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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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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.

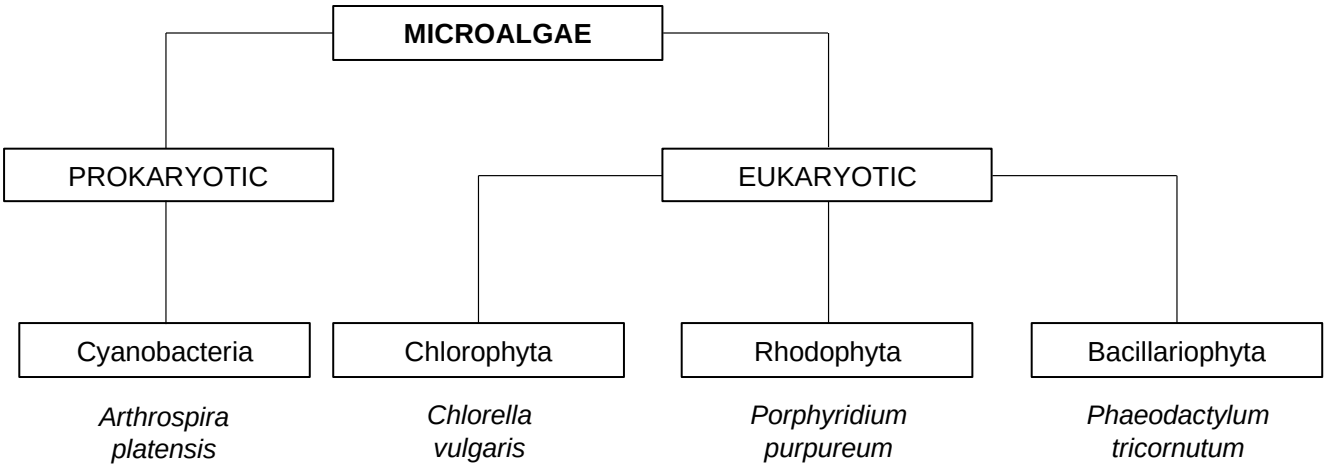

Figure 1. Microalgea represented by phylum and cell structure.
Şekil 1. Filum ve hücre yapısıyla temsil edilen mikroalgler.

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).

