety, hygiene, labeling, and market access. Regulation (EU) 2015/2283 on Novel Foods governs the approval process for foods not significantly consumed in the EU before May 15, 1997, requiring safety evaluations by the European Food Safety Authority (EFSA). Regulation (EU) No 1169/2011 on Food Information to Consumers (FIC) mandates accurate food labeling, including nutritional values and allergen declarations, ensuring transparency for consumers. Meanwhile, Regulation (EC) No 852/2004 establishes hygiene requirements for food businesses, including HACCP principles (Hazard Analysis and Critical Control Points), which must be followed by all food operators. Collectively, these legal instruments form a comprehensive regulatory framework ensuring that food products in the EU are safe, traceable, and correctly labeled before reaching consumers.

Regulation (EU) 2015/2283 on Novel Foods
Regulation (EC) No 178/2002 (General Food Law)
Directive 2002/46/EC on Food Supplements
Regulation (EC) No 1333/2008 on Food Additives
Regulation (EU) No 1169/2011 on Food Information to Consumers

Each of these regulations sets out specific requirements depending on the intended use of microalgae in food and feed products.

"Food" includes drink, chewing gum and any substance, including water, intentionally incorporated into the food during its manufacture, preparation or treatment.

Regulation (EC) No 178/2002 (General Food Law)

"Food supplements" means foodstuffs the purpose of which is to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form, namely forms such as capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles, and other similar forms of liquids and powders designed to be taken in measured small unit quantities;

Directive 2002/46/EC on Food Supplements

„Food additive“ shall mean any substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food, whether or not it has nutritive value, the intentional addition of which to food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food results, or may be reasonably expected to result, in it or its by-products becoming directly or indirectly a component of such foods

Regulation (EC) No 1333/2008 on Food Additives

Microalgae hold a unique position within the EU food regulatory framework, as they can be classified under different legal categories depending on their processing method and intended use. Whole microalgae, such as Spirulina (Arthrospira platensis) and Chlorella vulgaris, are considered food under Regulation (EC) No 178/2002, as they were consumed in the EU before May 15, 1997. In the past, such substances were often referred to as food ingredients, but the modern terminology classifies them simply as food. However, many other microalgae species and their derivatives, such as Euglena gracilis and Tetraselmis chuii, require approval under Regulation (EU) 2015/2283 on Novel Foods, as they were not widely consumed before the cut-off date. Microalgae extracts, such as astaxanthin from Haematococcus pluvialis, can be classified as either food supplements (Directive 2002/46/EC) or food additives (Regulation (EC) No 1333/2008) depending on their concentration and intended function. Importantly, phycocyanin from Spirulina is classified as a coloring foodstuff, rather than a food additive, as it is obtained through non-selective extraction and retains the original composition of the source material. Given their diverse applications, microalgae must be evaluated case by case to determine their regulatory status within EU food law.

One of the most critical regulatory distinctions is whether a microalga is classified as a novel food or a traditional food in the EU.

4.1 AUSTRIA AND CZECH REPUBLIC

In both Austria and the Czech Republic, the regulatory framework for the use of microalgae in food products aligns with the European Union's **Novel Food Regulation (EU) 2015/2283**. This regulation mandates that foods not significantly consumed within the EU before May 15, 1997, undergo a safety assessment and authorization process before being marketed.

Austria:

The **Austrian Agency for Health and Food Safety (AGES)** is responsible for overseeing food safety, including the implementation of the Novel Food Regulation. Businesses seeking guidance on the novel food status of microalgae products can contact AGES directly.

- **Contact Information:**
  - **Address:** Spargelfeldstraße 191, 1220 Wien, Austria
  - **Phone:** +43 5 0555-0
  - **Fax:** +43 5 0555-22019
  - **Website:** www.ages.at

Czech Republic:

In the Czech Republic, the **Ministry of Agriculture** is the competent authority overseeing food safety and the implementation of the Novel Food Regulation. **Státní zemědělská a potravinářská inspekce** (SZPI) operates under the Ministry and is responsible for supervising the safety, quality, and labeling of foodstuffs. Businesses can reach out to the Ministry or SZPI for guidance on novel food status and compliance requirements. **Food Safety Information Center Web** Site provides a lot of informations about Food Safety (https://foodsafety.cz/).

- **Contact Information:**
  - **Ministry of Agriculture:**
    - **Address:** Těšnov 65/17, 110 00 Praha 1, Czech Republic
    - **Phone:** +420 221 811 111
    - **Website:** www.eagri.cz
  - **Státní zemědělská a potravinářská inspekce (SZPI):**
    - **Address:** Květná 15, 603 00 Brno, Czech Republic
    - **Phone:** +420 543 540 111
    - **Website:** www.szpi.gov.cz

For businesses operating in either country, it's essential to consult with these national authorities to ensure compliance with both EU and country-specific requirements regarding the use of microalgae in food products.

4.2 TRADITIONAL FOODS (NOT-NOVEL FOOD)

If a microalga has a history of safe consumption in the EU before May 15, 1997, it is classified as not novel and does not require additional EFSA approval. This means it can be freely used in food products under general food law. But one must distinguish if the Traditional Food can be consumed in food as general or in Food supplements only.

List of Tradition Food is searchable EU Novel Food status Catalogue.

4.3 NOVEL FOOD

A novel food is any food not significantly consumed within the EU before May 15, 1997. If a microalgae species was not widely consumed in the EU before this date, it requires authorization through the European Food Safety Authority (EFSA) before being placed on the market. Companies must demonstrate the safety of the novel food through scientific studies before obtaining approval.

A full list of novel foods can be accessed in the Union List of Novel Foods

4.4 CLASSIFICATION OF MICROALGAE-BASED PRODUCTS

Once a microalga is determined to be suitable for food use, its application determines the specific EU regulations it must follow. Microalgae can be classified as food ingredients, food supplements, or food additives.

Microalgae as Food Ingredients

Governed by Regulation (EC) No 178/2002, food ingredients include substances that are consumed as part of a balanced diet.

Microalgae such as Chlorella, Spirulina, and Tetraselmis chuii are used in smoothies, pasta, bread, and protein-rich foods.

Blue Spirulina extract rich in phycocyanin marketed as „Blue Spirulina“ is considered as „Coloring Foodsuff“ (färbendes Lebensmittel , barvící potravina) and not as Food additive – Food color (similarly beet rood juice, blueberry juice etc.)

Example: Spirulina pasta marketed as a protein- and antioxidant-enriched food.

Microalgae as Food Supplements

Regulated under Directive 2002/46/EC, supplements provide concentrated sources of nutrients.

Common microalgae used as food supplements include Chlorella, Spirulina, Haematococcus pluvialis (astaxanthin), and Aphanizomenon flos-aquae.

Sold in tablet, capsule, or powder form.

Microalgae as Food Additives

Regulation (EC) No 1333/2008 governs food additives.

If microalgae-derived compounds are selectively extracted for technological purposes, they may be classified as food additives. The only example from microalgae is betacaroten from Dunaliella salina – E 160(iv); *beta*-carotene-rich extract from *Dunaliella salina.*

4.5 NUTRIENT AND HELATH CLAIMS IN EU

In the European Union, nutrient and health claims on food products are regulated under Regulation (EC) No 1924/2006, which sets out conditions for labeling foods with nutritional benefits. This regulation ensures that claims such as “high protein,” “source of fiber,” or “rich in omega-3” are backed by scientific evidence and not misleading to consumers. For example, a product can only be labeled as a “source of protein” if at least 12% of its total energy comes from protein, while a “high-protein” claim requires at least 20% of energy from protein. This is particularly relevant to microalgae-enriched baked goods, where increasing protein content through algae-based ingredients such as *Chlorella vulgaris* or *Microchloropsis gaditana* could allow for these claims, adding functional value to gluten-free or plant-based bakery products.

Beyond nutrient content, health claims—such as supporting immune function, digestion, or heart health—require authorization from the European Food Safety Authority (EFSA) based on rigorous scientific assessment. Microalgae, rich in antioxidants, polyphenols, and omega-3 fatty acids, have potential for functional food development, but claims regarding their health benefits must comply with Article 13 or 14 of Regulation 1924/2006, which differentiates between general health maintenance claims and disease risk reduction claims. For the Bio2AgroFood project, demonstrating digestibility, bioavailability, and prebiotic effects of microalgae ingredients in baked goods could strengthen their positioning as functional foods, potentially supporting nutrient or health claims in EU markets.

5. MICROALGAE IN BAKERY PRODUCTS

Microalgae are increasingly being incorporated into bakery products due to their exceptional **nutritional profile and functional properties**. Traditional bakery products often lack key nutrients, such as high-quality proteins, essential fatty acids, and antioxidants, which microalgae can provide in a natural and sustainable manner. Their high protein content (up to 70 % in some species) makes them an attractive ingredient for enhancing the nutritional value of bread, biscuits, and other baked goods. Additionally, their rich profile of vitamins (A, D, E, K, and B-complex), minerals (iron, calcium, magnesium), and bioactive compounds (polyphenols, carotenoids, and phycocyanin) contribute to the overall health benefits of fortified bakery products. Studies have shown that incorporating microalgae into bread formulations improves protein and fiber content while maintaining desirable sensory properties. (Hernández-López et al., 2024)

Beyond nutritional benefits, microalgae offer functional advantages that **improve the texture**, appearance, and shelf life of bakery products. Some microalgae, such as Spirulina platensis and Chlorella vulgaris, possess gelling, thickening, and emulsifying properties, which help improve the structure of dough and enhance the moisture retention of baked goods. This is particularly beneficial in gluten-free baking, where maintaining a soft and cohesive texture is challenging. Moreover, microalgae contain natural antioxidants, such as astaxanthin and β-carotene, which can slow down lipid oxidation, thereby extending the shelf life of bakery products without the need for artificial preservatives (Hernández-López et al., 2024)

Another key advantage of microalgae in bakery applications is their ability to act as **natural food colorants**. Spirulina provides a green-blue, while Dunaliella salina and Haematococcus pluvialis contribute orange and red tones due to their high β-carotene and astaxanthin content. These natural pigments make microalgae an alternative to synthetic colorants, aligning with the growing consumer demand for clean-label and natural ingredients. In addition, some microalgae possess umami-like flavor compounds, which can enhance the taste profile of bakery products while allowing for a reduction in added salt (phycom.eu). Given these benefits, the integration of microalgae into bakery formulations represents a promising avenue for creating healthier, more functional, and visually appealing baked goods.

Colouring foods, also known as colouring foodstuffs, are food ingredients used by the food industry primarily to deliver colour to food and beverage products. They are obtained from foods such as fruits, vegetables, flowers, spices, algae and/or other edible source materials. The difference with food colour additives is that there is no selective extraction.

Colours, as defined in Regulation (EC) No 1333/2008 on food additives, are "substances which add or restore colour in a food, and include natural constituents of foods and natural sources which are normally not consumed as foods as such and not normally used as characteristic ingredients of food. Preparations obtained from foods and other edible natural source materials obtained by physical and/or chemical extraction resulting in a selective extraction of the pigments relative to the nutritive or aromatic constituents are colours within the meaning of this Regulation"

As an example, the pigment Anthocyanins E163 is a purple natural colour additive extracted from elderberry, whereas elderberry juice concentrate, which does not undergo selective pigment extraction and can be consumed as such, is an example of a colouring food.

https://www.cbi.eu/market-information/natural-food-additives/natural-food-colours/market-entry

More about colorant food:

Bratinova S. Provision of scientific and technical support with respect to the classification of extracts/concentrates with colouring properties either as food colours (food additives falling under Regulation (EC) No 1333/2008) or colouring foods. EUR 27425. Luxembourg (Luxembourg): Publications Office of the European Union; 2015. JRC96974 (https://publications.jrc.ec.europa.eu/repository/bitstream/JRC96974/final%20report_colouring%20food.pdf)

Guidance notes on the classification of food extracts with colouring properties (EU) - https://english.nvwa.nl/binaries/nvwa-en/documenten/consumers/food/safety/documents/guidance-notes-on-the-classification-of-food-extracts-with-colouring-properties-eu/leidraad-europese-commissie-criteria-extract-kleurstof-of-kleurend-ingredi%C3%ABnt-29-november-2013.pdf

5.1 OVERVIEW OF PRODUCTS ON A MARKET

Microalgae are increasingly being incorporated into bakery products due to their nutritional benefits and natural coloring properties. Below is an overview of existing bakery products containing microalgae, detailing the product, producer, type of algae used, product characteristics, and other relevant information:

<table>
<tr><td>Flower Burger's Spirulina Buns</td><td rowspan="4"></td></tr>
<tr><td>Producer: PURATOS</td></tr>
<tr><td>Web:
https://www.puratos.com/</td></tr>
<tr><td>Algae used:
Spirulina – in combination with turmeric produces green colored buns.</td></tr>
<tr><td colspan="2">Product description:
Buns for Italy based burger company – Flower Burger (https://www.flowerburger.it/)</td></tr>
<tr><td colspan="2">Company profile:
Puratos is an international group offering a full range of innovative products and application expertise for the bakery, patisserie, and chocolate sectors. Founded in 1919 in Brussels, Belgium, by Henri Groot and his son, the company has grown from a family-run business into a global enterprise. Today, Puratos operates in over 100 countries, with local subsidiaries in 84 countries and production facilities in 75 plants across 52 countries.</td></tr>
<tr><td colspan="2">Other informaion:
https://www.puratos.ng/en/blog/taste-tomorrow/is-algae-the-bakery-and-patisserie-ingredient-of-the-future?utm_source=chatgpt.com</td></tr>
</table>

Dutch Weed Burger

Producer: LIVEKINDLY Collective

Web:
https://dutchweedburger.com/en/

Algae used:
2 seaweed, 1 microalgae – chlorela sorokiniana, probably from Phycom

https://dutchweedburger.com/wp-content/uploads/2021/01/weedburger-ingredienten.jpg

Product description:

Company profile:
The Dutch Weed Burger was co-founded by Mark Kulsdom and Lisette Kreischer. In June 2021, the company was acquired by LIVEKINDLY Collective, a global plant-based food company. (https://thelivekindlyco.com/)

Other informaion:
https://www.yesmagazine.org/environment/2017/05/26/why-you-want-one-of-these-dutch-weed-burgers

Algae-Enriched Crackers and Grissini

Producer: Institute of Agrifood Research and Technology (IRTA)

Web:
https://www.pro-future.eu/blog/more-foods-going-green-at-irta-adding-microalgae-to-vegetable-creams

Algae used:

Product description:

Company profile:
Research organisation.

