ITU Journal of Food Science and Technology

ITU/JFST

Investigation of the Use of Microalgae in Ice Cream Formulation

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Abstract: Microalgae have been used in human and animal nutrition since ancient times. Recently, the increase in consumer demands for healthy and nutritious food has led to the development of functional products in the market. The global market, especially for microalgae-based foods and supplements, has great growth potential. Since microalgae contain highly digestible proteins, minerals, vitamins, dietary fibers, carbohydrates and fats, their use as a healthy food supplement has become a trend. Microalgae find extensive applications across various industries owing to their abundant chemical composition and bioactive substance levels. Their abilities in gelling, thickening, and stabilizing have notably facilitated the creation of food additives like agar, alginate, and carrageenan. Furthermore, microalgae serve roles in the food sector as dietary supplements, additives, and natural colorants for functional food products. Ice cream is the most consumed milk dessert with a complex structure containing additives such as colorants, emulsifiers and stabilizers. In addition to its high sugar and fat content, the use of synthetic colorant, stabilizer and emulsifier additives negatively affects consumer preferences. For this reason, research on the use of alternative raw materials to replace fat and sugar by increasing the nutritional value of ice cream has increased recently. Microalgae are also used to improve health due to its functional properties such as antioxidant, anticancer and antiviral activities. The addition of microalgae to ice cream not only makes ice cream a rich source of nutrients, but also increases its preference as a natural colorant. Reducing or removing ice cream ingredients or adding unusual ingredients to the standard formulation should not impair the sensory properties and storage stability of the ice cream. This review has been prepared to bring a different perspective on the nutritional content of microalgae and their uses in the food industry, particularly in ice cream.

Keywords: Microalgae, ice cream, quality, stability.

Dondurma Formülasyonunda Mikroalglerin Kullanım Olanaklarının Araştırılması

Özet: Mikroalgler eski çağlardan beri insan ve hayvan beslenmesinde kullanılmaktadır. Son dönemde tüketicilerin sağlıklı ve besleyici gıdaya yönelik taleplerinin artması, pazarda fonksiyonel ürünlerin gelişmesine yol açmıştır. Özellikle mikroalg bazlı gıdalar ve takviyeler için küresel pazar büyük bir büyüme potansiyeline sahiptir. Mikroalgler yüksek oranda sindirilebilir proteinler, mineraller, vitaminler, diyet lifleri, karbonhidratlar ve yağlar içerdiğinden sağlıklı bir gıda takviyesi olarak kullanımları bir eğilim haline gelmiştir. Mikroalgler zengin kimyasal bileşimleri ve biyoaktif madde içerikleri nedeniyle endüstrinin birçok alanında kullanılmaktadır. Mikroalglerden agar, aljinat ve karragenan gibi jelleştirici, kıvam artırıcı ve stabilize edici katkı maddeleri geliştirilmektedir. Ayrıca mikroalgler gıda endüstrisinde gıda takviyesi ve fonksiyonel gıdalarda katkı maddesi ve renklendirici olarak kullanılmaktadır. Dondurma, renklendirici, emülgatör ve stabilizatör gibi katkı maddeleri içeren karmaşık yapısıyla en çok tüketilen sütlü tatlılardandır. Yüksek şeker ve yağ içeriğinin yanı sıra yapay renklendirici, stabilizatör ve emülgatör katkı maddelerinin kullanımı tüketici tercihlerini olumsuz yönde etkilemektedir. Bu nedenle son zamanlarda dondurmanın besin değerini artırarak yağ ve şeker yerine alternatif hammaddelerin kullanılmasına yönelik araştırmalar artmıştır. Mikroalgler antioksidan, antikanser ve antiviral gibi fonksiyonel özellikleri sayesinde sağlığı iyileştirmek için de kullanılmaktadırlar. Dondurmaya mikroalglerin ilavesi, dondurmayı zengin bir besin kaynağı haline getirmekle kalmayabilir, aynı zamanda doğal bir renklendirici olarak tercih edilmesini de sağlayabilir. Dondurma bileşenlerini azaltmak veya çıkarmak ya da standart formülasyona alışılmadık bileşenler eklemek, dondurmanın duyusal özelliklerini ve stabilitesini bozmamalıdır. Bu derleme, mikroalglerin besin içeriği ve bunların gıda endüstrisinde, özellikle dondurmadaki kullanım olanaklarına farklı bir bakış açısı getirmek amacıyla hazırlanmıştır.

Anahtar Kelimeler: Mikroalg, dondurma, kalite, dayanım.

Review

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Reference: Yalcin, B., Kamiloglu, S., & Kilic-Akyilmaz, M. (2024), Investigation of the use of microalgae in ice cream formulation, *ITU Journal of Food Science and Technology*, *2*(2), 47-56.

Submission Date: 26 February 2024
Online Acceptance: 10 April 2024
Online Publishing: 30 September 2024

1. Introduction

Ice cream is a frozen complex colloidal system consisting of a continuous aqueous phase in which air bubbles, ice crystals, carbohydrates, proteins, partially coalesced fat droplets and minerals are dispersed (Zaaboul et al., 2024). Ice cream mix, which is an important food product for human health, generally consists of a mixture of milk, stabilizers, emulsifiers, colorants and flavouring ingredients (Ozturk et al., 2018).