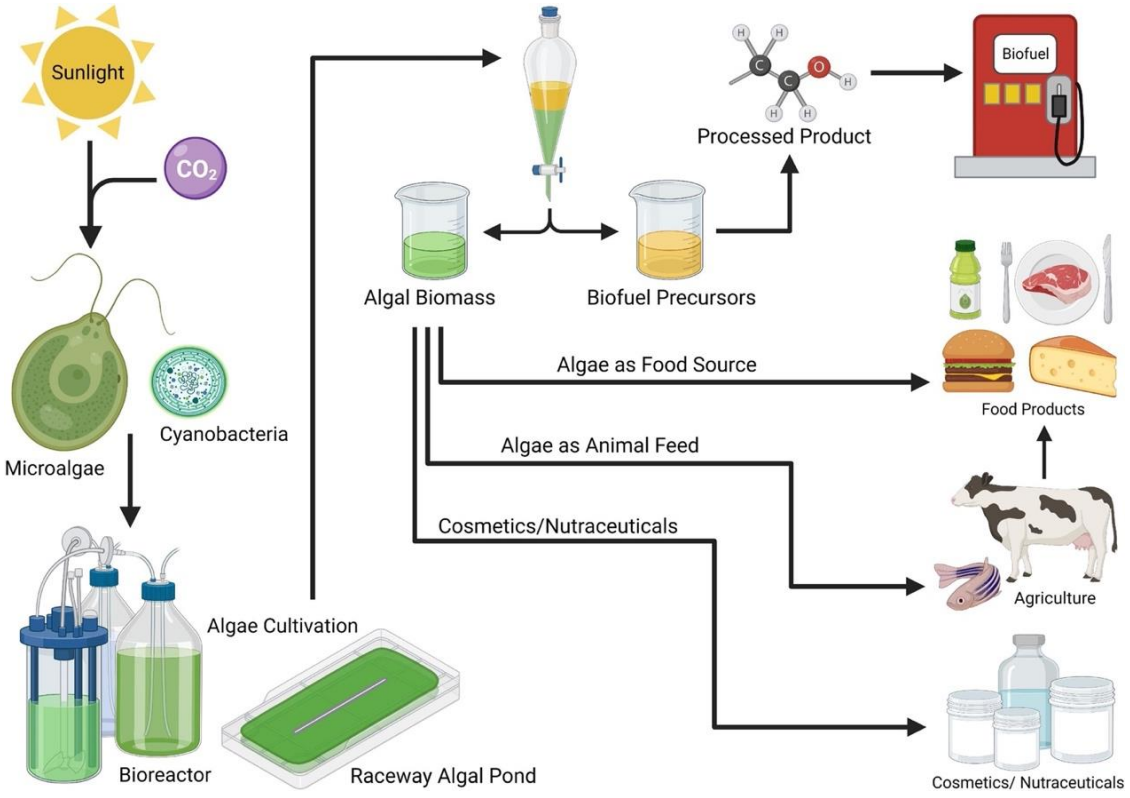

Figure 2. The versatile markets for algae products (Diaz et al., 2023).
Şekil 2. Mikroalg ürünleri için pazarlar (Diaz ve diğ., 2023).

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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Valorisation of Food By-products as a Source of Prebiotic

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Abstract: In light of the global food waste dilemma, it is important to promote a circular bio-economy model, stressing the transformational power of waste and by-products to reduce environmental impact and maximize resource use with a minimal carbon footprint. Based on the studies from the literature, this study highlights the need for global action to combat food waste, highlighting programs not only Turkey's zero-waste program but also the European Green Deal as examples of successful worldwide outreach. By increasing research about the introduction of food by-products into the food production cycle as useful prebiotic sources, the study not only emphasizes the benefit of waste reduction but also the economic and ecological relevance of such a strategy. Prebiotics are mostly composed of oligosaccharides, such as fructooligosaccharides and galactooligosaccharides, and polysaccharides, such as inulin, which are short-chain carbohydrates. These compounds may be extracted using various methods, and their impact on probiotic microorganisms is investigated. Among the methods used at this point, there are novel technologies as well as solvent extraction. In this context, it was aimed to review the literature to contribute to the debate on creating a sustainable food ecosystem.

Keywords: By-product, valorisation, extraction, prebiotic, probiotic.

Gıda Yan Ürünlerinin Prebiyotik Kaynağı Olarak Değerlendirilmesi

Özet: Küresel gıda atığı problemini dikkate alarak, çevresel etkiyi azaltmak ve minimum karbon ayak iziyle kaynak kullanımını en üst düzeye çıkarmak için atıkların ve yan ürünlerin tekrar kullanımıyla döngüsel biyoekonomi modelini desteklemek önemlidir. Bu nedenle, mevcut çalışmada, gıda israfıyla mücadele için yapılması gerekenleri içeren kaynaklar özetlenerek hem Türkiye'deki sıfır atık programı hem de Avrupa Yeşil Anlaşması vurgulanmaktadır. Aynı zamanda mevcut çalışmada, gıda yan ürünlerinin prebiyotik kaynağı olarak geri kazanılması ile ilgili çalışmalar ile hem ekonomik hem de ekolojik etkisine değinilmektedir. Prebiyotikler çoğunlukla fruktooligosakkaritler ve galaktooligosakkaritler gibi oligosakkaritler ve kısa zincirli karbonhidratlar olan inülin gibi polisakkaritlerden oluşmaktadır. Bu bileşikler, çeşitli yöntemlerle ekstrakte edilebilmekte ve probiyotik mikroorganizmalar üzerindeki etkileri araştırılmaktadır. Bu noktada kullanılan yöntemler arasında çözgen ekstraksiyonunun yanı sıra yeni teknolojiler de bulunmaktadır. Bu kapsamda, sürdürülebilir bir gıda ekosistemi yaratma konusundaki tartışmalara katkıda bulunmak üzere literatürde yer alan kaynakların incelenmesi hedeflenmiştir.