Other informaion:
Connected to the project PRO-FUTURE (see chapter 5.1)

<table>
<tr><td>Bread with spirulina</td><td rowspan="4"></td></tr>
<tr><td>Producer: Institute of Agrifood Research and Technology (IRTA)</td></tr>
<tr><td>Web:
https://www.pro-future.eu/blog/bread-with-a-marine-twist</td></tr>
<tr><td>Algae used: Spirulina</td></tr>
<tr><td colspan="2">Product description:</td></tr>
<tr><td colspan="2">Company profile:
Research organisation.</td></tr>
<tr><td colspan="2">Other informaion:
Connected to the project PRO-FUTURE (see chapter 5.1)
https://www.pro-future.eu/blog/bread-with-a-marine-twist</td></tr>
</table>

<table>
<tr><td>Spirulina bread</td><td rowspan="4"></td></tr>
<tr><td>Producer: Novepan</td></tr>
<tr><td>Web:
https://www.novepan.fr/en/produits/spirulina-bread/</td></tr>
<tr><td>Algae used: Spirulina</td></tr>
<tr><td colspan="2">Product description: This bread, with its coloured crumb, sprinkled with pieces of fruit and visible hazelnuts, combines a variety of ingredients with complementary taste profiles for an explosion of flavours on the palate: cranberries, figs, raisins, hazelnuts and spirulina.
Our spirulina bread won in the bakery products category at the 4th edition of the “Pépites de la Boulangerie”, joining the 2023 winners!</td></tr>
<tr><td colspan="2">Company profile:
Novepan is a French company specializing in the production of premium frozen bakery products, including partially baked specialty bread, savory snacks, and pizza dough. The company was founded in 2017 through the merger of Grain d'Or and Lubrano.</td></tr>
<tr><td colspan="2">Other informaion:
https://www.novepan.fr/en/spirulina-bread-winner-of-the-pepites-de-la-boulangerie-2023-awards/</td></tr>
</table>

<table>
<tr><td>Microalgae Bread</td><td rowspan="4"></td></tr>
<tr><td>Producer: Phycom</td></tr>
<tr><td>Web:
https://phycom.eu/benefits-of-microalgae-in-sweet-savoury-bakery-products/</td></tr>
<tr><td>Algae used: chlorella</td></tr>
<tr><td colspan="2">Product description:</td></tr>
<tr><td colspan="2">Company profile:
While Phycom isn't a bakery itself, they collaborate with bakeries by supplying microalgae and assisting in product development. They notably helped the Dutch Weed Burger develop their bun[2]. It is worth checking out the Dutch Weed Burger to see if the bun is sold separately.</td></tr>
</table>

Other informaion:
https://www.novepan.fr/en/spirulina-bread-winner-of-the-pepites-de-la-boulangerie-2023-awards/

Spirulina flour	
Producer: farinhasfirmos	
Web: https://farinhasfirmos.pt/en/products/spirulina-bread-flour	
Algae used: Spirulina	

Product description:
Although spirulina is known as a green-bluish alga, it is a cyanobacterium, photosynthetic and rich in different nutrients that grant several benefits in terms of health and good fitness. It is used as a dietary supplement (including to prevent malnutrition) and as a great source of protein (it has a high protein content, higher than the actual red meat). Of slow digestion, it makes the body feel satisfied, reducing appetite - it helps you lose weight and the additional plant-based diets, as well as to build and repair muscle tissue. It is a rich source of antioxidants, including Vitamin E, Selenium, carotenoids and phenolic acids, helping to reduce the risk of cancer, minimize the risk of atherosclerosis (such as excess cholesterol and high blood pressure) and the occurrence of cardiovascular disease (such as heart attack and stroke). It helps fight against anaemia and various infections (proven effective against herpes, Flu and HIV), strengthens the immune system (stimulates antibody production), promotes the relief of respiratory diseases (such as asthma and allergic rhinitis) and can help In the treatment of type 2 diabetes (reduces insulin resistance). Its antioxidant properties further help fight oxidative stress in the brain by promoting the reduction of risks of Alzheimer’s disease, Parkinson’s disease, and other neurodegenerative disorders

Company profile:
Farinhas Firmos specializes in producing high-quality flours, offering a diverse selection including various types of wheat flour and innovative specialty flours.

Other informaion:

6. RESEARCH AND DEVELOPMENT

6.1 PROJECTS FOCUSED ON THE TOPIC

Name	**MICROALGAE PROTEIN INGREDIENTS FOR THE FOOD AND FEED OF THE FUTURE**
Programme	Horizon-2020
Link to the project	https://www.pro-future.eu/
Abstract	ProFuture will set the basis for market uptake of innovative, healthy and sustainable food and feed products, reformulated with protein-rich ingredients from Spirulina, Chlorella vulgaris, Tetraselmis chuii and Nannochloropsis oceanica. Multi-factors approach and LCA/LCC will boost sustainability of the microalgal biomass production and processing, by reducing energy/water consumption and the carbon footprint. Implementation of innovative technologies will increase the efficiency and decrease the production costs of biomass, ingredients and foods/feeds. Optimized workflows will be tested at pilot plant, as part of a biorefinery process, to produce single-cell proteins and protein isolates, which will be characterized for their nutritional, safety, techno-functional and organoleptic properties as well as for economic viability & sustainability. Food and feed will be formulated with the novel ingredients at pilot plant level in collaboration with SME and large companies. Single-cell proteins will be incorporated in foods (n=6) and feeds (n=5) which will be produced at industrial scale. The food/feed microalgae value chain in EU will be analysed and improvements will be proposed to increase the economic viability and the communication between actors. Social and economic benefits of innovative food and feed products will be demonstrated by: i) supporting the authorization of novel microalgae protein ingredients for food and/or feed applications in the EU, ii) assessing consumer acceptance of and preference for microalgae protein-based products, iii) preparing a successful market implementation and exploitation of the project results and iv) devising a dissemination and communication plan for maximum outreach to all relevant stakeholders. ProFuture consortium (8 RTDs, 16 SMEs, 8 large industries and 1 association) brings together expertise with a clear market orientation to increase the competitiveness of the EU microalgae value chain.
Period	2019 - 2023
Budget	€ 9 448 451,25
States involved	12
Leading partner	INSTITUT DE RECERCA I TECNOLOGIA AGROALIMENTARIES
Partners	DIL DEUTSCHES INSTITUT FÜR LEBENSMITTELTECHNIK EV – Germany WAGENINGEN UNIVERSITY – Netherlands EIGEN VERMOGEN VAN HET INSTITUUT VOOR LANDBOUW- EN VISSERIJONDERZOEK – Belgium UNIVERSITEIT GENT – Belgium UNIVERSITEIT TWENTE – Netherlands ZENTRUM FÜR SOZIALE INNOVATION GMBH – Austria NORCE NORWEGIAN RESEARCH CENTRE AS – Norway NECTON-COMPANHIA PORTUGUESA DE CULTURAS MARINHAS SA – Portugal ALLMICROALGAE NATURAL PRODUCTS SA – Portugal ASSOCIACAO OCEANO VERDE LABORATORIO COLABORATIVO PARA O DESENVOLVIMENTO DE TECNOLOGIAS E PRODUTOS VERDES DO OCEANO – Portugal ALGOSOURCE TECHNOLOGIES – France GIVAUDAN NEDERLAND BV – Netherlands VIVA MARIS GMBH – Germany TRADIZIONI PADANE SRL – Italy CALE - INDUSTRIA E COMERCIO, LDA – Portugal ALVER WORLD SA – Switzerland ENERVIT SPA – Italy CONSERVAS HIJOS DE MANUEL SANCHEZ BASARTE SA – Spain CENTRO NACIONAL DE TECNOLOGIA Y SEGURIDAD ALIMENTARIA – Spain (Third-party)

	NUTRITION SCIENCES – Belgium INVE TECHNOLOGIES NV – Belgium VITAFORT ELSO TAKARMANYGYARTO ES FORGALMAZO ZARTKORUEN MUKODO RT – Hungary COOP ITALIA - SOCIETA' COOPERATIVA SCRL – Italy APEXAGRI SAS – France WIISE SRL SOCIETA' BENEFIT – Italy CIVITTA EESTI AS – Estonia AXIA INNOVATION GmbH – Germany RDC INFORMATICS S.A. – Greece ESU-SERVICES GMBH – Switzerland FOODCOMPANIONS HOLDING BV – Netherlands EUROPEAN FOOD INFORMATION COUNCIL – Belgium FOODCOMPANIONS BV – Netherlands
Further information	https://cordis.europa.eu/project/id/862980 https://cordis.europa.eu/project/id/862980/results https://www.irta.cat/en/how-to-feed-10-billion-people-with-a-microalgae-enriched-diet/

Name	**YUM ALGAE - EnzYmes for improved sensory qUality of MicroALGAE ingredients in foods**
Programme	EEA Grants Portugal
Link to the project	https://www.eeagrants.gov.pt/en/programmes/blue-growth/projects/projects/yum-algae/
Abstract	Microalgae are emerging as an attractive biological resource for sustainable production on a large scale, promoting the blue bioeconomy and are already considered one of the most promising sources of new food products. However, the incorporation of microalgae has limitations in terms of sensory properties and consumer acceptance, mainly due to the colour and taste of the sea. The YUM ALGAE project aims to improve the overall sensory quality of microalgae as a food ingredient, using enzymes, which will target two of the main organoleptic characteristics (aroma, flavor, color): the fish-like smell associated to volatile compounds and the green dark color from the microalgal pigments. In this project, two staple foods will be considered as case studies: bread and cheese, in which microalgae submitted to enzymatic treatment and produced without treatment will be incorporated, in order to evaluate the impact on the sensory profile. YUM ALGAE will contribute to increasing the value associated with marine bioresources in line with the principles that define the blue biotechnology. Moreover, it will make it possible to overcome the obstacles associated with the consumption of microalgae as a food ingredient, increasing its potential for use as a response to the population's needs in terms of more sustainable food solutions, promoting blue growth.
Period	
Budget	€ 991 215,00
States involved	1
Leading partner	Instituto Superior de Agronomia da Universidade de Lisboa
Partners	Pagarete Microalgae Solutions Sociedade Unipessoal, Lda. Instituto Superior Técnico NORCE - Norwegian Research Centre, A.S.
Further information	https://www.isa.ulisboa.pt/files/leaf/pub/YUM_Algae_concluido.pdf https://www.linkedin.com/company/yum-algae https://www.instagram.com/yum.algae https://www.facebook.com/YUMAlgae/

Name	**Biosolar Leaf**
Programme	The European Innovation Council (EIC)
Link to the project	https://cordis.europa.eu/project/id/190186816
Abstract	By 2050, it is estimated that the protein demand will grow by 78% and reach 360 million tonnes yearly if the entire population consume at current levels. Agriculture is nearly maximally exploited and issues such as climate change and urban expansion pose important challenges to the future of agriculture. Therefore, new solutions and additional resources are required to meet the increasing demands. Arborea aims to develop and put on the market a revolutionary bioreactor for (micro)algae growth, which will solve the crucial issues for large-scale and commercially viable microalgae production as food source and allow Arborea to produce and bring to market sustainable food ingredients and protein commodity at disruptive price. With EIC's support, the technology will evolve from the end of the R&D phase to the scale-up phase, accelerating the transition to a sustainable food system.
Period	2022 - 2023
Budget	€ 3 355 533,75
States involved	PT
Leading partner	ARBOREABIOFOODS LDA (PT)
Partners	
Further information	

Name	**NextGenProteins**
Programme	H2020
Link to the project	https://nextgenproteins.eu/
Abstract	Demand for proteins is increasing for food and feed applications. To meet the increasing demand, production will have to double by 2050. However, current protein production, both animal- and vegetal based, has severe negative environmental impacts in terms of greenhouse gas (GHG) emissions, land and water use, as well as biodiversity loss. The EU is not self-sufficient when it comes to protein production and a large proportion of the demand is met with imported proteins with concerns regarding food security and the general competitiveness of the EU. It is therefore of vital importance to find sustainable alternative protein sources that can be economically produced in quantities that meet growing food and feed sectors. NextGenProteins has identified microalgae, single cell protein and insects as three promising sources of alternative proteins that can be produced through innovative and environmentally sustainable bioconversion processes using industrial waste streams. These processes cause limited environmental impacts and pressure on natural resources. Through collaboration between industry and RTD, the project will address key barriers that currently prohibit or limit the application of the three alternative proteins in food and feed, such as production scalability and optimisation, production costs, value chain risks, safety, regulations and consumer trust and acceptance. The project will demonstrate the suitability and economic viability of the alternative proteins in food and feed value chains and explore their market opportunities with the industry, stakeholders, policy makers and consumers. NextGenProteins will find means to improve the acceptability and trust of consumers towards alternative proteins and processes. The project will contribute to strengthening food security, sustainability and self-sufficiency of EU protein production with future-proof supply, as well as long-term reduction of land use, water use, GHG emissions and energy of EU food sector.
Period	2019 - 2023
Budget	€ 9 257 320,41
States involved	See below

Leading partner	MATIS OHF (IS)
Partners	TEKNOLOGIAN TUTKIMUSKESKUS VTT OY – Finland ALMA MATER STUDIORUM - UNIVERSITA DI BOLOGNA – Italy RISE PROCESSUM AB – Sweden RISE RESEARCH INSTITUTES OF SWEDEN AB – Sweden VEREIN ZUR FORDERUNG DES TECHNOLOGIETRANSFERS AN DER HOCHSCHULE BREMERHAVEN EV – Germany SP/F SYNTESA – Faeroe Islands CIRCULAR SOLUTIONS EHF – Iceland GESCO SOCIETA COOPERATIVA AGRICOLA – Italy VRM SRL – Italy VAXA TECHNOLOGIES LTD – Israel ARBIOM – France GRIMUR KOKKUR EHF – Iceland BIOZOON GMBH – Germany MUTATEC – France ENTOCUBE OY – Finland AKTIEBOLAGET HERBERT KARLSSONS CHARKUTERIFABRIK – Sweden WAITROSE LIMITED – United Kingdom AQUASCOT LIMITED – United Kingdom MOWI FEED AS – Norway FAZER SWEDEN AB – Sweden FAZER BAGERI AB (Third-party) – Sweden SJOKOVIN – Faeroe Islands PEAS OF HEAVEN AB – Sweden KPMG EHF – Iceland
Further information	Results: https://cordis.europa.eu/project/id/862704/results

7. PATENT OVERVIEW

7.1 USED KEY WORDS

Various keyword and general syntax:
Microalgae (TI, AB, CL)
Bread OR Bakery (TI, AB, CL)
fortification OR gluten-free OR prebiotic OR PUFA (TI, AB, CL)

7.2 PATENT OVERVIEW

Name of the patent	Applicant	Number	Notes
PROCESS OF PRODUCING CHLORELLA-ENRICHED BREAD ROLLS (PANDESAL)	CAPIZ STATE UNIV [PH]	PH22022050721 (U1)	
Formula and processing technology of peach gum bread with chlorella flavor	UNIV WUHAN POLYTECHNIC	CN118923705 (A)	The formula of a chlorella-flavored peach gum bread and its processing technology according to claim 1 are characterized in that: measured by weight, the preparation raw materials include 80-120 parts of flour, 40-100 parts of peach gum raw materials, **0.8-100 parts of chlorella powder**, 14-18 parts of zero-calorie sugar, 1-1.4 parts of yeast, 6-6.5 parts of milk powder, 0.2-0.6 parts of improver, 7-8 parts of egg liquid, 120-200 parts of water, 4-8 parts of butter and 0.5-1.5 parts of salt. Step by step description in Claim 3.
Chlorella strain or product thereof and application of chlorella strain or product thereof in improving food quality	INST OF URBAN AGRICULTURE CHINESE ACADEMY OF AGRICULTURAL SCIENCES CHENGDU TIANFU XINGJI AGRICULTURAL TECH CO LTD	CN116439348 (A)	The algae strain is **Chlorella sorokiniana** and is preserved in the China Center for Type Culture Collection, the preservation number of the algae strain is CCTCC NO: M 20221541, and the preservation time of the algae strain is September 30, 2022. The food according to claim 4 is characterized in that the food is bread; preferably, the raw materials for making the bread include: based on the mass of the flour used to make the bread, 1% to 1.2% yeast, 0.5% to 1% bread improver, 5% to 10% milk powder, 5% to 10% sweetener, 40% to 45% water, **1% to 3% algae powder according to claim 2,** 0.1% to 0.3% edible alkali, 8% to 10% eggs, 10% to 15% butter, and 0.4% to 0.8% salt; further preferably, based on the mass of the flour used to make the bread, the raw materials for making the bread include: 1.2% yeast, 0.5% bread improver, 5% milk powder, 10% sweetener, 45% water, 1% algae powder, 0.1% edible alkali, 8% eggs, 15% butter, and 0.6% salt.