Increasing knowledge and research related to the relationship between food and health, together with the need for technological innovation, have resulted in new products with functional potential to benefit human health (Gremski et al., 2019). Ice cream, one of the most delicious foods, contains high amounts of fat and sugar. This makes ice cream an interesting product for researching alternative formulations. Enhancing the protein levels in ice cream and lowering its fat and sugar content, or opting for alternative ingredients, can enhance the functional and nutritional attributes of ice cream (da Silva Faresin et al., 2022).

Since synthetic additives are generally believed to be unsafe by the consumers, the food and beverage industry is looking for natural alternatives to improve products. This demand has arisen due to shifts in consumer preferences and emerging trends favouring natural foods with minimal processing and functional foods (Campos Assumpção de Amarante et al., 2020). Microalgae are one of the potential ingredients that can be added to formulations to increase the appeal of ice cream. Considering their macro components (polysaccharides as stabilizers) and micro components (polyunsaturated fatty acids as bioactive compounds and pigments as colouring agents), they have the potential to be used in dairy desserts, for example, functional ice cream (Imchen & Singh, 2023).

Marine algae are recognized for their numerous naturally existing colour compounds that have health-enhancing and sensory qualities. Colorants obtained from marine algae include pigments, proteins, phenolic derivatives, and glycosides. Additionally, various pigments such as fucoxanthin, zeaxanthin, *β*-carotene, lutein, anthocyanin, phlorotannins, and phycobiliproteins, have demonstrated several health advantages, including antioxidant properties and effects against diabetes (Durmaz et al., 2020).

2. Microalgea

The term microalgae include both microalgae and cyanobacteria. Although they are different, the production technologies of both are the same and they perform aerobic photosynthesis (Fernández et al., 2021). Microalgae are divided into two groups: prokaryotic cell microalgae represented by the cyanobacteria phylum, and eukaryotic microalgae including green microalgae (*Chlorophyta*), red microalgae (*Rhodophyta*) and diatom (*Bacillariophyta*) phylum (Ferreira de Oliveira & Bragotto, 2022) (Figure 1).

It is estimated that approximately 0.2 to 0.8 million microalgae species exist in nature, very few of which have been studied and characterized for commercial and research purposes (Mehariya et al., 2021). Microalgae primarily rely on sunlight as their energy source, boosting efficiencies reaching up to 10%. These rapidly proliferating microorganisms can double in less than a day and achieve impressive biomass productivities exceeding 100 tons per hectare per year by dry weight. For these reasons, they are considered essential for the development of sustainable processes, contributing to the global bioeconomy (Özçimen et al., 2018).

Recent proposals indicate that microalgae could serve as a viable source of edible proteins and therapeutic substances owing to their remarkable ecological adaptability. As a resource for industrial applications, microalgae offer the advantage of growing in non-arable water and areas unsuitable for traditional agriculture. In comparison to land-based crops, microalgae demonstrate notably higher productivity concerning surface area and photosynthetic efficiency. Nonetheless, microalgae lack intricate reproductive and support structures (Ahmad & Ashraf, 2023).

2.1. Chemical composition of microalgea

Microalgae are bioactive substances rich in nutrients, containing high-value proteins, long-chain polyunsaturated fatty acids, vitamins, carotenoids, phenolics and minerals, and can be considered a promising innovative food ingredient (Batista et al., 2017). Detailed information regarding these components is provided in the following sections.

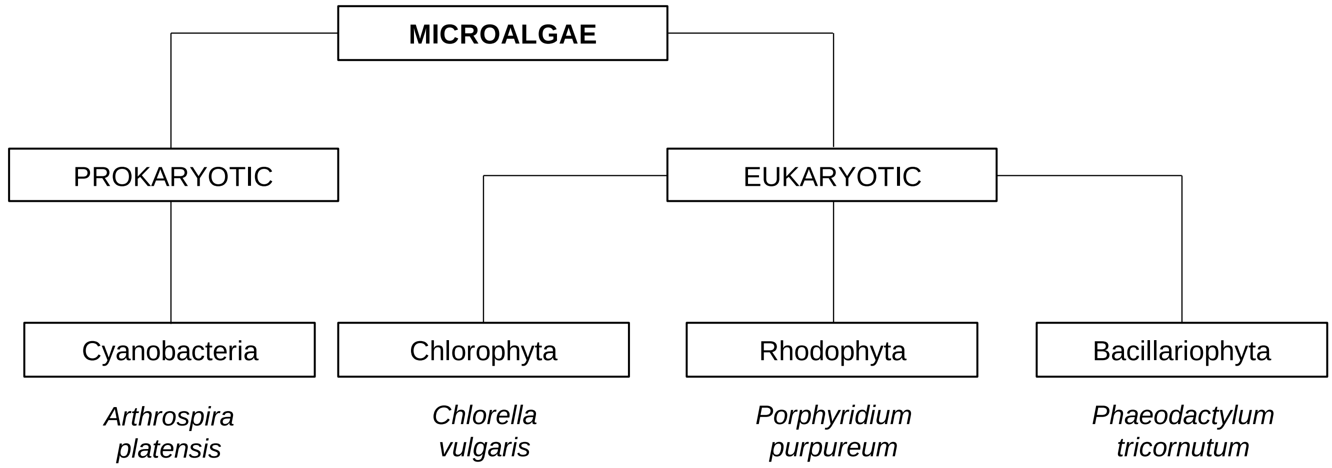

Figure 1. Microalgea represented by phylum and cell structure.
Şekil 1. Filum ve hücre yapısıyla temsil edilen mikroalgler.