Anahtar Kelimeler: Yan ürün, değerlendirme, ekstraksiyon, prebiyotik, probiyotik.

Review

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1. Introduction

It is crucial to emphasize the significance of reducing and treating food waste, and its management system has been widely improved to apply sustainability efficiently in use. With the increment in population that led to more and more consumption every year, the consequences that the world is faced with are becoming challenging. Food waste management is a global issue that is becoming more prevalent in many countries, with some countries accounting for over 50% of total waste (Han et al., 2022). Therefore, it is important to reduce food waste and take a big step to treat it so the industry can reuse it. The production, handling, and post-harvest phases of food production is highlighted by waste at different points in the supply chain. For this kind of waste, industrial waste treatment is significant, and vegetable by-products are incorporated and utilized in the manufacturing process and daily lives. To ensure sustainable waste management, it is necessary to consider each component's economic and environmental burden and operate this mechanism continuously.

Additionally, in the application of sustainability, there are some practices globally. It is known that without food safety and quality, it is unfeasible to apply efficient sustainability goals. Some of the objectives in the near future that aspire to these goals are to support sustainable agriculture by eradicating famine and ensuring food security and good nutrition. With the help of these, it is possible to ensure sustainable food safety, leading to sustainable development (Soylu, 2022). Another essential implementation that European countries contributed to is the Green Deal. The Green Deal aims to make the EU the first short-term climate-neutral country by 2050. The EU plans to adopt a new grand strategy and reshape policies in its second revision, with the Green Memorandum encompassing various sectors, including industry, finance, energy, transport, construction, and agriculture (*The European Green Deal - European Commission*, 2021). According to Food Loss, waste of up to $26.04 million per year is a problem in Türkiye, especially regarding the most wasted fruits and vegetables. Also, in Türkiye, nearly 18 million foods are thrown away every year (Hamzaoğlu & Göktuna, 2022). However, various methods exist to utilize the waste and by-products as probiotic and prebiotic sources. Bozdağ et al. (2023) reported that Türkiye launched a zero-waste initiative to isolate the waste and convert it into energy or crude resources, aiming to increase its reusing rate from 13% in 2019 to 35% in 2023. Consequently, all these applications for reducing waste and sustainably establishing a system are important in national development and catching up on European standards.

Food waste is a global issue affecting economies, the environment, and society's well-being. It leads to increased hunger, resource depletion, and environmental damage. Effective food waste management is crucial to end hunger, ensure food access, and reduce the environmental impact of wasted food. Good management techniques promote sustainable behaviours, environmental stewardship, and social responsibility, preventing food shortages and promoting sustainable practices. The precautions can impact the consumer's behaviour by adopting it in their daily diet. Therefore, increasing re-cycling of the inedible parts and being utilized by the industry with various applications such as investigated in this search, valorisation of by-products as prebiotics or even obtaining probiotics from them are just some of the solutions that exist. Moreover, paving the way out with several techniques applied to waste are some of the key criteria that not only help the sustainable food consumption but also boost the aims to prevent food loss. Some measures are applied by both the government and private corporations that help reduce waste. It is obvious that prospering implementations can be achieved with education, and then white hope consequences can be achieved. One of the practices performed in Türkiye is Zero Waste 'Sıfır Atık'. By encouraging a sustainable economy and using waste streams as raw materials for new goods, zero waste is a novel approach to waste management that sees garbage as a useful resource. By concentrating on waste and pollution avoidance at its source, this strategy engages a variety of stakeholders, fosters collaboration, and generates job possibilities locally (Bilgili et al., 2023). Besides, various practices are applied by the Ministry of Agriculture and Forestry, and various collaborations are made with some of the private corporations and chain stores in Türkiye. One of the plans is 'Gıdanı Koru' which aims to reduce food loss and waste and encourages the customers to 'buy as you need and protect your future'. It also makes collaborations with chain stores that help customers buy the products with the best before-date close ingredients cheaper. It also prevents sensitive products like fruits and vegetables from being thrown away. Also, one of the novel practices is 'Fazla Gıda', an application aiming for the customers to buy products from both restaurants and groceries more cheaply before the shop closure and the products become stale. In addition to these, before becoming waste, food products were also used for extraction from fruits, vegetables, cereals, etc. of bioactive compounds, biofuels as energy suppliers, animal feed, and many more (Akgün et al., 2019). It is inevitable that with the increased and fast consumption, more techniques should be developed with the help of technology to ensure sustainable and green food production.