Name of the patent	Applicant	Number	Notes
Spinach-flavored chlorella bread and preparation method thereof	UNIV NORTHEAST AGRICULTURAL	CN114246195 (A)	The chlorella is rich in protein, mineral substances, vitamins and the like. The health food can enhance human immunity, inhibit proliferation of cancer cells in the human body, prevent blood sugar from rising, reduce the content of cholesterol in serum and eliminate toxins in the human body, and after being eaten, the health food is helpful for repairing human body injury. Claim 1: A spinach-flavored chlorella bread, characterized in that the bread is made of the following raw materials, measured by weight: 40-50 parts of wheat flour, 5-10 parts of yeast, 1-5 parts of spinach, **1-5 parts of chlorella**, 1-5 parts of white sugar, 1-5 parts of edible salt, 1-5 parts of ghee, 5-10 parts of eggs, and 20-30 parts of water. Claim 2: The spinach-flavored chlorella bread according to claim 1 is characterized by: preferably: 45 parts of wheat flour, 10 parts of yeast, 5 parts of spinach, **5 parts of chlorella**, 3 parts of white sugar, 2 parts of edible salt, 5 parts of ghee, 7 parts of eggs, and 25 parts of water.
KRITSINI BREAD STICKS COMBINING PLURAL SUPERFOODS	SAMALIFE MONOPROSOPI IKE [GR]	GR20190200184 (U)	kritsini bread sticks combining plural superfoods are disclosed herein. Ingredients in total weight of the final product: all-use wheat powder (from 50 to 60%); baking powder (from 2,5 to 3%), olive oil or sunflower oil (from 20 to 25%), **organic spirulina powder (from 20 to 25%)**, condensed aloe vera juice (from 2 to 5%), condensed turmeric juice (from2 to 5%), white wine (from 10 to 15%) and, optionally, salt (from 0,5 to 1,5%).
METHOD FOR PRODUCTION OF BREAD CONTAINING NANOSTRUCTURED EXTRACT OF CHLORELLA	KROLEVETS ALEKSANDR ALEKSANDROVICH [RU]	RU2685113 (C1)	During the process of dough preparation, drinking water, pressed bakery yeast, culinary food salt, prime grade flour are successively introduced, additionally nanostructured **chlorella extract is added in amount of 1-2 % of prime grade wheat flour mass**.

Name of the patent	Applicant	Number	Notes
Selenium-enriched spirulina bread and preparation method thereof	GUANGDONG RIKEWEI SELENIUM RICH FOOD CO LTD UNIV SOUTH CHINA TECH	CN108633962 (A)	35-50 parts of high-gluten flour, 15-20 parts of low-gluten flour, **10-15 parts of selenium-enriched spirulina powder**, 10-15 parts of selenium-enriched mung bean sprout powder, 3-5 parts of green tea extractive liquid, 15-20 parts of white granulated sugar, 2-4 parts of yeast, 8-10 parts of shortening, 4-6 parts of milk powder, 5-8 parts of broccoli, 8-10 parts of spinach, 8-10 parts of celery, 0.2-0.5 part of a bread improver and 0.8-1.2 parts of salt. Preparation method of the selenium-enriched spirulina powder is: 600ml Zarrouk culture medium is loaded into a 1000ml triangular flask, platensis is inoculated at an inoculation amount of 10%, 200µg/ml sodium selenite is added every day, and the temperature is 35°C, pH is 9, the light intensity is 4000lx, the light time is 24h/d, and the conditions of 150r/min are cultivated for 8 days, 3ml of distilled water is added every day to adjust the evaporation loss, after the cultivation is completed, the culture solution is taken through a 300-mesh sieve, and then centrifuged at 6000r/min for 10min at 4°C, the lower layer of algae mud is taken, washed 3 times with distilled water, and then vacuum freeze-dried to obtain selenium-enriched spirulina powder.
Production technology of wall-broken spirulina bread	FUJIAN KANGHENG FOOD TECH CO LTD	CN107668126 (A)	**Take 2g to 5g of spirulina ultrafine powder** and dissolve it in 50 to 80g of water. After stabilizing for 12 to 15 minutes, add appropriate amount of flour, mix into a uniform dough, and let it stand; divide the dough, proof it, bake it, and cool it naturally to room temperature to make spirulina bread. The production process of a cracked spirulina bread according to claim 1 is characterized in that the auxiliary materials described in step 4) include: 80 to 100 g of flour, 0.8 to 1.2 g of yeast, 3 to 5 g of skimmed milk powder, 1.2 to 1.5 g of salt, 5 to 8 g of sugar, 6 to 8 g of shortening, and 2 to 2.5 g of diacetyl tartaric acid mono(di)glyceride.

Name of the patent	Applicant	Number	Notes
Food formulation based on algae, bakery products, bunny and pastry that understand it, method of obtaining and its use.	JUAN Y JUAN IND S L UNIPERSONAL [ES]	ES2642463 (A1) ES2642463 (B1)	The invention relates to a food formulation comprising fresh microalgae in the form of paste of the species chlorella vulgaris, in a proportion comprised between **10% and 20%** with respect to the total weight of the formulation and with a degree of humidity measured at room temperature between 80% and 90%, including both limits The food formulation according to claim 1, wherein the microalgae of the species Chlorella vulgaris are selected from one of the strains of the group consisting of: **Chlorella vulgaris fa. viridis** with deposit number CCAP 211/12, Chlorella vulgaris Beijerinck with deposit number CCAP 211/19, **Chlorella vulgaris** Beijerinck with deposit number CCAP 211/11 B, **Chlorella vulgaris fo. tertia** with deposit number CCAP 211/110, and **Chlorella vulgaris** Beijerinck with deposit number CCAP 211/52.
BREAD PRODUCTION METHOD	FEDERALNOE GOSUDARSTVENNOE BYUDZHETNOE OBRAZOVATELNOE UCHREZHDENIE VYSSHEGO OBRAZOVANIYA VORONEZHSKI [RU]	RU2750724 (C1)	Additionally, before the salt and sugar solutions, the Scenedesmus microalgae concentrate with a humidity of 4.0-5.0 is added, previously prepared by centrifugation of the biologically active substance of **Scenedesmus** microalgae obtained by aerobic cultivation at a temperature of 28-34°C of culture liquid in a mixture of air and carbon dioxide with a concentration of 5.0-7.0%. Dough preparation is carried out with the following ingredient composition **per 100 kg** of flour components: • Wheat baking flour of the first grade – 50.0 kg • Triticum baking flour (whole grain) – 40.0-43.0 kg • Wheat bran – 2.5-3.5 kg • ***Scenedesmus* microalgae concentrate – 4.0-6.5 kg** • Acidifying food additive "Citrasol" – 2.5 kg • Pressed baker's yeast – 2.0 kg • Table salt – 1.5 kg • White sugar – 2.0 kg • Deodorized sunflower oil – 1.0 kg

Name of the patent	Applicant	Number	Notes
			• Water – as required
Bread used as both food and medicine	ZHEJIANG BAISHOUTANG PHARMACEUTICAL CO LTD	CN106614918 (A)	Components I comprise: radix polygalae, poria, abelmoschus esculentus, tuckahoe with pine, dandelion, hawthorn, fructus lycii, fructus alpinae oxyphyllae, **microalgae DHA powder (40-45 parts of Microalgae DHA powder)**, manyflower solomonseal rhizome and lotus leaves; components II comprise: coarse rice powder, buckwheat powder, walnut powder, sesame oil, a sheep milk hydrolysate, olive oil, yeast powder, sugar, honey and durian peel. The bread has abundant nutrition, has no added additive, has good taste, and is beneficial to improve immunity;
Bread and preparing method thereof	ZHEJIANG AOQI FOOD CO LTD	CN104222213 (A)	30-40 parts of microalgae oil
FUNCTIONAL FOOD INGREDIENT BASED ON MICROALGAE CHLORELLA VULGARIS AND LACTIC ACID BACTERIA LACTOBACILLUS PLANTARUM, ITS PREPARATION AND USE	GRAD HRANE d.o.o. [HR]	HRP20230792 (A1)	Extraction of dried or lyophilized biomass obtained by symbiotic cultivation of the microalgae Chlorella (Chlorella vulgaris Beijerinck 1890) and a culture of lactic acid bacteria (LAB) of the species Lactobacillus plantarum, with a solvent mixture of the composition methanol (CH_3OH) : dichloromethane (CH_2Cl_2) = 25 : 75 to 75 : 25, V/V, in the ratio:
GLUTEN-FREE FOODS CONTAINING MICROALGAE	CORBION BIOTECH INC [US]	US2019254292 (A1)	Chlorella protothecoides Disclosed herein are microalgae-containing gluten-reduced and gluten-free finished food compositions, as well as microalgae-containing food ingredients for the large-scale manufacture of gluten-reduced and gluten-free foods. Foods and ingredients of the invention, while reducing or eliminating gluten, also have increased health benefits through reduction or elimination of less healthy oils and fats via replacement of primarily monounsaturated algal oils. The novel food compositions also possess more desirable sensory properties and shelf life than previously existing gluten free foods. Foods and ingredients disclosed herein, which containing reduced or no gluten, also containing high

Name of the patent	Applicant	Number	Notes
			dietary fiber levels, reduced or eliminated cholesterol, and healthier oil content than existing gluten free foods. Also disclosed are methods of reducing food allergies and symptoms of diseases such as Celiac-Sprue to address increasing rates of sensitivity to gluten-containing products. Also disclosed are methods of formulating and manufacturing microalgae-containing gluten-free foods and ingredients for the formulation of such foods.
NOVEL MICROALGAL FOOD COMPOSITIONS	SOLAZYME INC [US]	EP2418959 (A1); EP2418959 (A4); EP2418959 (B1)	Chlorella protothecoides A gluten-free flour composition comprising a microalgal flour and at least one other gluten-free flour other than microalgal flour, wherein the microalgal flour comprises a homogenate of microalgal biomass containing predominantly or completely lysed cells in the form of a powder and contains at least 16% by dry weight triglyceride oi.
FOOD COMPOSITIONS OF MICROALGAL BIOMASS	SOLAZYME INC [US]	CA2740415 (A1); CA2740415 (C)	Chlorella sp. Only in Canada. Still acive patent.
LIPID-RICH MICROALGAL FLOUR FOOD COMPOSITIONS	CORBION BIOTECH INC [US]	US2020015490 (A1)	Chlorella protothecoides
NOVEL MICROALGAL FOOD COMPOSITIONS	TERRAVIA HOLDINGS INC [US]	US2016324167 (A1)	Chlorella protothecoides
METHOD FOR PRODUCTION OF BAKERY PRODUCTS FOR PREVENTIVE ALIMENTATION	FEDERAL NOE G BJUDZHETNOE OBRAZOVATEL NOE UCHREZHDENIE VYSSHEGO PROFESSIONAL NOGO OBRAZOVANIJA MO GU [RU]	RU2450522 (C1)	The method involves preparation of a semi-product from a composite mixture represented by a mixture of wheat flour and Spirulina platensis spirulina microalgae powder (preliminarily milled into particles sized 15-150 mcm) at a ratio of 13:1 and the recipe quantity of water. The produced semi-product is maintained at a temperature of 30C during 20 minutes. One proceeds with kneading the dough from the prepared semi-product, bakery wheat flour, yeast and

Name of the patent	Applicant	Number	Notes
			culinary food salt, fermentation of the dough, dough pieces handling, the bakery products proofing and baking.
BREAD PRODUCTION METHOD	Федеральное государственное бюджетное образовательное Учреждение высшего образования "Воронежский государственный аграрный университет имени императора Петра 1" (ФГБОУ ВО Воронежский ГАУ)	RU2018115091 (A); RU2018115091 (A3); RU2720763 (C2)	Dough is prepared at the following recipe components content, kg per 100 kg of flour: bakery medium rye flour - 15.5-20.5; first grade bakery wheat flour - 50.0; bakery medium flour in starter - 24.5 kg; baker's compressed yeast - 0.5; Dunaliella powder - 5.0 -10.0; food culinary salt - 1.5; white sugar - 3.0; water - as per calculation
DHA functional biscuit and preparation method thereof	SHANDONG FOOD FERMENT INDUSTRY RES & DESIGN INSTITUTE	CN113812439 (A); CN113812439 (B)	A DHA functional biscuit, characterized by comprising 5-15 parts of microalgae containing DHA or microalgae cell residue after partial DHA oil is extracted, 20-30 parts of low-gluten flour, 0.1-0.2 parts of baking powder, 3-5 parts of butter, and 0.3-0.5 parts of baking yeast.
ALGAE-BASED FOOD FORMULATION, BREAD-MAKING, BAKERY AND CONFECTIONERY PRODUCTS CONTAINING IT, METHOD FOR OBTAINING THEREOF AND ITS USE	JUAN Y JUAN IND S L UNIPERSONAL [ES]	EP3243520 (A1); EP3243520 (B1)	The invention refers to a food formulation which comprises fresh microalgae in the form of paste of the species Chlorella vulgaris, in a proportion comprised between 10% and 20% of the total weight of the formulation and with an average degree of moisture at room temperature of between 80% and 90%, both inclusive
FOOD ADDITIVE COMPRISING ALGAE	ALGAE BEHEER B V [NL]	EP3967154 (A1); EP3967154 (B1); EP3967154 (C0)	Food additive comprising 10 to 95 wt% of one or more algae, chosen from the group of Chlorellaceae, Arthrospira, Monodopsidaceae and Skeletonemataceae, and 10 to 90 wt% of a fungi extract. The food additive can be a powder, solution, suspension or paste. Preferably, the food additive can be used in the preparation of bread and bread products.

Name of the patent	Applicant	Number	Notes
ALGAE-BASED FOOD FORMULATION, BREAD-MAKING, BAKERY AND CONFECTIONERY PRODUCTS CONTAINING IT, METHOD FOR OBTAINING THEREOF AND ITS USE	S L UNIPERSONAL JUAN Y JUAN IND [ES]	PT3243520 (T)	Exclusively of a microalgae paste fresh of the species Chlorella vulgaris, in a proportion comprised of 14% to 19% of the total weight of the formulation and with an average degree of humidity at room temperature of 81 % to 86%, both inclusive, and in which the microalgae of species Chlorella vulgaris are of the Chlorella strain vulgaris fo. tertia with deposit number CCAP 211/11D.
GLUTEN-FREE BREAD CONTAINING ALGAE FROM THE SPECIES HIMANTHALIA ELONGATA AND METHOD FOR PRODUCING SAID BREAD	UNIV LEON [ES]	WO2019162545 (A1)	Gluten-free bread characterized by comprising a flour of at least one gluten-free cereal, gluten-free sourdough 20-30%; an algae of the species Himanthalia elongata 5-8%; water 68-72%; salt 1.5-2.2%; dehydrated yeast 1.5-2%; corn starch 1%, milk powder 29-31%; sugar 4-6%; raising agent 1%; egg 9-11% and vegetable oil 5.5-6.25%; where these proportions are expressed by weight and referred to the quantity of flour.
Dietetic wholemeal bread	URBAN FERNAND [FR]	FR2617680 (A1); FR2617680 (B1)	**Dietary bread with magnesium**, characterized by being prepared from dough with the following composition: • Organic wholemeal flour 43.20% • Natural sourdough 25.90% • Water 25.60% • Liquid chicory extracts 2.20% • Magnesium chloride 0.83% • Wheat germ 0.83% • Nutrient-rich algae 0.60% • Sea salt 0.56% • Soy lecithin 0.28% • (Sunflower seeds for topping)

8. RESEARCH PAPERS

8.1 SELECTION

Extended search was done using Research Gate, Google Scholars and AI Perplexity tool.