2.1.1. Protein

In terms of overall production, brown algae have the lowest protein content, followed by green algae (Kadam et al., 2013). Microalgae proteins contain high amounts of essential amino acids, making them highly nutritious. Compared to some land crops like wheat and soybeans, which produce 1.1 tons/ha/year and 0.6-1.2 tons/ha/year of protein respectively, microalgae can yield between 4 to 15 tons/ha/year (Hosseinkhani et al., 2016). Microalgae genera largely used for human consumption due to their high content of essential nutrients and protein include *Arthrospira*, *Chlorella* and *Aphanizomenon*, as well as *Dunaliella* and *Haematococcus*, which are rich in antioxidant carotenoids (Niccolai et al., 2019). Microalgae protein (*Spirulina platensis* protein concentrate) has a high protein digestibility rate of 87.45-97.81%. Although the essential amino acid content of some microalgae species is very close to the levels found in eggs and soybeans, their protein content (510–710 g/kg dry powder) is higher than that in eggs and soybeans (132 and 370 g/kg) (Dineshbabu et al., 2019). Amino acid content comparisons between human dietary requirements and microalgae are presented in Table 1 (Diaz et al., 2023).

2.1.2. Carbohydrate

Microalgae produce energy storage components such as starch, which is primary carbon-containing metabolite due to photosynthesis. Different microalgae groups produce polysaccharides in diverse forms. *Cyanophytes*, for instance, store glycogen, whereas certain species create semi-amylopectin. *Rhodophyta* generate a carbohydrate polymer identified as fluoride starch, while *Chlorophyta* produce starch composed of two glucose polymers, amylopectin, and amylose. In both nutrient-rich and depleted conditions, a strain of *Tetraselmis suecica* has been documented to store between 11% and 47% of its dry weight as starch (Uzuner & Haznedar, 2020). *Porphyridium cruentum,* a single-celled red alga, is notable for producing sulfated galactan exopolysaccharide, serving as an alternative to carrageenans across various applications, making it a highly promising microalga for commercial use. This microalga has the capacity to synthesize valuable bioactive compounds, including extracellular polysaccharides and polyunsaturated fatty acids (PUFAs). Its characteristic red hue is attributed to phycobiliproteins such as phycocyanin, allophycocyanin, and phycoerythrin (Barkia et al., 2019).

2.1.3. Polysaccharides

Polysaccharides derived from microalgae can be categorized as either intracellular or structural, with the latter including exopolysaccharides released into the medium, polysaccharides bound to the cell, and those forming the cell wall. Exopolysaccharides produced by microalgae are generated as a result of physiological processes during cultivation or under stressful conditions. Extraction and purification procedures will need to be tailored based on the type of polysaccharide targeted for extraction and the cultivation conditions of the microalgae. Research has focused on the production of polysaccharides from *Porphyridium* sp., *Chlorella* sp., *Spirulina* sp., and *Nostoc* sp. Polysaccharides derived from microalgae have potential applications in nutraceuticals, food innovation, and as bioflocculants (Costa et al., 2021).

2.1.4. Lipids

From a nutritional standpoint, microalgae offer valuable profiles rich in nutritional and health-promoting components such as PUFAs. Especially ω3-PUFAs, such as *α*-linolenic acid, which cannot be synthesized by the human body, are essential fatty acids that must be provided through diet (Canelli et al., 2020). Recent research has confirmed that oily microalgae offer promising and sustainable alternatives to PUFAs found in fish oil. They can be efficiently cultivated on a large scale and accumulate substantial lipid content. Among various marine microalgae species examined, *Isochrysis galbana* stands out as a potential model microalga for its exceptional photosynthetic efficiency, high lipid production including PUFAs, and absence of a cell wall, facilitating oil extraction. Additionally, biomass derived from *I. galbana* has been integrated into traditional products like biscuits and pasta for its nutraceutical value, particularly in providing ω-3 PUFAs. These findings suggest that *I. galbana* oil could serve as a safe human dietary supplement, offering an alternative to ω-3 PUFAs sourced from fish oil (He et al., 2018).

Microalgae exhibit the capacity to synthesize triacyl glycerides (TAGs) with diverse fatty acid compositions, which vary among different species. For instance, *Chlorella* sp. was found to accumulate TAGs consisting of 21.6% linoleic acid, 25.1% palmitic acid, 23.1% oleic acid, and 8.9% *α*-linoleic acid based on total lipid mass, whereas *Nannochloropsis* sp. accumulated TAGs containing 5.1% myristic acid, 62.2% palmitic acid, 19.0% palmitoleic acid, 0.4% linoleic acid, and 0.9% eicosapentaenoic acid. *Chlorella* sp. and *Nannochloropsis* sp. are highlighted here due to their notable lipid content. Conversely, *Haematococcus*, *Dunaliella*, and *Spirulina* are recognized for their richness in astaxanthin, *β*-carotene, and proteins (De Bhowmick et al., 2023). Algae characterized by elevated lipid contents exhibited relatively heightened levels of volatile aldehydes.