It is known that the food industry is evolving due to overconsumption aspects of population and economy. The fruit, vegetable, and cereal sectors produce by-products with financial and ecological value. This has led to attempts in the circular bio-economy to increase the value of these by-products because of their higher nutritional content, which benefits health and encourages sustainability in the food production cycle (Alexandre et al., 2023). It is advantageous for developing industrial manufacturing and proving waste management to reduce the impacts. Besides preferring green food that is sustainable and eco-friendly, it is important to have healthy food that not only supports the gut system but also helps to maintain overall body health. Although a healthy gut system promotes a wholesome immune system, it needs to be supported by booster microorganisms such as probiotics and supporter prebiotics to enrich the microflora. When used with by-products or used in procuring the by-products from waste, these microorganisms have a significant effect on the industry. As mentioned by Alexandre et al. (2023), the increased focus on individual health has created a market for functional foods that, in addition to providing proper nutrition, also have health advantages. These foods use natural bioactive compounds and additional ingredients like probiotics and prebiotics, satisfying consumer demands for affordable

and palatable meals while highlighting their contribution to chronic disease prevention and quality of life preservation.

Depending on the context, this study aimed to review the research and applied methods examining the prebiotic properties of wastes as another alternative area of utilization.

2. Probiotic Microorganisms

Probiotic is derived from the Greek word "probios", which means "for life". Probiotics have a long history dating back to human history (Gasbarrini et al., 2016). The term "probiotic" has been defined in several ways. However, according to the World Health Organization and Food and Agriculture Organization, probiotics are live microorganisms that benefit the host if they are ingested in sufficient concentrations. It is important that the biological effects of probiotics may differ from strain to strain, and their success should be evaluated within their strain. Resistance to stomach and bile acids, complete safety for a host, positive effect on the immune system, antimicrobial effect, and prevention of intestinal diseases are the factors that play an essential role in evaluating a bacterial strain as a probiotic (Fijan, 2014). Probiotics have many applications that are beneficial to human health. Studies have shown that probiotics help lower serum cholesterol and lactose intolerance, reduce and prevent diarrhea and constipation problems, and reduce the risk of colon cancer (Villena & Kitazawa, 2017).

According to studies, microorganisms considered probiotics can be classified as *Lactobacillus* sp., *Bifidobacterium* sp., yeasts, and other microorganisms. They are considered probiotics because of their health benefits and ability to live in the intestines. Some probiotic bacteria species in the *Lactobacillus* genus are *Lactobacillus acidophilus*, *Lactobacillus fermentum*, *Lactobacillus plantarum, Lactobacillus casei,* and *Lactobacillus rhamnosus*. Some of the *Bifidobacterium* species that are considered probiotic are *Bifidobacterium infantis, Bifidobacterium adolascentis, Bifidobacterium longum, Bifidobacterium bifidum* and *Bifidobacterium breve* (Williams, 2010). In addition, *Saccharomyces boulardii, Saccharomyces cerevisiae, Saccharomyces carlsbergensis*, and *Saccharomyces lactis* are yeast species with probiotic properties (Das et al., 2022). The morphological structures of probiotic bacteria are spore-free, Gram (+), and bacilli traits (Fijan, 2014). Although more has been known about the probiotic properties of bacteria in the past, yeast species such as *S. boulardii* have also been shown to have probiotic properties. The necessary studies have been carried out for this purpose (Yıldıran et al., 2017).