Which microalgae species were used?
Were Was biomass or an extract used?
What was What was the concentration/amount of the algal component in the product (e.g., bread)? (e.g. bread)?
What technology was used for incorporating microalgae into the product (e.g. bread)?
Were benefits on health observed?
What was results?
Other information relevant to our project.

Application of Spirulina as an innovative ingredient in pasta and bakery products ((Nejatian et al., 2024)

1. Which microalgae species were used?
The study focuses on *Spirulina* (*Arthrospira spp.*), particularly *Arthrospira maxima* and *Arthrospira platensis*. These species are commercially known as Spirulina and are widely used due to their rich nutritional profile.

2. Was biomass or an extract used?
The research incorporated both whole *Spirulina* biomass (powder) and ethanol-treated Spirulina in various bakery and pasta products. The ethanol-treated Spirulina was used to mitigate its strong sensory impact, particularly its odor and taste.

3. What was the concentration/amount of the algal component in the product (e.g., bread)?
The amount of *Spirulina* used varied across different products:

- **Pasta:** 2–20% (optimum levels were below 20% for acceptable sensory and technological properties)
- **Bread:** 1–10% (optimum level ~10%)
- **Cookies:** 1–5% (acceptable up to 5%)
- **Biscuits:** 0.5–3% (higher levels affected taste and texture)
- **Cakes:** 0.5–1.5%

The study emphasized that excessive inclusion (>10% in most cases) negatively impacted sensory properties such as taste, odor, and color.

4. What technology was used to incorporate microalgae into the product (e.g., bread)?

- *Kneading and mixing:* Spirulina powder was mixed directly into dough formulations.
- *Encapsulation:* Encapsulation was explored as a strategy to mask the undesirable taste and odor.
- *Ethanol treatment:* Ethanol-washed *Spirulina* was used to reduce its strong odor and pigment effect.
- *Baking and drying:* The impact of heat on bioactive compounds such as phycocyanin and polyphenols was studied.

5. Were any health benefits observed?
Several health benefits were linked to *Spirulina* incorporation in bakery products:

- **Rich in proteins and essential amino acids** (protein content increased significantly in fortified products)
- **High in minerals** (iron, zinc, calcium, magnesium)
- **Antioxidant properties** (phenolic compounds and phycocyanin contributed to reducing oxidative stress)
- **Potential to lower cholesterol and blood sugar levels** (noted in other studies related to *Spirulina* consumption)
- **Anti-inflammatory effects** (polyphenols and omega-6 fatty acids present in *Spirulina*)

6. Results

- *Nutritional enhancement:* All *Spirulina*-fortified products showed significant improvements in protein content, minerals, and antioxidants.
- *Textural changes:* Products with higher *Spirulina* content (>10%) experienced altered dough consistency, making them denser or firmer.
- *Color changes:* High *Spirulina* levels caused an intense green color, which was less acceptable in cookies and bread but acceptable in pasta.
- *Sensory acceptability:* Encapsulation and ethanol treatment improved the overall acceptability, but the fishy odor and taste remained an issue at high levels.

7. Other Relevant Information for Bio2AgroFood Project

- **Targeted products:** Pasta, bread, and cookies were the best candidates for *Spirulina* incorporation due to their ability to mask some of its sensory issues.
- **Consumer acceptability:** Encapsulation techniques and complementary flavors (such as cocoa) were proposed solutions for overcoming taste issues.
- **Industrial applications:** The study supports the idea that *Spirulina* can be used in functional foods but suggests further research on optimizing sensory and processing methods.

Consumer perception and acceptability of microalgae based breadstick (García-Segovia, García Alcaraz, Tárrega, & Martínez-Monzó, 2020)

1. Which microalgae species were used?
The study used *Tetraselmis chuii*, a microalgae species that was approved as a novel food in the European Union in 2013. It has a nutritional profile similar to *Chlorella*, but with a distinctive marine flavor.

2. Was biomass or an extract used?
The research incorporated whole *Tetraselmis chuii* biomass (powder) into the breadstick formulation.

3. What was the concentration/amount of the algal component in the product (e.g., bread)?
The study tested different concentrations of *Tetraselmis chuii*, with the final product containing:

- **0.5%**
- **1%**
- **1.5%** (based on wheat flour weight)

4. What technology was used to incorporate microalgae into the product (e.g., bread)?

- The microalgae powder was directly **mixed into the dough**.
- The dough was **kneaded twice** for uniform distribution.
- **Fermentation at 33°C for 60 minutes**.
- **Pre-baking at 180°C for 4 minutes** followed by **baking at 160°C for 10 minutes**.

5. Were any health benefits observed?
Consumers associated the microalgae breadsticks with **higher nutritional value**:

- **Healthier perception**: Considered more nutritious and beneficial due to fiber and other bioactive compounds.
- **Rich in marine-derived nutrients**: Potential benefits from minerals and antioxidants.
- **Potential as a functional food**: Seen as a sustainable and healthy snack option.

6. Results

- **Consumer acceptance**: Despite initial skepticism, *Tetraselmis chuii* breadsticks were **as acceptable as the control breadsticks** after tasting.
- **Sensory properties**:
  - More noticeable differences in **color (greenish tint), flavor (marine/vegetal), and odor**.
  - Some consumers **perceived an off-flavor** that negatively influenced their acceptance.
- **Expectations vs. Reality**:
  - Consumers initially **expected greater differences** from regular breadsticks.
  - However, **after tasting, differences were perceived as lower** than expected.
- **Willingness to pay**: Consumers understood that these breadsticks might be more expensive due to added health benefits.

7. Other Relevant Information for Bio2AgroFood Project

- **Potential market positioning**: The product was seen as a **vegetarian snack**, **functional food**, and **suitable for vending machines**.
- **Challenges**: The **marine-like flavor and color** may require **further sensory optimization** to increase broader acceptance.
- **Marketing insights**:
  - Focus on **health-conscious consumers** who are interested in **natural, sustainable, and nutrient-rich foods**.
  - Targeting **snack and functional food categories** could increase commercial success.

Development and optimization of high-protein and low-saturated fat bread formulations enriched with lupin and microalgae (Pereira et al., 2024)

1. Which microalgae species were used?
The study used *Chlorella vulgaris*, specifically two variants:

- *Chlorella vulgaris White*
- *Chlorella vulgaris Smooth*
  The selected strains had reduced chlorophyll content to minimize the grassy flavor, making them more suitable for food applications.

2. Was biomass or an extract used?
Whole biomass of *Chlorella vulgaris* was used, in a **4:1 ratio of C. vulgaris White to C. vulgaris Smooth*.

3. What was the concentration/amount of the algal component in the product (e.g., bread)?

- **Microalgae concentration in flour mix: 1.9–3.9%** (w/w of flour)
- The final optimized breads contained:
  - **2.4% microalgae (w/w of flour) in rye-lupin bread**
  - **2.9% microalgae (w/w of flour) in spelt-lupin bread**
  - **1.9% microalgae (w/w of flour) in oats-carob-lupin bread**

4. What technology was used to incorporate microalgae into the product (e.g., bread)?

- **Dough preparation:**

    - Microalgae powder was directly mixed into dry ingredients.
    - Kneaded for **10 minutes** using a food processor.
- **Fermentation: 60 minutes at 35°C**
- **Baking: 30 minutes at 200°C**
- **Optimization via Response Surface Methodology (RSM):**
    - Used to determine ideal water and microalgae content for the best sensory properties.

5. Were any health benefits observed?
- **High in protein:** Increased protein content by **82–91%** compared to regular wheat bread.
- **Low in saturated fat:** All formulations met regulatory standards for "low in saturated fat" labeling.
- **Improved fatty acid profile:** Higher monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA).
- **Potential mineral enrichment:** Higher ash content suggested increased mineral content (not analyzed in detail).

6. Results
- **Nutritional improvements:**
    - Higher protein, healthy fats, and lower carbohydrates compared to wheat bread.
- **Sensory acceptability:**
    - Rye-lupin formulation had the highest acceptance.
    - Spelt-lupin formulation had the lowest scores due to bitterness.
    - Water content significantly influenced texture and acceptability.
- **Shelf-life:**
    - Microbial stability confirmed for **5 days**.
    - Increasing hardness and decreasing moisture over storage, similar to conventional bread.

7. Other Relevant Information for Bio2AgroFood Project
- **Alternative flours (lupin, rye, oats, spelt, carob) can help balance taste and nutrition in microalgae bread.**
- **Optimization of water content is crucial** to mitigate bitterness and improve texture.
- **Further research suggested** on mineral content and antioxidant properties.

Development of new microalgae-based sourdough "crostini": functional effects of Arthrospira platensis (spirulina) addition (Niccolai et al., 2019)

1. Which microalgae species were used?
The study used *Arthrospira platensis* (commonly known as Spirulina), specifically the strain *A. platensis F&M-C256*.

2. Was biomass or an extract used?
Whole biomass of *A. platensis* was used in powdered form. The biomass was cultivated, harvested, washed, and dried at **low temperature (33°C) for 20 hours** before being incorporated into the dough.

3. What was the concentration/amount of the algal component in the product (e.g., bread)?
Three concentrations of *A. platensis* were tested:
- **2% (w/w)**
- **6% (w/w)**
- **10% (w/w)**

These were added by replacing an equivalent proportion of wheat flour in the formulation.

4. What technology was used to incorporate microalgae into the product (e.g., bread)?

- **Sourdough fermentation**: Used as the leavening and fermentation method, with *Lactobacillus farciminis* and *Saccharomyces cerevisiae* as the main microbial cultures.
- **Kneading**: Ingredients mixed in a **twin-arm mixer** at **50 rpm for 10 minutes**.
- **Proofing**: Doughs were proofed at **25°C for 25 minutes**.
- **Fermentation**: Conducted at **30°C for 2 hours**.
- **Baking**: **160°C for 11 minutes**.
- **Toasting**: **140°C for 14 minutes** to obtain the final "crostini".

5. Were any health benefits observed?

- **High protein content**: *A. platensis*-enriched crostini had **44–69% more protein** than the control.
- **Rich in bioactive compounds**:
    - **Phycocyanin** (a blue antioxidant protein) was retained in the product.
    - **Phenolic compounds** significantly increased, boosting antioxidant properties.
- **Improved nutritional claims**:
    - Crostini with **≥6% *A. platensis*** qualified as a "**source of protein**" per **EU nutrition regulations**.
- **Antioxidant properties**: Increased radical-scavenging activity, primarily due to **phycocyanin and phenolic compounds**.

6. Results

- **Technological properties**:
    - Doughs with higher *A. platensis* levels showed **lower volume increase** after fermentation, likely due to interference with the gluten network.
    - The microbial community was not negatively affected by microalgae supplementation.
- **Nutritional impact**:
    - Higher **ash content** (+50–61%) due to residual minerals from *A. platensis*.
    - No significant changes in **fat or carbohydrate content**.
    - Energy content **429–450 kcal/100 g**.
- **Digestibility**:
    - In vitro **protein digestibility was lower** in *A. platensis* crostini than in control crostini.
    - Despite this, crostini with 10% *A. platensis* still had **higher absolute protein digestibility** due to its high protein content.
- **Color and sensory evaluation**:
    - Higher *A. platensis* levels resulted in **darker color** with a reduced yellow hue.
    - **2% *A. platensis* crostini had the best sensory acceptance**, rated higher than the control.
    - **6% and 10% crostini had lower acceptability**, with more than 25% of panelists saying they "would not buy" them.

7. Other Relevant Information for Bio2AgroFood Project

- **Sourdough fermentation can enhance microalgae-enriched bakery products** by improving digestibility and flavor.
- **High *A. platensis* concentrations negatively affect gluten structure**, requiring further optimization.
- **Educational marketing strategies** could help increase consumer acceptance of microalgae-enriched baked goods.

Effect of Arthrospira platensis (spirulina) incorporation on the rheological and bioactive properties of gluten-free fresh pasta (Fradinho et al., 2020)

1. Which microalgae species were used?
The study used *Arthrospira platensis* (commonly known as Spirulina), with two variants:

- **F&M-C256 strain** (provided by Società Agricola Serenissima, Italy)
- **Ox Nature commercial strain** (spray-dried, sourced from China)

2. Was biomass or an extract used?
Whole biomass of *A. platensis* was used, in powder form. The two variants were processed differently:

- **F&M-C256**: Cultivated in **photobioreactors**, harvested, centrifuged, washed, frozen (-20°C), **lyophilized**, powdered, and stored at -20°C.
- **Ox Nature**: Commercially available **spray-dried** product.

3. What was the concentration/amount of the algal component in the product (e.g., bread)?

- *A. platensis* was incorporated into gluten-free pasta at **1%, 2%, and 3% (w/w)**.
- The pasta dough was made with **rice flour and Psyllium gel** as a gluten substitute.
- Levels above 3% led to **high adhesiveness, poor manufacturing abilities, and strong fishy odor**, making processing impractical.

4. What technology was used to incorporate microalgae into the product (e.g., bread)?

- **Dough preparation**:
  - Psyllium husk and rice flour were mixed in a food processor for **3 minutes**.
  - Dough was **covered in foil and rested for 15 minutes at 25°C**.
  - Dough was **laminated into tagliatelle** shape.
- **Cooking and drying**:
  - Pasta samples were **cooked for 1 minute**, then **lyophilized and powdered** for biochemical analyses.
- **Texture, rheology, and digestibility tests** were performed.

5. Were Benefits on Health Observed?
Yes, the study identified several nutritional and functional benefits:

- **Increased protein content** (up to **8.9%** in 3% *A. platensis* pasta, compared to **3.9%** in the control).
- **High antioxidant activity** due to **phenolic compounds, chlorophylls, carotenoids, and phycocyanin**.
- **Improved nutritional profile**, with reduced carbohydrates and higher bioactive compounds.
- **Good consumer acceptability at 2% incorporation**.

6. What Were the Results?

- **Cooking quality**:
  - *A. platensis* pasta absorbed **more water** than the control.
  - Cooking loss was higher but within acceptable ranges.
- **Texture and rheology**:
  - Increased *A. platensis* led to **less elasticity**.
  - Texture remained acceptable at **2% incorporation**.
- **Sensory acceptance**:
  - **2% *A. platensis*-enriched pasta had the best consumer acceptance**.
  - **Higher levels (3%) reduced acceptability** due to fishy flavor.
  - **58% of panelists would buy the 2% pasta**.
- **Nutritional composition**:
  - Higher **protein and mineral content** than the control.
  - Significant presence of **bioactive pigments** (chlorophyll, carotenoids, phycocyanin).

7. Were any tests conducted on bioactivity, bioavailability, digestion, or prebiotic effects?

- **In vitro digestibility tests** showed:
    - **Protein digestibility decreased at 3% incorporation** for *A. platensis* F&M-C256.
    - **Carbohydrate digestibility remained stable** across formulations.
    - The **Ox Nature strain improved protein digestibility** slightly.
- **Antioxidant capacity tests**:
    - **DPPH radical scavenging activity was significantly increased** in *A. platensis* pasta.
    - **Phenolic content was higher** in spray-dried *A. platensis* pasta.

8. Other Information Relevant to the Bio2AgroFood Project

- **Optimized incorporation level for functional gluten-free pasta is 2% *A. platensis***.
- **Spray-dried *A. platensis* retained more phenolic compounds** than lyophilized biomass.
- **Microalgae incorporation in pasta requires balancing texture, digestibility, and sensory properties**.
- **Further studies on improving sensory properties (masking fishy odor) are needed**.

Effects of baking on the biochemical composition of Chlorella vulgaris (Gelgör, Ozcelik, & Haznedaroglu, 2022)

1. Which microalgae species were used?
The study focused on *Chlorella vulgaris* (strain CCAP 211/11B), a widely consumed microalga recognized for its high nutritional value.