Table 1. Amino acid profile of some important microalgae species (Diaz et al., 2023).
Tablo 1. Bazı önemli mikroalg türlerinin amino asit profili (Diaz ve diğ., 2023).

Amino acid	**Human requirement** (mg/kg)	***Dunaliella bardawil*** (g/100 g protein)	***Spirulina*** (g/100 g protein)	***Chlorella vulgaris*** (g/100 g protein)
Histidine	10	1.8	1.8-2.2	2
Isoleucine	20	4.2	6.0-6.7	3.8
Leucine	39	11	8.0-8.9	8.8
Lysine	30	7	4.6-4.8	8.4
Methionine	10.4	2.3	1.4-2.5	2.2
Phenylalanine	25	5.8	4.9-5.3	5
Threonine	15	5.4	4.6-6.2	4.8
Tryptophan	4	0.7	1.4	2.1
Valine	26	5.8	6.5-7.1	5.5

Linear aldehydes originate from the chemical oxidation of lipids, while branched/aromatic aldehydes stem from the enzymatic oxidation of lipids and proteins. Consequently, *Rhodomonas* and *Tetraselmis*, known for their higher concentrations of PUFAs, emitted aromas reminiscent of 'rancid, fatty odor', 'fresh marine, fishy', and 'cooked shrimp/cooked seafood'. Conversely, *Botryococcus*, *Nannochloropsis*, and *Chlorella* emitted fragrances described as 'grassy, vegetable, cucumber', and 'fruity', owing to their distinct lipid profiles (Colonia et al., 2023).

2.1.5. Dietary fiber

The total dietary fiber content of most of the microalgae such as *Aphanizomenon flos-aquae*, *Spirulina platensis*, *Nostoc sphaeroides*, *Chlorella sorokiniana*, *Chlorella vulgaris* Allma, *Tetraselmis suecica*, *Porphyridium purpureum*, *Phaeodactylum tricornutum*, *Tisochrysis lutea* and *Nannochloropsis oceanica* (33-75%) was reported to be significantly higher than cooked grains such as white rice (0.3%) and oatmeal (1.7%), raw vegetables such as tomatoes (1.3%) and lettuce (1.0%), and raw fruits such as bananas (1.8%) pineapple (1.5%) (Niccolai et al., 2019).

2.1.6. Vitamins and minerals

Some of the important vitamins found in microalgae are vitamin A, B1, B2, B6, B12, C, E, K, niacin, biotin, and folic acid. Some microalgae varieties like *Spirulina*, *Chlorella*, and *Scenedesmus* contain higher levels of vitamins A, B1, B2, niacin, and E compared to spinach and baker's yeast (De Jesus Raposo et al., 2013). Additionally, microalgae are abundant in minerals like calcium, phosphorus, magnesium, potassium, sodium, zinc, iron, copper, and sulphur. The composition and type of minerals vary depending on the composition of the growing medium, strain type and environmental conditions. Minerals constitute approximately 2.2 to 4.8% of the total dry weight of microalgae (Dineshbabu et al., 2019).

2.1.7. Pigments

Three main pigment groups found in microalgae include chlorophylls, carotenoids and phycobilins (phycobiliprotein). Phycobilins are water-soluble, while chlorophyll and carotenoids are generally fat-soluble (Sasa et al., 2020). As new generation of consumers prefer natural products over synthetic ones (especially in response to allergic reactions and health concerns), carotenoids such as lutein, *β*-carotene, lycopene and astaxanthin are used primarily as natural colorants in dietary supplements, food supplements and beverages. For these reasons, microalgae extracts and biomass are used as dietary supplements and food additives such as flavour enhancers, colour additives, preservatives, emulsifiers and antioxidants (Mendes et al., 2022).

Carotenoids derived from microalgae play a vital role in maintaining health. Among them, astaxanthin stands out for its potent antioxidant properties, surpassing other carotenoids in effectiveness. Studies have shown that *β*-carotene sourced from *Dunaliella salina* effectively inhibits angiogenesis in laboratory settings (Guruvayoorappan vd., 2007). Additionally, astaxanthin from *Haematococcus pluvialis* has been noted for its ability to reduce blood pressure (Hussein vd., 2005). Carotenoids, by absorbing harmful UV light and other solar radiation, contribute to healthy eye cells, thereby mitigating oxidative damage and potential vision impairment. Moreover, carotenoids have shown promise in managing diabetes, with blood *β*-carotene levels inversely linked to fasting blood sugar levels and insulin resistance (Fernández et al., 2021).

Phycobiliproteins, consisting of apoproteins and chromophores, are light-capturing complexes predominantly present in red algae and cyanobacteria. Presently, commercially available microalgal phycobiliproteins include C-phycocyanin from *Spirulina* sp. and B-phycoerythrin from single-celled red microalgae (*Porphyridium* sp.). The formulation of the growth medium including carbon and nitrogen sources, environmental factors such as light and temperature, the method of nutrition (autotrophic, mixotrophic, etc.), and the choice of bioreactor can influence the synthesis of phycobiliproteins (Ji et al., 2023). Phycocyanins, besides serving as food additives, also function as water-resistant natural colorants employed in various industries such as food, cosmetics, and immunological tests. However, factors such as the presence of alcohols, low pH, high ionic strength, high temperature, and other conditions render them susceptible to instability when exposed to light. Marine species offer a highly efficient and minimally toxic source for isolating phycocyanin (Ravi et al., 2023). *Spirulina*-derived phycocyanins have been shown to lower blood pressure and reduce the risk of heart attack, diabetes, and stroke (Folarin et al., 2017).