3. Prebiotic compounds

The gastrointestinal system is a major key to a healthy diet and digestion. Additionally, carbohydrates are known as the primary energy source for the body and can affect the overall diet influencing entire well-being. In contrast, non-digestible carbohydrates significantly alter the gut microbiota's appearance and function, enabling the fermentation of prebiotics by intestinal microorganisms and providing energy for survival by breaking down indigestible bonds (Davani-Davari et al., 2019). Prebiotic complexes that are not possible to digest have favourable traces affecting the host with the help of selected revitalizing development and activity of a distinguished number of bacteria in the gut system, affecting gut health. Prebiotics can be classified as prebiotics according to a few factors, such as both the stomach and small intestine cannot hydrolyse or absorb the substrate. Apart from this, beneficial colonic bacteria, such as *bifidobacteria*, must be the only type of substrate used, and fermentation must have positive luminal and systemic effects on the host (Manning & Gibson, 2004). As a result, prebiotics are fundamental because they not only help to grow but also support the development of useful microorganisms such as probiotics within the intestine, cultivating a more advantageous microbiome and helping in assimilation and general well-being.

Additionally, prebiotics in the carbohydrate category comprise lactulose, galactooligosaccharides (GOS), and short- and long-chain β-fructans, or fructooligosaccharides (FOS) (Table 1). Additional substances under consideration include whole grains, pectin, arabinoxylan, resistant starches, and polyphenols that do not include carbohydrates (Valcheva & Dieleman, 2016). According to You et al. (2022), prebiotics convert into short-chain fatty acids (SCFAs) through fermentation, contributing to intestine health. Prebiotics stimulate intestinal obstruction, reduce inflammation, and prevent harmful substances from entering the system. They also balance the intestinal microbiota, potentially reducing the risk of certain infections and diseases. Prebiotics must be absorbed, promote beneficial microorganisms, be fermentable, safe for use, and have proven health benefits. Classification of prebiotics varies however, some types need to be mentioned such as fructans: β(2→1)-linked linear chains of fructose; and a significant class of prebiotics is GOS, nonetheless, oligosaccharides that don't include carbohydrates like flavanols obtained from cocoa promote lactic acid bacteria; resistant starch (RS) is useful for generating large amounts of butyrate improving health; polydextrose is an oligosaccharide produced from glucose; pectic oligosaccharides (POS) sourced by the pectin (Davani-Davari et al., 2019). It is known that prebiotics has numerous benefits, including improved digestion, immunity, bowel movement regulation, weight management support, reduced inflammation, improved mineral absorption, blood sugar levels, blood balancing, and potential support for mental health, all of which contribute to overall health by nourishing a healthy gut microbiome. Furthermore, they also serve as dietary fibers and low-calorie components added to diverse food products, enhancing their nutritional value, promoting health, and positively impacting their taste and texture (Hurtado-Romero et al., 2020). As a consequence, the use of these prebiotics is inevitably essential for a healthy microflora for overall well-being. However, it also cannot be ignored that innovative applications of prebiotics extend beyond their role in human health, finding utility in waste treatment processes, where their ability to stimulate beneficial microbial activity holds promise for enhancing organic waste decomposition; for instance, prebiotics like inulin have shown potential in optimizing anaerobic digestion systems by promoting the growth of methane-producing bacteria, aiding in the efficient waste breakdown (Rahul et al., 2014). Inulin, oligo-fructose, and FOS are nutritional supplements and functional food components in beverages, yogurts, biscuits, and spreads (Kelly, 2008).

Table 1. Studies conducted on prebiotics extracted by different methods.
Tablo 1. Farklı yöntemler ile ekstrakte edilen prebiyotik çalışmaları.