2. Was biomass or an extract used?
Whole biomass of *C. vulgaris* was used. The biomass was cultivated in **Bold 3N medium**, harvested at the **early stationary phase**, freeze-dried, and subjected to baking.

3. What was the concentration/amount of the algal component in the product (e.g., bread)?

- The paper does not specify the exact amount of *C. vulgaris* used in a final food product.
- However, the study tested the effects of **baking at 125°C for 15 minutes** on the biochemical composition of the biomass.

4. What technology was used to incorporate microalgae into the product (e.g., bread)?

- **Baking at 125°C for 15 minutes** was applied to *C. vulgaris* biomass to assess the impact on biochemical stability.
- The **baked and raw (control) samples** were analyzed for carbohydrates, proteins, lipids, and vitamins.

5. Were Benefits on Health Observed?
Yes, *C. vulgaris* retained its **nutritional value** after baking:

- **High protein content** (~21% of dry weight (DW) after baking, 22.7% in raw biomass).
- **Rich in carbohydrates** (~25.7% DW after baking, 27.3% in raw biomass).
- **Good lipid content** (~9.3% DW after baking, 7.3% in raw biomass).
- **High in vitamins**, particularly B-complex and vitamin C.

6. What Were the Results?

- **Nutritional impact of baking:**
    - No **significant changes** in carbohydrate, protein, and lipid contents.
    - **Vitamin B1, B2, and B3 levels increased after baking**—possibly due to better extraction from the cell wall.
    - **Vitamin B3 saw the highest increase (from 237.6 to 1943.1 µg/100 g)**.
    - **Vitamin C slightly decreased (~21% loss).**

- **Thermal stability of nutrients:**
    - Most **vitamins remained stable**, except **B3 increased**, and **vitamin C slightly degraded**.
- **Potential as a functional ingredient:**
    - Retains **high protein and vitamin B12 content**, making it **a strong candidate for plant-based diets**.

7. Were any tests conducted on bioactivity, bioavailability, digestion, or prebiotic effects?
- The study **did not test digestion or bioavailability**, but it suggested that baking might enhance vitamin extraction.
- The **increase in B1, B2, and B3** after baking suggests **improved nutrient accessibility** due to **cell wall breakdown**.

8. Other Information Relevant to the Bio2AgroFood Project
- *C. vulgaris* can be **baked at 125°C for 15 minutes** without losing key nutrients, making it **suitable for bakery applications** like **cookies, granola bars, and crackers**.
- Further research should explore **protein digestibility, bioactivity, and sensory optimization**.

Enhancing the Protein, Mineral Content, and Bioactivity of Wheat Bread through the Utilisation of Microalgal Biomass: A Comparative Study of Chlorella vulgaris, Phaeodactylum tricornutum, and Tetraselmis chuii (Mahmoud, Ferreira, Raymundo, & Nunes, 2024)

1. Which microalgae species were used?
The study used three microalgae species:
- *Chlorella vulgaris*
- *Phaeodactylum tricornutum*
- *Tetraselmis chuii*

2. Was biomass or an extract used?
Whole **freeze-dried** biomass of all three microalgae species was used.

3. What was the concentration/amount of the algal component in the product (e.g., bread)?
- The microalgae were incorporated at **4% (w/w) of wheat flour** in bread formulations.

4. What technology was used to incorporate microalgae into the product (e.g., bread)?
- **Dough preparation:**
    - The microalgae biomass was mixed directly into wheat flour.
    - The dough was kneaded using a **thermomixer** (Bimby Vorwerk) at **37°C for 30s**.
    - Additional kneading in **"ear position" mode for 150s**.
- **Fermentation: 60 minutes at 35°C**.
- **Baking: 180°C for 20 minutes**.

5. Were Benefits on Health Observed?
Yes, multiple benefits were identified:
- **Increased protein content** (up to **14.7% higher** in *C. vulgaris*-enriched bread).
- **Higher mineral content**, including:
    - **Iron (Fe)**
    - **Calcium (Ca)**
    - **Magnesium (Mg)**
    - **Phosphorus (P)**
- **Rich in bioactive compounds**, particularly in *Tetraselmis chuii*, which increased **total phenolic content (TPC) and antioxidant capacity**.

- **Improved antioxidant activity**:
    - FRAP values increased from **117.29 to 591.96 µg TEAC/g** (T. chuii bread).
    - DPPH radical scavenging activity also increased significantly.

6. What Were the Results?

- **Textural and structural changes:**
    - *C. vulgaris* caused **a significant reduction in bread volume (-22.7%)** and **increased firmness**.
    - *T. chuii* and *P. tricornutum* did not affect bread volume.
    - All microalgae caused **dark green coloration** of the bread.
- **Rheology and dough properties:**
    - *T. chuii* strengthened the dough network, leading to better texture.
    - *C. vulgaris* disrupted the gluten network, weakening dough stability.
    - All microalgae increased **water absorption capacity**.
- **Moisture and shelf-life factors:**
    - Bread with *T. chuii* had **lower moisture content**, potentially improving shelf life.
    - Water activity (aw) was slightly lower in *T. chuii*- and *P. tricornutum*-enriched bread, which could help in microbial stability.

7. Were any tests conducted on bioactivity, bioavailability, digestion, or prebiotic effects?

- **Bioactivity tests:**
    - **Total phenolic content (TPC) increased significantly**, especially in *T. chuii*.
    - **Antioxidant assays (DPPH and FRAP) confirmed increased antioxidant activity**.
- **No direct bioavailability or digestion studies were performed**.

8. Other Information Relevant to the Bio2AgroFood Project

- *T. chuii* was the best-performing microalgae in terms of **texture, bioactivity, and antioxidant content**.
- *C. vulgaris* caused **dough weakening and lower bread volume**, which might require **process optimization**.
- The **4% incorporation level was optimal for functional benefits without major sensory issues**.
- Further studies on **digestibility, sensory optimization, and consumer acceptance are recommended**.

Evaluation of nutritional and sensory properties of bread enriched with Spirulina (Hafsa, Amel, Samia, & Sidahmed, 2014)

1. Which microalgae species were used?

- *Arthrospira fusiformis* (Spirulina)

2. Was biomass or an extract used?

- Whole **dry biomass** of *Spirulina* was used.
- The biomass was **milled into powder** before incorporation into bread.

3. What was the concentration/amount of the algal component in the product (e.g., bread)?

- Two enrichment levels were tested:
    - **1% (w/w)**
    - **3% (w/w)**

4. What technology was used to incorporate microalgae into the product (e.g., bread)?

- **Dough preparation:**
    - Flour, yeast, sugar, salt, water, fat, and *Spirulina* biomass were **mixed directly**.
    - Kneading was performed in a **BOMANN automated mixer at 90 rpm**.
- **Fermentation:**
    - First fermentation: **45 minutes**.
    - Second fermentation (proofing): **1 hour**.
- **Baking:**
    - **250°C for 20 minutes** in an electric oven.
- **Cooling & Storage:**
    - Bread loaves were cooled at room temperature for **1 hour** before packaging in polyethylene bags.

5. Were Benefits on Health Observed?
Yes, several health benefits were observed:
- **Protein content increased significantly**:
    - **8.18% in control bread → 9.98% in 3% *Spirulina* bread** (+22% increase).
- **Higher mineral content**:
    - **Ash content** increased from **1.76% to 2.32%**.
- **Fat content increased** slightly with *Spirulina* addition.
- **No major changes in carbohydrate and fiber content**.
- **Energy value increased**, with **3% *Spirulina* bread providing 254.23 kcal/100g**.

6. What Were the Results?
- **Physical properties:**
    - **Slight reduction in bread volume** was observed (**specific volume decreased significantly**).
    - **Density remained stable** across all formulations.
    - **Crust thickness remained unaffected**.
- **Sensory evaluation:**
    - **1% *Spirulina* bread was the most accepted**.
    - **3% *Spirulina* bread had a strong green color, making it less appealing**.
    - **Taste, texture, and aroma were well accepted** in both enriched breads.
    - **Characteristic spirulina odor was masked, even at 3% supplementation**.

7. Were any tests conducted on bioactivity, bioavailability, digestion, or prebiotic effects?
- No direct **bioavailability** or **digestion** tests were performed.
- The study focused on **protein, fat, and mineral improvements** as indicators of enhanced
- **nutritional bioactivity**.

8. Other Information Relevant to the Bio2AgroFood Project
- *Spirulina*-enriched bread showed **good nutritional benefits** with **acceptable sensory properties at 1% inclusion**.
- **3% inclusion resulted in strong green coloration**, which **affected consumer perception**.
- **Further research could explore optimization of color-masking strategies** and **enhanced bioavailability testing**.

Evaluation of the Prospects for the Use of Microalgae in Functional Bread Production (Sukhikh et al., 2022)

1. Which microalgae species were used?
- *Arthrospira platensis* (Spirulina)
- *Chlorella vulgaris*

2. Was biomass or an extract used?

- Whole **dry biomass** of both *A. platensis* and *C. vulgaris* was used.
- The biomass was cultivated, dried at **30–40°C for 12 hours**, and ground into powder.

3. What was the concentration/amount of the algal component in the product (e.g., bread)?

- The bread was formulated with **three different levels of microalgae**:
  - **1% (0.5% Spirulina, 0.5% Chlorella)**
  - **3% (1.5% Spirulina, 1.5% Chlorella)**
  - **5% (2.5% Spirulina, 2.5% Chlorella)**

4. What technology was used to incorporate microalgae into the product (e.g., bread)?

- **Dough preparation:**
  - The microalgae mixture was incorporated directly into wheat flour.
  - The dough was prepared using a **straight dough method**.
- **Fermentation:**
  - **First fermentation: 30 minutes**.
  - **Proofing: 50 minutes**.
- **Baking:**
  - **210°C for 25 minutes**.
- **Post-baking handling:**
  - Bread was cooled before testing and sensory evaluation.

5. Were Benefits on Health Observed?

Yes, several health benefits were identified:

- **Increased polyphenol content**, leading to **higher antioxidant capacity**.
- **Higher protein and mineral content**.
- **Lower pH (more acidic) at 5% microalgae**, which might aid digestion.
- **Moisture content increased with higher microalgae concentration**, improving softness and shelf-life.

6. What Were the Results?

- **Physical properties:**
  - Bread with **1% and 3% microalgae had good volume and texture**.
  - Bread with **5% microalgae had lower volume** and **denser texture**.
  - **Increased moisture content** at higher microalgae levels.
- **Sensory evaluation:**
  - **1% and 3% microalgae breads were well accepted**.
  - **5% microalgae bread had a stronger fishy smell and slight sour taste**.
  - **Greenish color intensified with more microalgae**.
- **Antioxidant properties:**
  - **Higher polyphenol content** in 3% and 5% microalgae bread.
  - **FRAP and DPPH antioxidant assays confirmed increased antioxidant capacity**.

7. Were any tests conducted on bioactivity, bioavailability, digestion, or prebiotic effects?

- **Bioactivity tests**:
  - **Total phenol content was measured** and found to be significantly higher in 3% and 5% microalgae bread.
  - **FRAP and DPPH assays confirmed high antioxidant activity**.
- **No direct digestion or prebiotic effect tests were conducted**, but the **increase in polyphenols and antioxidant activity suggests potential functional benefits**.

8. Other Information Relevant to the Bio2AgroFood Project

- **1–3% inclusion is optimal for balancing nutritional benefits and sensory properties**.
- **5% microalgae addition negatively impacted taste and consumer acceptance**.
- **Further research could explore optimizing the sensory attributes** and **evaluating digestibility improvements**.

Exploring the Nutritional Potential of Microalgae in the Formulation of Bakery Products
(Hernández-López et al., 2024)

1. Which microalgae species were used?

- *Spirulina* (*Arthrospira platensis*)
- *Chlorella* (*Chlorella vulgaris*)
- *Tetraselmis* (*Tetraselmis chuii*)

2. Was biomass or an extract used?

- Whole **dry biomass** of each microalgae species was used.
- The biomass was cultivated, dried, and ground into powder.

3. What was the concentration/amount of the algal component in the product (e.g., bread)?

Microalgae were incorporated into two types of baked products:

- **Crackers** and **Grissini** (breadsticks)
- Substitution of flour with microalgae:
    - **1.5% (w/w)**
    - **2.5% (w/w)**
    - **3.5% (w/w)**

4. What technology was used to incorporate microalgae into the product (e.g., bread)?

- **Dough preparation:**
    - Ingredients were mixed with a **bread dough mixer**.
    - The dough was kneaded at **low speed for 4 minutes** and then at **medium speed for 3 minutes**.
    - The dough was rested before shaping.
- **Baking:**
    - **Crackers:** 170°C for **14 minutes**.
    - **Grissini:** 170°C for **18 minutes**.

5. Were Benefits on Health Observed?

Yes, several nutritional benefits were observed:

- **Increased protein content**, especially in Spirulina and Chlorella-enriched products.
- **Higher antioxidant capacity** (FRAP assay).
- **Increased total phenolic content (TPC)**.
- **Improved amino acid composition**, with higher levels of:
    - **Alanine, aspartate, and tryptophan (Spirulina crackers)**
    - **Isoleucine, leucine, lysine, and valine (Chlorella grissini)**
- **Potential immune-boosting and anti-inflammatory properties** from β-glucans (especially in Chlorella).
- **High levels of omega-3 and omega-6 fatty acids**, especially in Spirulina.

6. What Were the Results?

- **Sensory evaluation:**

    - **Crackers with 1.5% Spirulina** and **Grissini with 3.5% Chlorella** had the best overall acceptance.
    - **Tetraselmis was less accepted** due to its dark green color and salty taste.
    - Higher microalgae content (3.5%) resulted in a **stronger marine flavor**, which some consumers found unappealing.
- **Texture and structure:**
    - **Spirulina crackers became softer at higher concentrations.**
    - **Chlorella grissini became denser and harder** with increasing concentration.
    - **Tetraselmis increased hardness** in both products.
- **Color changes:**
    - Higher microalgae concentration led to a **darker green color**.
    - **Spirulina and Tetraselmis resulted in darker shades**, while Chlorella caused a **lighter green hue**.
- **Antioxidant properties:**
    - FRAP and TPC tests showed **significant increases** in polyphenol content and antioxidant activity.
    - **Crackers with 1.5% Spirulina** and **Grissini with 3.5% Chlorella** had the highest antioxidant values.

7. Were any tests conducted on bioactivity, bioavailability, digestion, or prebiotic effects?
- **In vitro gastrointestinal digestion assay was conducted.**
    - **Antioxidant activity increased significantly after digestion.**
    - **Polyphenol content increased after digestion, suggesting good bioavailability.**
- **Essential amino acid profiling was performed.**
    - **Spirulina crackers were rich in alanine, aspartate, and tryptophan.**
    - **Chlorella grissini had higher isoleucine, leucine, lysine, and valine.**
- **No specific prebiotic effects were tested,** but β-glucans in Chlorella suggest potential gut health benefits.

8. Other Information Relevant to the Bio2AgroFood Project
- **1.5–3.5% inclusion is optimal for functional benefits while maintaining acceptability.**
- **Spirulina and Chlorella performed best in sensory evaluations.**
- **Further studies could explore masking marine flavors for broader consumer acceptance.**

Chlorella sorokiniana: A new alternative source of carotenoids and proteins for gluten-free bread
(Diprat, Silveira Thys, Rodrigues, & Rech, 2020)

1. Which microalgae species were used?
- *Chlorella sorokiniana*

2. Was biomass or an extract used?
- Whole **dry biomass** of *C. sorokiniana* was used.
- The biomass was milled into powder before incorporation into gluten-free bread.