2.1.8. Antioxidants

Microalgae possess antioxidant organic compounds and enzymes that mitigate oxidative damage stemming from diminished oxygen states. These antioxidants alleviate oxidative stress on the gut microbiome by curbing reactive oxygen species within the digestive tract. The array of antioxidant compounds found in algae harbours potential for anti-aging, dietary enhancement, anti-inflammatory, antibacterial, antifungal, cytotoxic, anti-malarial, antiproliferative, and anticancer applications (Folarin ve Sharma, 2017). A research endeavour carried out on the Yucatan Peninsula assessed the antioxidant capabilities of selected microalgae using the DPPH (2,2 diphenyl-1-picrylhydrazyl) method, alongside the evaluation of phenolic content in each extract. All species demonstrated DPPH radical scavenging activity. Notably, three species, *Avrainvillea longicaulis*, *Chondria baileyana*, and *Lobophora variegata*, demonstrated strong antioxidant abilities with very low oxidation levels. These findings underscore the substantial antioxidant potential of certain macroalgae, holding promise for applications in medicine, food production, and the cosmetic industry (Zubia et al., 2007).

3. Application of Microalgae in Ice Cream

Ice cream represents a sophisticated food matrix comprising emulsified fat, colloidal protein, air bubbles, and a lactose solution. Generally, ice cream encompasses 8–16% fat content, a factor crucial for determining its texture, shape maintenance post-freezing, and resistance to melting. The fat component plays a pivotal role in establishing a fat network, stabilizing bubbles and foam structures, and enhancing melting attributes (Jin et al., 2024). In response to consumers seeking healthier lifestyles, new formulations of ice cream are emerging to meet this trend. These formulations incorporate a diverse range of raw materials and flavours while aiming for lower fat and sugar content (Villaró-Cos et al., 2023).

The emerging functional ice cream market is anticipated to experience rapid growth, with an estimated $319.8 million in purchases expected by 2028. Nutraceutical ingredients such as probiotics, antioxidants, dietary fibre, and bioactive peptides are utilized in the reformulation of functional ice cream. However, developing functional ice cream entails addressing specific physical and chemical constraints to ensure consumer acceptability (Genovese et al., 2022).

In a study by Szmejda et al. (2018), antioxidant activity and carotenoid content of ice cream was enhanced by fortification with *Spirulina.* Preliminary results from this study suggest that ice cream formulations enriched with algae extract reached up to 39.7% inhibition in mint-flavored samples, compared to 32.8% inhibition in control samples. This indicates a significantly higher level of inhibition compared to algae-free samples. Additionally, *Spirulina*-fortified versions of ice cream in various other flavors (milk, pistachio) also exhibited enhanced antioxidant activities, as demonstrated by increased free radical scavenging potential and carotenoid content.

The melting speed of ice cream, considered one of the most important features for consumers, depends on various factors including the air trapped in the structure, the ice crystal network, and the fat structure during the freezing process (Yosefiyan et al., 2024). Microencapsulated *Spirulina* with maltodextrin or gum Arabic was used in handmade ice cream. *Spirulina platensis* presented 35% to 53% more proteins compared to the standard formulation. Ice creams without microcapsules melt slower because they lack encapsulators that accelerate melting. Consumer survey indicated that, on average, 76.5% of tasters would purchase these ice cream (Tiepo et al., 2021). Another investigation by da Silva Faresin et al. (2022) aimed to diminish sugar and fat levels by introducing inulin, *Spirulina platensis*, or phycocyanin into ice cream. In the standard ice cream formulation, the melting rate was measured as 2.26 ml/min, while in the formulation with a 50% reduction in fat and 25% less sugar combined with inulin and *Spirulina*, the melting rate was 2.46 ml/min. The melting profile exhibited similar behaviour in terms of the volume of ice cream drained over time. Ice creams with phycocyanin extract, without industrial emulsifiers, exhibited a smoother and softer texture along with higher volume increase values. The addition of inulin (2%) and *Spirulina* (1%) enabled up to a 50% reduction in fat and a 25% reduction in sugar. Addition of 2% inulin to ice cream caused an increase in the complete melting time due to the hygroscopic properties of inulin. Inulin reduces the free circulation of water, which increases viscosity (Akin, 2005). A significant reduction in overrun values of ice cream formulations was observed with an increase in inulin content above 4% as a fat replacer. This occurs because the excess inulin interacts with the aqueous phase in ice cream, reducing the concentration of free water and resulting in a thicker ice cream mix (Narala et al., 2022).