Prebiotics	Source	Extraction method	Microorganism	Key outcomes	References
Carbohydrates, inulin, oligofructose	Rice bran	Ultrasound-assisted extraction (UAE)	Lactobacilli strains	In the extracts, no significant differences in prebiotic activity were observed between carbohydrates. Probiotic growth and prebiotic activity values were similar to or higher than those carbohydrates in *Lactobacillus acidophilus*, etc. compared to inulin and oligofructose.	(Antunes et al., 2023)
Cellulose	Banana peel	Enzymatic and diluted-acid hydrolysis	*Lactobacillus plantarum*, *Lactobacillus casei*	Water-soluble cellulose showed a higher prebiotic activity index than commercial prebiotics line inulin by increasing the growth of *L. plantarum*.	(Phirom-on & Apiraksakorn, 2021)
Dietary fiber	Bagasse and date seed	Alkaline hydrogen-peroxide extraction	*Lactobacillus acidophilus*	Probiotics increase the rate of cell death by consuming nutrients from the environment. The dietary fiber samples of both sources (5%) showed the greatest prebiotic activity.	(Afrazeh et al., 2021)
FOS, inulin	Edible mushroom	Solvent extraction (SE)	Lactobacilli strains	In comparison to commercial prebiotics like FOS and inulin, mushroom polysaccharides greatly encouraged the development of *L. acidophilus* and *L. plantarum*.	(Sawangwan et al., 2018)
Inulin	*Jerusalem artichoke* tubers	Hot distilled water (HWE)	The mixture of probiotic culture in yoghurt	Depending on the degree of polymerization of inulin, its effect on probiotics was remarkable.	(Li et al., 2015)
Oligosaccharide	Sweet potato (*Ipomoea batatas* L.)	Ultrasound-microwave-assisted extraction (UMAE)	*Bifidobacteria adolescentis*	Signifying that sweet potato oligosaccharides promote the growth of *B. adolescentis* with more efficiency with the help of UMAE compared with HWE, UAE.	(Guo et al., 2019)
	Dragon fruit (Pitaya)	SE, HWE	*Bifidobacterium bifidum, Lactobacillus delbrueckii*	Prebiotic characteristics of pitaya oligosaccharides include partial resistance to salivary α-amylase, acid resistance in the human stomach, and the capacity to promote the development of lactobacilli and bifidobacteria.	(Wichienchot et al., 2010)
Pectin-type Polysaccharide	Lotus leaf	Hot Water Reflux, Medium-Temperature Alkali, Ultrahigh pressure-assisted deep eutectic solvents (DES), high pressure homogenization-assisted dual enzyme extraction	*Eubaterium rectale, Bacteroides thetaiotaomicron, Roseburia intestinalis, Faecalibacterium prausnitzii* and other bacterial strains	The study discovered that lotus leaf polysaccharides with potent *in vitro* prebiotic activity may be prepared efficiently using reflux and high-pressure homogenization-assisted dual enzyme extraction while other methods were not appropriate.	(Ke et al., 2023)

Table 1. Studies conducted on prebiotics extracted by different methods (continue).
Tablo 1. Farklı yöntemler ile ekstrakte edilen prebiyotik çalışmaları (devamı).

Prebiotics	Source	Extraction method	Microorganism	Key outcomes	References
Polysaccharide	Bamboo shoots	SE, UAE, HWE, enzyme assisted (EAE), microwave-assisted extraction (MAE)	Bifidobacterial and *Lactobacillus* strains	UAE and EAE from bamboo shoots showed better prebiotic effects than FOS, but not as good. They have lower total carbohydrate levels, potentially exhibiting similar proliferative effects.	(Chen et al., 2019)
	Zizyphus jujube	DES, HWE, ultrahigh pressure extraction (UD)	Lactobacilli strains	While *Zizyphus jujube* polysaccharide (JP)-UD had the highest prebiotic response, all JPs demonstrated varying proliferative effects on the four Lactobacillus strains at different doses.	(Zou et al., 2022)
	Soy hulls	MAE	*Lactobacillus bulgaricus*	MAE of soy polysaccharides revealed more robust prebiotic activity.	(Yang et al., 2019)
Xylooligosaccharides	Rice Straw	Alkaline extraction	*Lactobacillus rhamnosus, Lactobacillus casei*	*L. rhamnosus* and *L. casei* growth were positively influenced by autohydrolysate treatments, particularly regarding xylobiose, xylotriose, and neutral XOS, with pure xylobiose having the greatest effect.	(Kaur & Mankoo, 2021)

3.1. Inulin

Inulin-type prebiotic compounds found in root vegetables like Jerusalem artichokes, burdock, chicory, leeks, and onions belong to the broader group of "fructans". These substances are natural plant oligo- and polysaccharides primarily characterized by glycosidic bonds predominantly formed by fructosyl-fructose connections. Fructans typically contain at least one fructosyl-glucose bond, often serving as the initial link in the polymer chain. Inulin is also a bifidogenic substance, which promotes the growth of *Bifidobacteria* species in the gut. It is resistant to digestion by enzymes in the upper gastrointestinal system and makes it to the colon intact, where bacteria ferment it (Kelly, 2008).