3. What was the concentration/amount of the algal component in the product (e.g., bread)?
- The bread was formulated with:
    - **2.5% (M2.5)**
    - **5.0% (M5.0)**

      These percentages replaced **pea flour** in a gluten-free formulation made of **rice flour and corn starch (30:70 ratio).**

4. What technology was used to incorporate microalgae into the product (e.g., bread)?

- **Dough preparation:**
    - The ingredients were mixed with a **bread dough mixer**.
    - Dough portions (180 g) were placed into baking pans and proofed at **32°C, 80% relative humidity for 1 hour**.
- **Baking:**
    - Two temperature-time conditions were tested:
        - **180°C for 15 minutes**
        - **220°C for 12 minutes**
    - The **higher temperature (220°C) resulted in better nutrient retention**.
- **Post-baking handling:**
    - Bread samples were **cooled at room temperature for 1 hour** before analysis.

5. Were Benefits on Health Observed?

Yes, several nutritional and functional benefits were observed:

- **Increased protein content**:
    - **6.7% in control → 7.6% in M2.5 → 8.5% in M5.0**.
- **Higher lipid content**, especially omega-3 fatty acids:
    - **3.5% in control → 4.4% in M2.5 → 5.7% in M5.0**.
- **Rich in bioactive carotenoids**:
    - Lutein increased **from 1.6 µg/g (control) to 57.5 µg/g (M5.0) at 220°C**.
    - The **bread could be considered a functional food** due to its high lutein content.
- **Increased omega-3 fatty acids**:
    - **5.0% in control → 6.1% in M5.0**.
- **Potential antioxidant and anti-inflammatory benefits**.

6. What Were the Results?

- **Physical properties:**
    - **Bread volume remained stable** despite microalgae addition.
    - **Hardness and chewiness were unaffected**.
    - **Green color intensified with more *C. sorokiniana***.
- **Sensory evaluation:**
    - **M2.5 bread had good acceptance (>70%)**.
    - **M5.0 had lower acceptability due to strong green color and slightly bitter taste**.
    - **Some panelists noted a "seaweed" aroma**.
- **Nutritional impact:**
    - **10- to 40-fold increase in carotenoids**, depending on baking conditions.
    - **Baking at 220°C preserved more lutein**.
    - **Polyunsaturated fatty acids remained stable** after baking.

7. Were any tests conducted on bioactivity, bioavailability, digestion, or prebiotic effects?

- **Carotenoid retention analysis**:
    - Lutein content was measured before and after baking.
    - Baking at **220°C retained more lutein than 180°C**.
- **No direct digestion or bioavailability tests** were conducted, but **antioxidant and nutritional benefits were demonstrated**.

8. Other Information Relevant to the Bio2AgroFood Project

- *C. sorokiniana* **is a promising ingredient for gluten-free functional bread**.
- **2.5% inclusion provided the best balance between nutrition and sensory acceptance**.
- **Higher levels (>5%) may require formulation adjustments** to improve taste and appearance.
- **Further research on digestibility and consumer adaptation is recommended**.

Impact of Chlorella vulgaris on the rheology of wheat flour dough and bread texture (Graça, Fradinho, Sousa, & Raymundo, 2018)

1. Which microalgae species were used?

- *Chlorella vulgaris*

2. Was biomass or an extract used?

- Whole **dry biomass** of *Chlorella vulgaris* was used.
- The biomass was **milled into powder** before incorporation into wheat-based bread.

3. What was the concentration/amount of the algal component in the product (e.g., bread)?

- Bread formulations included:
  - **1.0 g per 100 g wheat flour**
  - **2.0 g per 100 g wheat flour**
  - **3.0 g per 100 g wheat flour**
  - **4.0 g per 100 g wheat flour**
  - **5.0 g per 100 g wheat flour**

4. What technology was used to incorporate microalgae into the product (e.g., bread)?

- **Dough preparation:**
  - *C. vulgaris* powder was mixed into wheat flour.
  - Kneading was performed in a **thermo-processor**.
  - **Fermentation: 60 minutes at 37°C** in an electric oven.
- **Baking:**
  - **160°C for 30 minutes**.
- **Storage:**
  - Breads were stored in **sealed plastic bags at room temperature** for texture analysis over time.

5. Were Benefits on Health Observed?

Yes, *C. vulgaris*-enriched bread provided multiple benefits:

- **Higher protein content**, due to *C. vulgaris* (~60% protein content).
- **Rich in vitamin B12 (220 µg/100 g)**.
- **Increased iron (120 mg/100 g) and chlorophyll content**.
- **Antioxidant potential from bioactive compounds**.

6. What Were the Results?

- **Dough properties:**
  - Up to **3.0 g *C. vulgaris*/100 g wheat flour**, the gluten network was strengthened.
  - Above **3.0 g *C. vulgaris*/100 g**, the gluten network was disrupted, leading to **lower dough elasticity and bread volume**.
- **Texture and sensory evaluation:**
  - Bread containing **up to 3.0 g *C. vulgaris*** had **good texture and volume**.
  - Higher levels **(4–5 g)** resulted in **denser, firmer bread**.
  - **Green color intensified** with increasing *C. vulgaris* content.
  - **A mild marine taste was detected**, more noticeable at higher concentrations.
- **Fermentation and baking properties:**
  - No significant changes in **fermentation time**.
  - **Higher *C. vulgaris* levels (4–5 g) accelerated bread aging** (firmer texture over time).

7. Were any tests conducted on bioactivity, bioavailability, digestion, or prebiotic effects?

- **Dough rheology tests were conducted**:
  - **Farinograph analysis** showed increased water absorption at **up to 3.0 g *C. vulgaris***.
  - **Alveograph tests** confirmed weakened gluten networks at **>3.0 g inclusion**.
- **Bread aging analysis:**
  - Higher *C. vulgaris* levels accelerated bread **firmness over 72 hours**.
- **No digestion or prebiotic effect tests were performed**.

8. Other Information Relevant to the Bio2AgroFood Project

- **3.0 g *C. vulgaris*/100 g wheat flour is the optimal inclusion level** for maintaining **dough elasticity, bread volume, and consumer acceptability**.
- **Higher concentrations (>3%) negatively impact texture, appearance, and aging**.
- **Potential applications for microalgae-enriched bakery products beyond bread** (e.g., biscuits or crackers).

Improving the Nutritional, Structural, and Sensory Properties of Gluten-Free Bread with Different Species of Microalgae (M. W. Qazi, de Sousa, Nunes, & Raymundo, 2022)

1. Which microalgae species were used?

- *Tetraselmis chuii* (Tc)
- *Chlorella vulgaris* (Cv)
- *Nannochloropsis gaditana* (Ng)

2. Was biomass or an extract used?

- Whole **dry biomass** of all three species was used.
- Ethanol-treated biomass was also tested to **remove pigments and odor compounds**.

3. What was the concentration/amount of the algal component in the product (e.g., bread)?

- **4% (w/w) replacement of flour with microalgae biomass**.
- The same percentage was tested for ethanol-treated biomasses (**TcT, CvT, NgT**).

4. What technology was used to incorporate microalgae into the product (e.g., bread)?

- **Dough preparation:**
  - Algal biomass was mixed with gluten-free (GF) ingredients (**buckwheat, rice flour, potato starch**).
  - Dough was mixed in a **Micro-doughLab 2800 (Perten Instruments)**.
- **Fermentation:**
  - Proofing at **30°C for 1 hour**.
- **Baking:**
  - **180°C for 20 minutes**.

5. Were Benefits on Health Observed?

Yes, several nutritional and health benefits were observed:

- **Higher protein content** (especially with *Chlorella vulgaris* and *Nannochloropsis gaditana*).
- **Increased mineral content**:
  - *Tetraselmis chuii* was particularly rich in **calcium (6× higher than other algae species)**.
  - Iron, zinc, and phosphorus levels also increased.
- **Higher antioxidant activity** (especially in *Tetraselmis chuii* and *Nannochloropsis gaditana*).
- **Improved dietary fiber content**.

6. What Were the Results?

- **Texture and dough structure:**

- **Ethanol-treated biomass improved dough strength**, making the bread **softer (23–65% improvement)** and **higher in volume (12–27% increase)**.
    - **Non-treated biomass weakened dough elasticity**.
- **Sensory acceptance:**
    - **Ethanol-treated algae breads received similar scores to control GF bread**.
    - **Untreated algae breads had stronger green color and odor**, reducing acceptance.
    - **Nannochloropsis (NgT) performed the best in sensory evaluation**.
- **Bioactivity and antioxidant capacity:**
    - **Higher total phenolic content (TPC) and antioxidant activity** in untreated algae breads.
    - **Ethanol treatment reduced antioxidant activity** but **improved sensory and technological properties**.

7. Were any tests conducted on bioactivity, bioavailability, digestion, or prebiotic effects?

- **Antioxidant capacity tests (DPPH, FRAP) confirmed increased bioactivity**.
- **Ethanol treatment significantly reduced chlorophylls and pigments**.
- **No direct digestion or prebiotic effect tests were conducted**, but the **increase in polyphenols suggests potential functional benefits**.

8. Other Information Relevant to the Bio2AgroFood Project

- **Ethanol treatment is a promising method for improving sensory acceptance** of microalgae-enriched baked goods.
- **4% inclusion of ethanol-treated *Nannochloropsis gaditana* (NgT) provided the best balance between nutrition and consumer acceptability**.
- **Further research is recommended on digestion and sensory optimization**.

Interaction of dough acidity and microalga level on bread quality and antioxidant properties
(Garzon, Skendi, Antonio Lazo-Velez, Papageorgiou, & Rosell, 2021)

1. Which microalgae species were used?

- *Chlorella vulgaris*

2. Was biomass or an extract used?

- Whole **dry biomass** of *C. vulgaris* was used.

3. What was the concentration/amount of the algal component in the product (e.g., bread)?

- Bread was enriched with **1%, 2%, and 3% (w/w) *C. vulgaris***.
- Two types of acidification were tested:
    - **10% sourdough**
    - **Chemically acidified dough** (with lactic and acetic acids)

4. What technology was used to incorporate microalgae into the product (e.g., bread)?

- **Dough preparation:**
    - Microalgae biomass was mixed directly into wheat flour.
    - Doughs were fermented with **baker's yeast, sourdough, or chemical acidification**.
- **Fermentation:**
    - Proofing at **30°C until volume doubled** (~50-60 min).
- **Baking:**
    - **180°C for 15 minutes**.
- **Post-baking handling:**
    - Breads were cooled for **45 minutes at room temperature** before testing.

5. Were Benefits on Health Observed?
Yes, multiple nutritional and health benefits were observed:

- **Higher protein content**:
  - **12.17% in control → 13.75% in 3% *C. vulgaris* bread**.
- **Increased mineral content**:
  - Iron, zinc, calcium, and phosphorus levels were higher.
- **Higher antioxidant capacity**:
  - **Total phenolic content (TPC) increased significantly**.
  - **Antioxidant activity (FRAP, DPPH, ABTS assays) was higher in *C. vulgaris*-enriched breads**.
- **Potential benefits from dough acidification**:
  - Acidified doughs showed **even higher antioxidant activity**.

6. What Were the Results?

- **Dough and bread properties:**
  - Water absorption increased with *C. vulgaris* addition.
  - Higher algae levels led to **denser and firmer bread**.
  - **Acidified doughs produced softer breads with better texture**.
- **Color and sensory evaluation:**
  - **Greenish color intensified with more *C. vulgaris***.
  - **Acidified doughs produced lighter crumb color**.
  - **Sourdough and chemically acidified breads had better acceptability** than neutral pH doughs.
- **Bioactivity and antioxidant capacity:**
  - **Bread antioxidant levels were highest in acidified *C. vulgaris* breads**.
  - **Dough acidification increased polyphenol extraction and bioactivity**.

7. Were any tests conducted on bioactivity, bioavailability, digestion, or prebiotic effects?

- **Bioactivity tests were conducted**:
  - **Total phenolic content (TPC) increased with *C. vulgaris* and acidification**.
  - **FRAP, DPPH, and ABTS assays confirmed higher antioxidant activity**.
- **No direct digestion or prebiotic effect tests were performed**, but results suggest improved polyphenol bioavailability due to **acidification**.

8. Other Information Relevant to the Bio2AgroFood Project

- **Dough acidification (sourdough or chemical) enhances antioxidant properties** of *C. vulgaris*-enriched breads.
- **Sourdough and chemically acidified doughs improved texture, making microalgae bread more acceptable**.
- **Further research on prebiotic potential and digestibility of microalgae in sourdough bread is recommended**.

Microalgae biomass as an additional ingredient of gluten-free bread: Dough rheology, texture quality and nutritional properties (Khemiri et al., 2020)

1. Which microalgae species were used?

- *Nannochloropsis gaditana* L2
- *Chlamydomonas* sp. EL5

2. Was biomass or an extract used?

- Whole **dry biomass** of both species was used.

- The biomass was cultivated in artificial seawater, harvested by centrifugation, and freeze-dried before incorporation into gluten-free bread.

3. What was the concentration/amount of the algal component in the product (e.g., bread)?

- Two levels of incorporation were tested:
  - **1.0 g per 100 g flour (1%)**
  - **3.0 g per 100 g flour (3%)**

4. What technology was used to incorporate microalgae into the product (e.g., bread)?

- **Dough preparation:**
  - Microalgae biomass was mixed into a gluten-free flour blend (rice flour, buckwheat flour, and potato starch).
  - The dough was mixed for **10 minutes** and proofed for **50 minutes at 40°C**.
- **Baking:**
  - **180°C for 30 minutes**.
- **Post-baking handling:**
  - Bread samples were cooled at room temperature, packed in plastic, and stored for further analysis.

5. Were Benefits on Health Observed?

Yes, significant nutritional benefits were observed:

- **Increased protein content**:
  - **5.6% (control) → 6.6% (3% microalgae-enriched bread)**.
- **Higher mineral content**:
  - **Iron content doubled** (from **1.9 mg/100 g in control** to **8.3–10.5 mg/100 g in enriched bread**).
  - **Calcium content increased more than fourfold** (from **12 mg/100 g to 51.6 mg/100 g**).
- **Improved fatty acid profile**:
  - Higher **omega-3 (18:3 ω3) and omega-6 fatty acids**.
  - **Reduced ω3/ω6 ratio**, which is beneficial for health.

6. What Were the Results?

- **Dough and bread properties:**
  - Microalgae addition **did not significantly alter dough mixing behavior**.
  - **Bread texture improved**, with increased firmness and adhesiveness.
  - **Green coloration intensified**, especially with *Chlamydomonas* sp.
- **Sensory evaluation:**
  - **Bread with 3% *Nannochloropsis gaditana* had the highest consumer acceptance**.
  - **Panelists appreciated the green color and improved texture**.
  - **No strong marine odor was detected**.
- **Bioactivity and antioxidant capacity:**
  - Higher **polyphenol content and antioxidant activity** were reported.
  - **Significant increases in iron and calcium suggest improved nutritional functionality**.

7. Were any tests conducted on bioactivity, bioavailability, digestion, or prebiotic effects?

- **Fatty acid analysis confirmed increased omega-3 content**.
- **No direct digestion or prebiotic effect tests were conducted**, but the **high iron and calcium content suggests good bioavailability**.

8. Other Information Relevant to the Bio2AgroFood Project

- **3% *Nannochloropsis gaditana* inclusion provided the best balance between nutrition and sensory acceptance**.
- **No strong fishy flavor detected**, making it a promising gluten-free bread ingredient.
- **Further studies could focus on bioavailability and digestibility improvements**.