Malik et al. (2013) found that enrichment of ice cream with *Spirulina* increased overrun and penetration value, improved the nutritional profile, and decreased whipping rate. Additionally, a natural light green colour was observed in the ice cream. Ice cream prepared by replacing 50% of the stabilizer with *Spirulina* showed sensory parameters comparable to the control. The increased melting resistance may be attributed to *Spirulina*'s water absorption capacity of 1.45 g/g protein and fat absorption capacity of 3.73 g/g protein. This study demonstrated a decrease in viscosity with an increase in the level of replacement of stabilizer with *Spirulina*. The results obtained in this investigation showed that ice cream prepared by replacing the stabilizer with *Spirulina* at a 100% level recorded an increase in overrun to 95% from the 90.6% overrun recorded for the control. Winarni Agustini et al. (2016) determined that the addition of 1.2% *S. platensis* was optimum for freezing. The addition of *S. platensis* had a significant effect on protein, total solids, fat, total sugar, volume increase, melting point, and sensory aspects of the ice cream. The overrun of ice cream with the addition of *S. platensis* powder tends to be higher compared to that without the addition of *S. platensis* powder. Additionally, the addition of *S. platensis* increases the melting point of the ice cream.

A study by de Amarante et al. (2020) showed that food-grade C-phycocyanin from *S. platensis* was a reliable blue colorant for ice cream over a period of 182 days. In addition, fortification with C-phycocyanin enhanced antioxidant resilience post simulated *in vitro* digestion. This study suggested that the use of this protein source in food and beverages is limited due to its susceptibility to heat, light, and acidic conditions. The quality of phycocyanin deteriorates when exposed to heat, leading to a decrease in antioxidant activity and colour. Encapsulation may be employed to prevent such changes in substance quality (Hadiyanto et al., 2018).

Nannochloropsis oculata, *Porphyridium cruentum*, and *Diacronema vlkianum* were introduced into ice cream formulations at varying concentrations (0.10-0.30 g/100 g) (Durmaz et al., 2020). The flow behaviour of the ice cream mixtures was effectively described by the Ostwal de Waele model. The consistency index rose with the concentration of *P. cruentum* biomass but declined with the application of *N. oculata* and *D. vlkianum* biomasses. Generally, both the type and concentration of microalgae significantly influenced the colour of the ice creams, with *N. oculata* and *D. vlkianum* exhibiting a more pronounced impact. *P. cruentum* algae contributed to a pinkish hue, while the others presented a greenish tint. Concerning sensory attributes, ice cream samples incorporating *P. cruentum* were preferred over others. Moreover, the phenolic content of ice cream samples increased with the incorporation of microalgae. The addition of microalgae did not significantly influence the melting behaviour, which offers an advantage for using microalgae in ice cream formulation. While increasing melting parameters with the addition of microalgae can provide stability or resistance to melting during consumption, it may also negatively affect sensory characteristics.

According to the Turkish Food Codex Food Additives Regulation, the addition of microalgae additives to ice cream is not allowed. Additionally, dyestuffs originating from blue-green algae are not defined, rendering their current use in ice cream and edible ice products prohibited. However, the use of concentrated *Spirulina* as a colorant with the addition of sauce is permitted within specified limits (Turkish Food Codex, 2023). Since there are not many applications for microalgae production and their use in food products in Türkiye yet, regulations regarding the use of algae as additives are not yet established. At this stage, it may be advisable to adhere to the definitions and limit values set by internationally recognized authorities. FDA classifies any organism, food, substance, or chemical suitable for consumption by all humans as 'Generally Recognized as Safe (GRAS)'. In this context, algae such as *Spirulina platensis*, *Chlamydomonas reinhardtii*, *Auxenochlorella protothecoides*, *Chlorella vulgaris*, *Dunaliella bardawil*, *Euglena gracilis*, and some products derived from them are considered GRAS (Torres-Tiji et al., 2020).

4. Microalgae in Food Industry

Microalgae are important sources of functional ingredients with unique structures that provide opportunities for the development of healthier foods with their nutritional and therapeutic activities (Samani et al., 2021). Since algae are the most diverse organisms, they are excellent candidates to replace existing animal products to meet different environmental and production needs, as well as to design new food products (Figure 2) (Diaz et al., 2023).

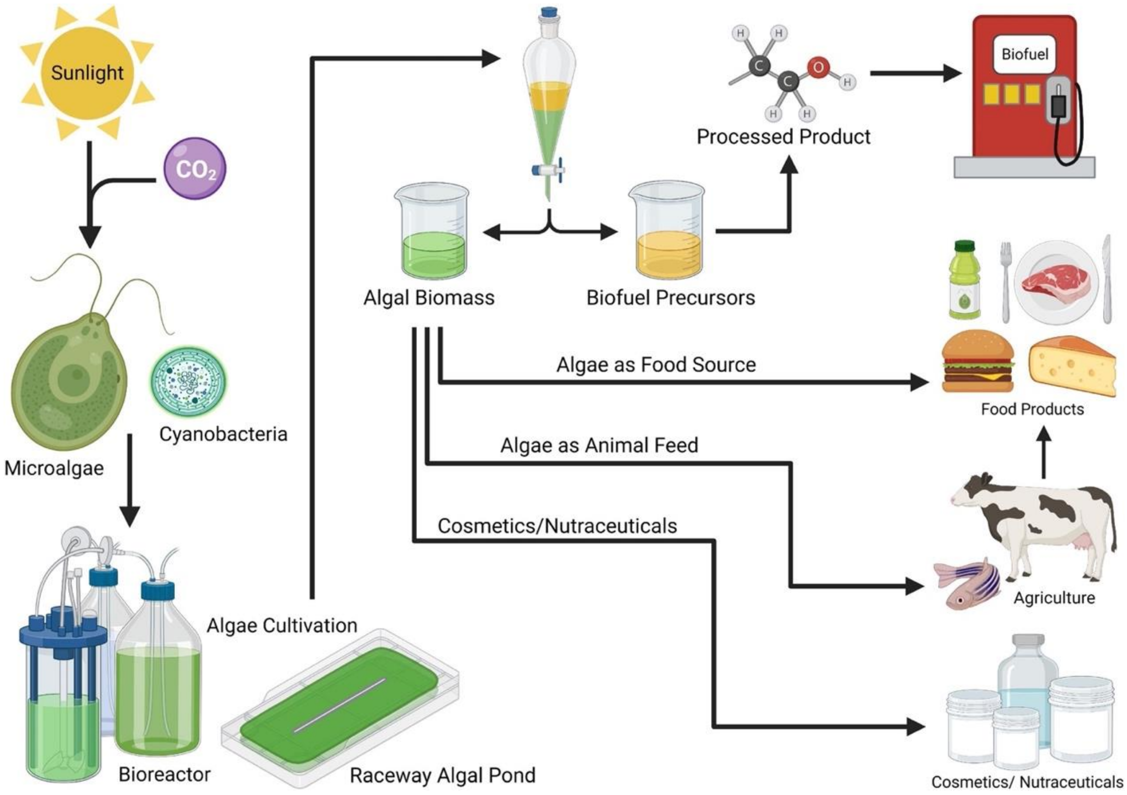

Figure 2. The versatile markets for algae products (Diaz et al., 2023).
Şekil 2. Mikroalg ürünleri için pazarlar (Diaz ve diğ., 2023).

The historical practice of incorporating algae into diets across the Far East and Asia highlights its long-standing culinary tradition. Conversely, Western nations have only recently shown interest in algae-based products. In Europe, attention has historically centred on utilizing algae for producing gelling agents, thickeners, and stabilizers in the food industry. Algae are favoured by vegetarians and are commonly featured as main dishes or appetizers (Ścieszka and Klewicka, 2019). Microalgae can be used as a potential food ingredient that can enrich the bioactive content of foods due to their biochemical composition (Durmaz et al., 2020). Algae boost abundant levels of protein and lipids, which are not only rich in nutrition but also highly digestible (Diaz et al., 2023).

Between 2015 and 2019, around 13,090 novel food products globally incorporated algae or its derivatives, with 79% being food items and 21% beverages (Boukid & Castellari, 2021). Phycocyanin, a significant pigment derived from microalgae, serves as a colouring agent in various food products such as chewing gums, popsicles, candies, soft drinks, dairy items, and cosmetics like lipsticks and eyeliner. *Spirulina* and the red alga *Porphyridium* are the primary organisms utilized for producing phycocyanin and phycoerythrin, respectively (Alam et al., 2018).

The literature documents the development and characterization of several food products enriched with *Spirulina*; for example, beverages, biscuits, dairy products, and breads containing *Spirulina* have been investigated. The majority of research findings indicate that *Spirulina* can augment the nutritional and bioactive advantages of foods, especially by increasing protein and essential amino acid levels, along with enhancing antioxidant capacity (Hei et al., 2024).

Algae serve as food additives in meat processing facilities to enhance both the shape and flavour of the products. With the introduction of artificial meat, algae, known for their high protein content, have emerged as a new and reliable source of quality protein. Researchers frequently explore plant proteins derived from microalgae like *Spirulina* and *Chlorella* to create meat alternatives. *Spirulina*, in particular, is recognized by nutritionists as an exceptional natural protein source, boosting a protein content ranging from 60% to 70%, with a remarkable human absorption rate of up to 95% (Wu et al., 2022). Using algae and their extracts in meat products not only enhances their quality and flavour but also offers consumers healthy options with reduced salt and fat content and bioactive components. The antibacterial and antioxidant properties of algae contribute to prolonging the shelf life of these products as well. Moreover, algae are being investigated for various other applications including serving as substitutes for animal fats, supplements for protein and minerals, and even as packaging materials for meat products (Wang et al., 2023).

The primary challenge for plant-based meat producers lies in traditional plant-based pigment sources like soy, peas, and almonds, which fail to provide the desired red-brown colour (hem) found in meat, instead often resulting in a dull gray-brown hue. Many plant-based pigment sources also suffer from stability issues. However, in certain food applications, these challenges can be mitigated by utilizing pigments derived from algal biomass. Notably, the projected global increase in the value of carotenoids and other new algae-based natural colours is 6% (Nayar et al., 2023).

For microalgae to be used as flavouring ingredients, it is essential that aroma and flavour compounds are present in sufficient concentrations. To mitigate undesirable flavours in algae-based food products, it is essential to carefully choose algae species, optimize cultivation conditions, conduct phytochemical studies, and thoroughly characterize odor compounds. These measures play an important role in the successful development of algae-infused food items. There are concerns that most consumers do not prefer microalgae-added foods on the market due to their fishy odor, grassy taste, and intense green colour (Matos et al., 2022).