Microalgae Incorporated in Bakery Products: Application to Millet Pretzel (Maiti, Lal, & Masih, 2024)

1. Which microalgae species were used?

- *Chlorella vulgaris*

2. Was biomass or an extract used?

- Whole **dry biomass** of *Chlorella vulgaris* was used.
- The biomass was obtained from **Amedeo Ventures Private Limited, India**.

3. What was the concentration/amount of the algal component in the product (e.g., bread)?

- Three concentrations were tested:
  - **0.6% (w/w)**
  - **0.8% (w/w)**
  - **1.0% (w/w)**
- Two different flour blends were used:
  - **CW (80% foxtail millet, 20% little millet)**
  - **TS (75% foxtail millet, 25% little millet)**

4. What technology was used to incorporate microalgae into the product (e.g., bread)?

- **Dough preparation:**
  - Microalgae biomass was mixed directly into a millet flour blend.
  - Yeast was proofed in **lukewarm sugar water**.
  - Psyllium husk and xanthan gum were used to enhance structure.
  - Dough was kneaded and left to **rise for 70 minutes**.
- **Baking Process:**
  - Pretzels were shaped and dipped in **hot water with baking soda for 30 seconds**.
  - **Baking at 200–210°C for 40–45 minutes**.

5. Were Benefits on Health Observed?

Yes, multiple nutritional benefits were observed:

- **Protein content increased with higher *C. vulgaris* inclusion**:
  - **1.75% in control → 1.93% in 1.0% *C. vulgaris* pretzels**.
- **Higher mineral (ash) content**:
  - **1.65% in control → 1.95% in 1.0% *C. vulgaris* pretzels**.
- **Potential functional food benefits**:
  - Increased protein makes pretzels **a viable protein-rich snack**.
  - No significant moisture loss was observed.

6. What Were the Results?

- **Physical properties:**
  - Moisture content **decreased with increasing *C. vulgaris* concentration**.
  - **Green color was well-accepted** by sensory panelists.
- **Sensory evaluation:**
  - **Pretzels with 0.8% *C. vulgaris* had the highest overall acceptance**.
  - The **fishy off-flavor was neutralized by millet flour**, making the product more appealing.

    - **1.0% *C. vulgaris* had higher protein but lower sensory acceptance**.
- **Nutritional analysis:**
    - **Ash content increased with microalgae inclusion**.
    - **Protein content was significantly higher in 1.0% *C. vulgaris* pretzels**.

7. Were any tests conducted on bioactivity, bioavailability, digestion, or prebiotic effects?
- **Protein content was analyzed using the Kjeldahl method**.
- **No digestion or prebiotic effect tests were conducted**.

8. Other Information Relevant to the Bio2AgroFood Project
- **0.8% *C. vulgaris* is the optimal level for balancing nutrition and sensory properties**.
- **No strong off-flavors were detected**, making this formulation promising.
- **Further studies on digestibility and consumer preference in different markets are recommended**.

Nutritional and functional assessment of fortified bread with psychrotolerant and mesophilic microalgae (Görünmek, Kibar, Çakmak, & Çakmak, 2025)

1. Which microalgae species were used?
- *Chlorella vulgaris* (Psychrotolerant strain ASYA27 from Antarctica)
- *Micractinium simplicissimum* (Psychrotolerant strain ASYA46 from Antarctica)
- *Chlorella vulgaris* (Mesophilic strain ARAS102 from a volcanic lake in Türkiye)

2. Was biomass or an extract used?
- Whole **lyophilized biomass** was used.
- The microalgae were **cultivated in photobioreactors**, centrifuged, washed, and **freeze-dried** into a powder.

3. What was the concentration/amount of the algal component in the product (e.g., bread)?
- **1% (w/w) and 3% (w/w)** microalgae were added to:
    - **Whole wheat bread** formulations
    - **Gluten-free bread** formulations (using oat flour)

4. What technology was used to incorporate microalgae into the product (e.g., bread)?
- **Dough preparation:**
    - Microalgae biomass was **mixed with dry ingredients**.
    - Yeast fermentation followed at **30°C**.
- **Baking:**
    - **180°C for 20 minutes**.
- **Post-processing:**
    - Baked bread was **lyophilized and stored in cryo-boxes** for analysis.

5. Were Benefits on Health Observed?
Yes, significant nutritional benefits were observed:
- **Increased protein content**:
    - **Control: 9.2 g/100 g → 12.3 g/100 g in 3% *C. vulgaris* bread**.
- **Higher mineral content**:
    - Iron content **increased from 2.8 mg to 5.8 mg/100 g**.
    - Magnesium, calcium, and phosphorus levels significantly improved.
- **Higher vitamin content**:
    - **Vitamin B1, B2, and B6 were enriched**.

- **Rich in dietary fiber**:
  - **Gluten-free bread with *Micractinium simplicissimum* (3%) had a 74% increase in fiber content**.
- **Lower glycemic index**:
  - **GI reduced by 23% in gluten-free bread with 3% *Micractinium simplicissimum***.
- **Improved fatty acid profile**:
  - **PUFA (polyunsaturated fatty acids) increased significantly**.
  - **Omega-3 levels increased by 142% in whole wheat bread with *M. simplicissimum***.
- **Higher antioxidant activity**:
  - Bread with **3% *M. simplicissimum* showed a 38% increase in antioxidant content**.

6. What Were the Results?

- **Texture and structure:**
  - Increased water absorption.
  - Bread with **3% microalgae was firmer but still acceptable**.
- **Sensory evaluation:**
  - **1% microalgae bread was more accepted** due to milder color and taste.
  - **Green color intensified at 3% but was not strongly disliked**.
- **Bioactivity improvements:**
  - **Lower glycemic index** → Could help manage blood sugar levels.
  - **Rich amino acid profile** → Comparable to eggs in nutritional value.

7. Were any tests conducted on bioactivity, bioavailability, digestion, or prebiotic effects?

- **In vitro glycemic index test confirmed a significant GI reduction**.
- **Total dietary fiber content analysis was conducted**.
- **FAME analysis showed a strong omega-3 and PUFA increase**.
- **Antioxidant capacity (DPPH test) confirmed higher bioactivity**.
- **Amino acid profiling showed increased essential amino acids**.
- **Mineral analysis confirmed increased iron, calcium, and magnesium bioavailability**.

8. Other Information Relevant to the Bio2AgroFood Project

- **Psychrotolerant microalgae strains (from Antarctica) had the best nutritional impact**.
- **3% inclusion gave the highest nutritional benefits but altered bread texture**.
- **1% inclusion was the best compromise between nutrition and sensory acceptance**.
- **Further studies on prebiotic potential and consumer acceptance needed**.

Phenolic compounds in dietary target the regulation of gut microbiota: Role in health and disease (Zhang et al., 2024)

1. Which microalgae species were used?

- No microalgae species were used in this study.
- However, the research **discusses the impact of plant-derived phenolic compounds**, which are **also present in some microalgae**.

2. Was biomass or an extract used?

- The study focuses on **dietary phenolic compounds (PCs)** found in plants, fermented foods, and grains.
- No microalgal biomass or extracts were used, but the insights **apply to microalgae-derived bioactive compounds**.

3. What was the concentration/amount of the algal component in the product (e.g., bread)?

- Not applicable, as the study does not include microalgae-based products.

4. What technology was used to incorporate microalgae into the product (e.g., bread)?
- Not applicable to this study.

5. Were Benefits on Health Observed?
Yes, the paper **strongly supports** the role of phenolic compounds in promoting **gut health and overall well-being**:
- **Gut microbiota modulation**:
  - PCs can **increase beneficial bacteria** like **Lactobacillus, Bifidobacterium, and Akkermansia muciniphila**.
  - PCs **reduce harmful bacteria** and help maintain **gut microbial balance**.
- **Metabolic health**:
  - PCs improve **insulin sensitivity, lipid metabolism**, and **reduce obesity risk**.
  - **Lower glycemic response** was observed in some studies.
- **Cardiovascular benefits**:
  - PCs help reduce **hypertension and atherosclerosis risk** by modulating gut microbiota.
- **Neurological protection**:
  - PCs **reduce neuroinflammation**, **lower oxidative stress**, and **support cognitive function**.
- **Cancer prevention**:
  - PCs have **anti-tumor effects**, modulate gut bacteria, and may **enhance immune response**.

6. What Were the Results?
- **Bioavailability challenges**:
  - **Only 5-10% of PCs are absorbed in the small intestine**; most reach the colon, where **gut bacteria metabolize them into bioactive compounds**.
  - Gut microbiota **enzymatically transform** phenolics, enhancing their activity.
- **Bioactivity improvement**:
  - **Phenolic metabolites produced by gut bacteria are more bioavailable and potent** than the original compounds.
- **Short-chain fatty acid (SCFA) production**:
  - PCs stimulate **SCFA-producing bacteria**, improving **gut barrier function and immunity**.
- **Inflammation reduction**:
  - PCs can **regulate immune cells (T-cells, macrophages)** and **reduce inflammation via NF-kB inhibition**.

7. Were any tests conducted on bioactivity, bioavailability, digestion, or prebiotic effects?
Yes, the study **provides a comprehensive review of bioavailability and gut microbiota interactions**:
- **Gut microbiota plays a key role in metabolizing phenolics** into bioavailable forms.
- **Metabolic pathways** such as **hydrolysis, reduction, and fermentation** were reviewed.
- **SCFA production was analyzed** in relation to phenolic intake.
- **Prebiotic effects were confirmed** as PCs enhanced beneficial gut bacteria.

8. Other Information Relevant to the Bio2AgroFood Project
- **These insights are crucial for microalgae research**, as **some microalgae species contain polyphenols and flavonoids**.
- **Microalgae-enriched functional foods could be designed** to **support gut microbiota and health**.

- **Future research should investigate how microalgae-derived phenolics impact digestion, SCFA production, and gut health**.

Polyunsaturated Fatty Acids as Prebiotics: Innovation or Confirmation? (Rinninella & Costantini, 2022)

1. Which microalgae species were used?

- No specific **microalgae** were studied, but **PUFAs (omega-3 and omega-6) from dietary sources** were analyzed.
- Since **microalgae (e.g., *Nannochloropsis*, *Schizochytrium*, *Chlorella*) are key sources of omega-3 PUFAs**, these findings are applicable.

2. Was biomass or an extract used?

- The study did **not** use biomass or extracts but focused on **PUFA metabolism in gut microbiota**.

3. What was the concentration/amount of the algal component in the product (e.g., bread)?

- The paper reviewed **500 mg/day omega-3 PUFA supplementation** (EPA/DHA).
- Compared it with **20 g/day inulin (a known prebiotic)** in a **6-week human intervention study**.

4. What technology was used to incorporate microalgae into the product (e.g., bread)?

- **Dietary intervention study in humans**.
- **PUFA metabolism by gut microbiota was analyzed in vivo**.

5. Were Benefits on Health Observed?

Yes, multiple health benefits were observed, especially related to **gut microbiota and inflammation regulation**:

- **Microbiota modulation**:
  - **Omega-3 PUFAs increased Coprococcus and Bacteroides**.
  - **Inulin increased Bifidobacterium and Lachnospiraceae**.
- **Short-chain fatty acid (SCFA) production**:
  - Both inulin and omega-3 PUFAs **increased iso-valerate and iso-butyrate production**, which have **anti-inflammatory effects**.
- **Anti-inflammatory properties**:
  - PUFA metabolites reduced **TNF-α, IL-6, and IL-1β**, lowering chronic inflammation.
- **Potential metabolic benefits**:
  - PUFA-derived gut metabolites **regulated glucose homeostasis and insulin sensitivity**.
  - Improved lipid metabolism by activating **G-protein coupled receptor (GPR40 and GPR120)** pathways.

6. What Were the Results?

- **Prebiotic effects were confirmed for PUFAs**:
  - PUFA metabolism by gut microbiota produced **anti-inflammatory lipid mediators**.
  - **Reduced lipid absorption and increased gut motility** via EP receptor activation.
- **PUFA metabolism products had diverse health effects**:
  - **10-hydroxy-cis-12-octadecenoic acid (HYA)** and **10-oxo-trans-11-octadecenoic acid (KetoC)** reduced **oxidative stress and inflammation**.
  - **KetoA activated TRPV1 receptors**, enhancing energy metabolism and reducing obesity risk.
- **Gut microbiota played a critical role**:

- **Lactobacillus species metabolized omega-6 PUFAs** into beneficial compounds.
- **More studies are needed on omega-3 PUFA metabolism**.

7. Were any tests conducted on bioactivity, bioavailability, digestion, or prebiotic effects?
Yes, the study included:

- **SCFA production analysis (iso-valerate, iso-butyrate) in humans**.
- **Gut microbiota changes following PUFA supplementation**.
- **Metabolite analysis of omega-6 PUFA transformation into bioactive compounds**.
- **No direct bioavailability or digestion tests**, but metabolic transformations suggest **enhanced bioactivity**.

8. Other Information Relevant to the Bio2AgroFood Project

- **PUFA-rich microalgae could serve as functional prebiotic ingredients**.
- **Further research is needed on omega-3 PUFA metabolism by gut microbiota**.
- **Combination of microalgae-derived PUFAs with traditional prebiotics (e.g., fiber) could enhance gut health benefits**.

Potential of the microalgae Nannochloropsis and Tetraselmis for being used as innovative ingredients in baked goods(Lafarga et al., 2019)

1. Which microalgae species were used?

- *Nannochloropsis* sp.
- *Tetraselmis* sp.

2. Was biomass or an extract used?

- Whole **dry biomass** of both species was used.
- Biomass was incorporated as **a flour substitute**.

3. What was the concentration/amount of the algal component in the product (e.g., bread)?

- **Breads**:
    - *Nannochloropsis*: **1.0% (w/w)**
    - *Tetraselmis*: **2.0% (w/w)**
- **Crackers**:
    - Both *Nannochloropsis* and *Tetraselmis*: **2.5% (w/w)**

4. What technology was used to incorporate microalgae into the product (e.g., bread)?

- **Dough preparation**:
    - The microalgae biomass was **mixed with wheat flour**.
    - Doughs were kneaded and proofed.
- **Baking**:
    - **Bread**: **180°C for 20 minutes**.
    - **Crackers**: **Baked at 200°C**.
- **Post-processing**:
    - Products were **analyzed for sensory, nutritional, and bioactivity properties**.

5. Were Benefits on Health Observed?
Yes, several **nutritional and functional benefits** were reported:

- **Increased polyphenol content**:
    - Crackers:
        - **Control: 24.6 mg/100 g → 34.2 mg/100 g (Nannochloropsis)**
        - **Control: 24.6 mg/100 g → 32.4 mg/100 g (Tetraselmis)**

- **Higher antioxidant capacity**:
    - Bread and crackers with microalgae **showed significantly higher FRAP and DPPH antioxidant activity**.
- **Improved bioaccessibility**:
    - **More bioavailable polyphenols** after simulated gastrointestinal digestion.
- **Higher emission of volatile compounds**:
    - *p-Cymene* and *(Z)-2-heptenal*, which contribute **fresh, citrus, and fruity notes**, were more present in microalgae-containing crackers.

6. What Were the Results?

- **Physicochemical properties**:
    - **Color**: Microalgae incorporation resulted in **darker, greener color**.
    - **Texture**: No major differences in texture or structure.
    - **Moisture**: Bread with *Tetraselmis* had slightly lower moisture content.
    - **Shelf-life stability**: No major changes in color or texture over **10 days of storage**.
- **Sensory acceptance**:
    - **Bread with 2.0% *Tetraselmis* and 1.0% *Nannochloropsis* had high acceptability (>70%)**.
    - **Crackers with 2.5% of both microalgae had even better acceptance (85.9% for *Tetraselmis*, 79.8% for *Nannochloropsis*)**.
    - **No strong fishy odor or off-flavor was reported**, making these formulations viable.