Spirulina platensis was added at a level of 10% in white bread resulted in an increase in the protein content 7.40-11.63% calcium, magnesium, and iron compared to conventional bread (Ak et al., 2016). Some seaweed taste was noted but this did not affect acceptability. Additionally, *Spirulina* added bread stored under room conditions had reduced mold growth.

Incorporating microalgae into both plain and probiotic fermented milk is intended to elevate the functionality of these products. The goal is to amplify their influence on the viability of probiotic microorganisms within the product and the gastrointestinal tract, while also enhancing their direct health benefits. In one study, *Spirulina platensis* and *Chlorella vulgaris* were added to yogurt, at three concentrations (0.25%, 0.50%, and 1.00%) (Beheshtipour et al., 2012). The samples containing *C. vulgaris* and the control demonstrated a more rapid pH decrease, a slower increase in acidity, a shorter incubation time, and a lower final titratable acidity in comparison to those containing *A. platensis*. In treatments with 0.5% or 1% microalgae, viability remained consistently higher than 10^7 cfu/mL throughout the refrigerated storage period. In another study, addition of 0.25% *Spirulina* to yoghurt expedited fermentation and improved water holding capacity during 28 days of storage (Barkallah et al., 2017). In addition, antioxidant activity of yoghurt was enhanced by the added algae.

Recently, microalgae have been utilized as natural nutritional additives to enhance the nutritional quality of fish-transformed products. In a particular investigation, fish burgers with 1% *Spirulina platensis* exhibited superior texture, increased swelling and water and fat retention capacity, and sensory properties (Barkallah et al., 2017). These can be attributed to the notable levels of dietary fiber and polysaccharides present in *S. platensis*. The incorporation of *S. platensis* notably boosted the antioxidant properties of freshly prepared fish burgers owing to the presence of polysaccharides and pigments such as chlorophylls, carotenoids, and phycocyanin (Barkallah et al., 2019).

Microalgae biomass has a notable impact on the rheological characteristics of novel food items. A study by Letras et al. (2022) examined 3D printed cookies, integrating microalgae species like *Spirulina platensis* and *Chlorella vulgaris*, resulting in structurally sound products with enhanced resistance to baking. Similarly, another study by Hlaing et al. (2020) revealed that chocolate bars displayed improved oxidative stability and lower peroxide values after being enriched with lyophilized and encapsulated microalgae, particularly *Scenedesmus obliquus*. Additionally, an investigation indicated that chocolate milk powder fortified with *Spirulina*-LEB-18 exhibited enhanced sedimentation rates, increased solubility, and reduced hygroscopicity, with levels below 10% (De Oliveira et al., 2021).

Microalgae are rich sources of diverse compounds, offering high protein content from species of *Spirulina platensis* and low fibre content from species of *Chlorella* (Ferreira de Oliveira & Bragotto, 2022). *Chlorella* is utilized for human consumption in various forms, including soups, millet, juices, biscuits, ice creams, and smoothies by Portuguese companies such as Allma and Necton, which specialize in algae products. Furthermore, *Chlorella vulgaris* biomass has been incorporated into traditional oil biscuits as a colorant, resulting in improvements in the biscuits' textural properties. *Dunaliella* powder, ranging from red to orange in colour, contains 1-3% *β*-carotene. This oil-based *β*-carotene extract finds application as a colouring agent in margarine and beverages (Uzuner & Haznedar, 2020).

5. Conclusions and Future Perspectives

Many countries have seen an increase in protein consumption due to population growth and the adoption of protein-rich diets. Unfortunately, traditional protein product has negative environmental impacts that may worsen with increasing demand. Therefore, it is crucial to find sustainable alternative protein sources.

With the increasing awareness among people about the importance of healthy food and the need for protein-rich diet, the increasing demand for algae with anti-obesity, anti-cancer and anti-diabetic and antioxidant properties is expected to increase the demand for algal ingredients and the growth of the market. The high cost of the algae extraction method hinders the growth of the market by increasing the production cost. Microalgae farming is a promising alternative to combine anthropic emissions with food and feed production. Some microalgae show protein content twice as high as traditional protein sources. This is an important factor in the development of functional foods.

Ice cream is a milk dessert enjoyed by all age groups. The low protein ratio and high fat and sugar content negatively affect consumer preferences. Especially in the last 10 years, research on improving the nutritional composition of ice cream has increased. The most interesting of these are studies using microalgae. In studies where algae isolated in powder form or by microencapsulation technique were added to ice cream, it was reported that the protein content of ice cream increased. In addition, it has been determined that the melting and overrun properties of ice cream are improved, microalgae positively affect the stability of ice cream and acts as an emulsifier. Additionally, its use as a natural colorant in ice cream increases consumer reliability.

Studies on the use of algae in ice cream are very limited. There is no study yet that includes the toxic properties of microalgae and their microbiological properties originating from their production. Additionally, there is only one study on industrial ice cream with a prescription. Industrial equipment was not used in that study. In consumer tests, negative effects such as fishy smell and seaweed taste, which microalgae naturally contain, are observed at optimum levels that increase the protein content as a result of the use of microalgae in ice cream. Techniques that will eliminate these adverse effects need to be investigated.

6. Conflicts of Interest

The authors declare no conflict of interest.

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