7. Were any tests conducted on bioactivity, bioavailability, digestion, or prebiotic effects?

- **In vitro gastrointestinal digestion was performed**:
    - **Bioaccessible polyphenols were higher** than in control products.
    - **Antioxidant capacity increased after digestion**, suggesting enhanced bioactivity.
- **Volatile compound analysis**:
    - Microalgae-containing products had increased **terpenic and citrus aroma compounds**.
- **No direct prebiotic or digestion effect tests were conducted**, but **higher bioactive compound availability was confirmed**.

8. Other Information Relevant to the Bio2AgroFood Project

- **2.0% *Tetraselmis* and 1.0% *Nannochloropsis* are optimal inclusion levels for bread**.
- **2.5% inclusion in crackers showed strong nutritional and sensory benefits**.
- **No major structural or sensory drawbacks** were found, making these microalgae promising **functional ingredients for baked goods**.
- **Further studies on digestibility and gut microbiota effects are recommended**.

Protein Enrichment of Wheat Bread with Microalgae : Microchloropsis gaditana, Tetraselmis chui and Chlorella vulgaris (W. M. Qazi et al., 2021)

1. Which microalgae species were used?

- *Microchloropsis gaditana* (previously *Nannochloropsis gaditana*)
- *Tetraselmis chui*
- *Chlorella vulgaris*

2. Was biomass or an extract used?

- Whole **cell wall-disrupted, dried biomass** was used.
- Biomass was tested in **raw form** and **ethanol-treated** to remove pigments and strong odors.

3. What was the concentration/amount of the algal component in the product (e.g., bread)?

- Microalgae replaced **12% of wheat flour** to **increase bread protein content by 30%**.
- Ethanol-treated biomass was used to **improve sensory acceptance**.

4. What technology was used to incorporate microalgae into the product (e.g., bread)?

- **Dough preparation:**
  - Microalgae biomass was mixed with **high-protein wheat flour**.
  - Dough kneaded using a **Farinograph DoughLab**.
- **Fermentation:**
  - Proofing at **30°C until volume doubled (~60 min)**.
- **Baking:**
  - **180°C for 20 minutes**.
- **Post-processing:**
  - Bread was stored for **sensory and nutritional analysis**.

5. Were Benefits on Health Observed?

Yes, several nutritional and functional benefits were observed:

- **Increased protein content**:
  - **Control: 13.14 g/100 g → 19.16 g/100 g in 12% *Microchloropsis*-enriched bread**.
- **Higher mineral content**:
  - **Iron, magnesium, and calcium levels increased significantly**.
- **Improved amino acid profile**:
  - **Lysine content increased (~30%)**, improving **protein quality**.
- **Higher digestible indispensable amino acid score (DIAAS)**:
  - **Control: 46% → *Microchloropsis* bread: 66% DIAAS**.
- **Rich in omega-3 fatty acids** (especially from *Microchloropsis gaditana*).

6. What Were the Results?

- **Dough and bread properties**:
  - **Microalgae weakened dough strength** (especially *Chlorella* and *Tetraselmis*).
  - **Bread with *Microchloropsis* retained good volume and texture**.
  - **Ethanol-treated biomass improved texture and elasticity**.
- **Sensory evaluation**:
  - **Untreated microalgae caused strong odors and dark colors**.
  - **Ethanol-treated *Microchloropsis* had the best acceptance**.
  - **Tetraselmis-enriched bread had a mild seaweed taste but was acceptable**.
- **Bioactivity improvements**:
  - **Higher antioxidant activity** (DPPH, FRAP assays).
  - **No significant lipid oxidation observed after baking**.

7. Were any tests conducted on bioactivity, bioavailability, digestion, or prebiotic effects?

- **In vitro protein digestibility tests confirmed improved digestibility**.
  - **Protein digestibility (DSEC method) was highest in *Microchloropsis*-enriched bread**.
- **No direct gut microbiota or prebiotic effect studies were conducted**, but **higher bioactive compound retention was confirmed**.

8. Other Information Relevant to the Bio2AgroFood Project

- **Ethanol treatment improves microalgae sensory acceptance without major nutritional loss**.
- **12% *Microchloropsis* is optimal for protein fortification with good baking performance**.
- **Further studies on consumer acceptance and digestion needed**.

Tetraselmis chuii as a sustainable and healthy ingredient to produce gluten-free bread: Impact on structure, colour and bioactivity (Nunes et al., 2020)

1. Which microalgae species were used?

- *Tetraselmis chuii*

2. Was biomass or an extract used?

- Whole **freeze-dried biomass**.
- Biomass was **pre-treated by bead milling** to **disrupt the cell wall**, improving bioactive compound release.

3. What was the concentration/amount of the algal component in the product (e.g., bread)?

- *T. chuii* was incorporated at **1%, 2%, and 4% (w/w)** in gluten-free (GF) bread formulations.

4. What technology was used to incorporate microalgae into the product (e.g., bread)?

- **Dough preparation:**
  - *T. chuii* was **mixed with GF flour blend (buckwheat, rice flour, and potato starch)**.
  - Dough was mixed using a **thermoprocessor** for 10 minutes.
  - Proofing at **37°C for 50 minutes**.
- **Baking:**
  - **180°C for 50 minutes**.
- **Post-processing:**
  - Breads were cooled for **2 hours** before analysis.

5. Were Benefits on Health Observed?

Yes, multiple nutritional benefits were observed:

- **Higher protein content**:
  - *T. chuii* contains **47.7% protein**, improving GF bread nutritional value.
- **Increased total phenolic content (TPC)**:
  - **Control bread: 0.11 mg/g gallic acid equivalents**.
  - **4% *T. chuii* bread: 0.24 mg/g (2.2× increase)**.
- **Higher antioxidant capacity**:
  - **DPPH assay: +17% in 4% *T. chuii* bread**.
  - **FRAP assay: +42% in 4% *T. chuii* bread**.
- **Omega-3 fatty acids and vitamin E**:
  - *T. chuii* contributes **EPA (3.6%) and α-tocopherol**.

6. What Were the Results?

- **Dough and bread properties**:
  - **1% and 2% *T. chuii* weakened the dough structure** (lower elasticity and higher firmness).
  - **4% *T. chuii* led to structure recovery**, increasing volume and softening crumb texture.
- **Texture and structure:**
  - **Bread firmness increased up to 2% *T. chuii*, then decreased at 4%**.
  - **4% *T. chuii* resulted in improved cohesiveness and better air retention**.
- **Sensory evaluation:**
  - **1% *T. chuii* had the best overall acceptance (score: 3.84/5)**.
  - **4% *T. chuii* bread had a green color and seaweed-like aroma**, reducing acceptability to **3.25/5**.
  - Some panelists suggested **it would pair well with fish dishes**.
- **Color changes:**
  - **Darker greenish crumb and crust at higher *T. chuii* levels**.

- *Total color difference (ΔE) exceeded 64**, meaning a **visible color shift**.

7. Were any tests conducted on bioactivity, bioavailability, digestion, or prebiotic effects?

- **Total phenolic content (TPC) and antioxidant assays (DPPH, FRAP) were performed**.
- **No direct digestion or prebiotic effect tests were conducted**, but **bioactive compound retention was confirmed**.

8. Other Information Relevant to the Bio2AgroFood Project

- **4% *T. chuii* improves bioactivity while maintaining acceptable bread structure**.
- **1% *T. chuii* is better for general consumer acceptance**.
- **Further studies could focus on improving sensory properties** to increase consumer preference.

The chemistry and bioactive properties behind microalgae-enriched gluten-free breads (Freitas, Ferreira, Nunes, & Raymundo, 2024)

1. Which microalgae species were used?

- *Tetraselmis chuii*
- *Chlorella vulgaris*
- *Microchloropsis gaditana* (formerly *Nannochloropsis gaditana*)

2. Was biomass or an extract used?

- Whole **freeze-dried biomass** was used.
- Biomass was **ethanol-treated** in some formulations to remove pigments and improve sensory attributes.

3. What was the concentration/amount of the algal component in the product (e.g., bread)?

- **4% (w/w)** replacement of gluten-free flour with microalgae biomass.
- Both **raw** and **ethanol-treated biomass** were tested for each species.

4. What technology was used to incorporate microalgae into the product (e.g., bread)?

- **Dough preparation:**
  - *T. chuii, C. vulgaris, and M. gaditana* were mixed with a **gluten-free flour blend (buckwheat, rice flour, and potato starch).**
  - The dough was mixed and fermented at **30°C for 50 minutes.**
- **Baking:**
  - **180°C for 50 minutes.**
- **Post-processing:**
  - Breads were cooled for **2 hours** before analysis.

5. Were Benefits on Health Observed?

Yes, multiple **nutritional and functional benefits** were observed:

- **Higher protein content**:
  - **Control: 8.35 g/100 g DW → Microalgae-enriched breads: up to 12.3 g/100 g DW**.
  - *Microchloropsis gaditana*-enriched bread had the highest protein increase.
- **Higher mineral content**:
  - *Tetraselmis chuii*-enriched bread had the **highest calcium (Ca) and iron (Fe) content**.
  - *C. vulgaris* and *M. gaditana* contributed **higher phosphorus (P), magnesium (Mg), and zinc (Zn).**
- **Rich in omega-3 fatty acids and lipophilic bioactives**:
  - **9,12-octadecadienoic acid** was the most abundant fatty acid.

- **Antioxidant properties**:
  - *Microchloropsis gaditana* had **24% higher terpenes**, including **γ-terpinene**.
  - **DPPH and FRAP assays confirmed increased antioxidant capacity**.

6. What Were the Results?

- **Protein and mineral bioaccessibility after digestion**:
  - **Protein bioaccessibility decreased by ~55%** after **in vitro digestion**.
  - **Iron bioaccessibility was only ~12–26%**, indicating low intestinal absorption.
  - **Phytic acid in microalgae may contribute to reduced mineral bioavailability.**
- **Texture and structure**:
  - **Bread firmness increased slightly with microalgae addition**.
  - **Color changes**: Higher microalgae levels resulted in a **darker greenish color**.
- **Sensory evaluation**:
  - **Ethanol-treated biomass improved sensory acceptance**.
  - **4% *Microchloropsis gaditana* and *Tetraselmis chuii* resulted in good consumer acceptance**.
  - **Raw *Chlorella vulgaris* had stronger marine odor**, affecting preference.
- **Bioactivity improvements**:
  - **Antioxidant capacity increased significantly with all three microalgae species**.
  - **Volatile analysis confirmed lower odor intensity with ethanol-treated biomass**.

7. Were any tests conducted on bioactivity, bioavailability, digestion, or prebiotic effects?

- **In vitro digestion assay confirmed reduced protein and mineral bioaccessibility**.
- **Antidiabetic potential was tested using α-amylase inhibition assay**:
  - **No significant α-amylase inhibition was detected**, indicating **no direct antidiabetic effect**.
- **Total phenolic content (TPC) and antioxidant capacity (DPPH, FRAP) were analyzed**:
  - **Bioactive retention was confirmed after baking**.
- **Lipophilic bioactives were analyzed (fatty acids, alcohols, terpenes)**.

8. Other Information Relevant to the Bio2AgroFood Project

- **Ethanol treatment significantly improved sensory acceptance without reducing bioactivity**.
- **4% inclusion is ideal for balancing nutritional benefits and consumer acceptability**.
- **Phytic acid and fiber in microalgae may impact protein and mineral digestibility**.
- **Further studies needed on prebiotic effects and gut microbiota interactions**.

8.2 COMPARATIVE SUMMARY OF MICROALGAE-ENRICHED BAKERY PRODUCTS RESEARCH

1. Which microalgae species were used?

Microalgae	Studies
Chlorella vulgaris	Most studied; used in bread, crackers, and gluten-free products
Tetraselmis chuii	Found in gluten-free bread and crackers
Microchloropsis gaditana (*Nannochloropsis gaditana*)	Used in high-protein bread and gluten-free formulations
Arthrospira platensis (*Spirulina*)	Used in pasta, sourdough crostini, and breadsticks
Nannochloropsis sp.	Used in functional wheat and GF bread

Micractinium simplicissimum	Found in gluten-free formulations
Chlamydomonas sp.	Used in GF bread and crackers

2. Biomass or Extracts Used

- **Whole biomass (most common)**: Freeze-dried, spray-dried, or bead-milled to improve digestibility.
- **Ethanol-treated biomass**: Used in some studies to remove strong pigments and odors (e.g., *Tetraselmis chuii, Microchloropsis gaditana*).
- **Cell wall-disrupted biomass**: Applied to *Chlorella vulgaris* to improve nutrient bioavailability.

3. Concentration/Volume of Microalgae in Products

Microalgae	Optimal Inclusion (%)	Products
Chlorella vulgaris	1–4%	Bread, crackers, pasta
Tetraselmis chuii	1–4%	Gluten-free bread, crackers
Microchloropsis gaditana	4–12%	High-protein wheat bread
Arthrospira platensis	2–10%	Pasta, sourdough crostini, breadsticks
Nannochloropsis sp.	3–5%	Functional bread
Micractinium simplicissimum	3%	Gluten-free bread
Chlamydomonas sp.	1–3%	Gluten-free bread, crackers

Higher concentrations (>5%) negatively impacted texture and sensory acceptability **due to green coloration and off-flavors.**

4. What technology was used to incorporate microalgae into the product (e.g., bread)?

Process	Effect
Direct mixing with flour	Most common method; simple but can lead to pigment retention and taste issues
Bead milling	Improves digestibility and bioactivity of cell-walled microalgae
Ethanol treatment	Reduces chlorophyll content, improving color and odor
Sourdough fermentation	Enhances digestibility and flavor masking (e.g., *Spirulina* in crostini)
Encapsulation	Suggested in some studies to mask off-flavors

5. Were any health benefits observed?

Benefit	Microalgae Species	Key Findings
Higher protein content	*Chlorella, Microchloropsis, Tetraselmis*	30–50% protein increase in fortified breads
Rich in omega-3 and omega-6	*Microchloropsis, Nannochloropsis, Tetraselmis*	PUFA increase improves anti-inflammatory properties
Mineral enhancement	*Chlorella, Tetraselmis, Spirulina*	Higher **iron, magnesium, calcium, zinc** levels
Higher antioxidant activity	*Chlorella, Microchloropsis, Spirulina*	FRAP/DPPH assays confirmed increased bioactivity

Lower glycemic index	*Micractinium simplicissimum*	23% GI reduction in gluten-free bread
Antidiabetic potential	*Tetraselmis, Chlorella*	α-Amylase inhibition was **not** significant

Combination of microalgae with sourdough fermentation or ethanol treatment improved sensory attributes while retaining nutritional benefits.

6. Key Results Across Studies

Parameter	Optimal Outcome
Texture	Up to **4% inclusion** had minimal negative effects; higher amounts led to firmer textures
Color	Greenish hue intensified with **higher inclusion**, affecting sensory acceptance
Moisture	Increased in most microalgae breads, prolonging freshness
Sensory acceptance	**Best at 1–3% inclusion**; ethanol treatment improved consumer preference

7. Bioactivity, Bioavailability, and Digestion Tests

Test	Findings
In vitro digestion assays	**Protein bioaccessibility decreased ~55% post-digestion**
SCFA production (prebiotic potential)	Not tested, but fiber content suggests potential benefits
Antioxidant assays (DPPH, FRAP)	**Confirmed increased bioactivity** with *Tetraselmis* and *Microchloropsis*
Fatty acid profiling	Increased **PUFAs, linoleic acid, and omega-3 fatty acids** in *Nannochloropsis*
Antidiabetic α-amylase inhibition	**Not significantly altered** in microalgae-enriched breads

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