3.2. Fructooligosaccharides

Fructooligosaccharides known as FOS, are an inulin-type prebiotic that helps increase gut flora, especially advantageous bacteria such as *Bifidobacterium*. It is a fact that FOS intake can boost *Bifidobacteria* numbers while lowering Bacteroides, Clostridia, and Fusobacteria whereas the amount of FOS consumed correlates with higher *Bifidobacteria* numbers. Additionally, FOS glycosidic links are mostly beta (2-1) linkages generated by transfructosylation with the fungal enzyme beta-fructosidase, and they are estimated as soluble dietary fiber; however, FOS may be made by partially hydrolyzing inulin or glucose, resulting in short-chain, low-molecular-weight inulin-type fructans with degree of polymerization values ranging from 2-4. Consequently, FOS manufacturing includes the application of specialized technology to fructan-containing dietary sources, resulting in a wide range of inulin-type prebiotic products (Kelly, 2008).

3.3. Galactooligosaccharides

Galactooligosaccharides (GOS), synthesized by lactose extension, are classified as excess galactose at C3, C4, or C6 and produced from lactose through enzymatic trans-glycosylation. They promote *Bifidobacteria* and *Lactobacilli*, stimulate Enterobacteria, *Bacteroidetes*, and *Firmicutes*, and some are prebiotics, while others, like raffinose family oligosaccharides, are yet to be discovered (Davani-Davari et al., 2019). GOS are soluble dietary fibers that may have some positive effects on health by encouraging the growth of *Bifidobacteria* in the gut. They have also been studied for their effects on infant infections and allergy symptoms, as well as adult plasma triacylglycerol concentrations and hepatic lipogenesis. GOS are frequently used as functional food ingredients in food products in order to support health claims and produce a harmonious sweetness profile. Lactose can be used to make them through enzymatic synthesis (Kelly, 2008).

3.4. Dietary fibers

Dietary fibers are indigestible carbohydrates that may be extracted from plants, offering physiological advantages for human health. Substances are classified as soluble and insoluble due to the inability of human small intestinal enzymes to break them down. Dietary fibers promote digestive health by providing the colon with a carbon source for fermentation. Colonic microbiota has identified them as fermentable substances, and they may be found in marine plants, mushrooms, cereal grains, and algae. The importance of prebiotic dietary fibers in intestinal health and general well-being is well-established. Examples of these fibers include FOSs, inulin, GOSs, and beta-glucan. They are classified into numerous groups, each with its own set of health advantages (Carlson et al., 2018).

4. Conclusion

As a result, this study emphasizes how important it is to manage waste sustainably in the face of rising food waste worldwide. To minimize waste and maximize resource usage, the research promotes a circular bio-economy by emphasizing the value-adding of waste and by-products. Initiatives like the European Green Deal and Türkiye's zero-waste responsibility demonstrate a worldwide dedication to decreasing food waste. By solving waste issues and enhancing gut health, the incorporation of waste by-products—which are prime examples of possible prebiotic sources—into the food production cycle provides a dual benefit. The study highlights the role that prebiotic substances and probiotic bacteria play in promoting general health. Prebiotics' creative use in waste treatment procedures, such as enhancing anaerobic digestion systems, demonstrates how well they may be used to solve environmental and health issues. By taking into account social, environmental, and economic factors, this all-encompassing strategy supports sustainability goals and opens the door for a more accountable and environmentally friendly global food system.

It is important to approach waste management from both an industrial and a global standpoint. Businesses should spend money on research to find the best ways to remove by-products from plants, so they may be used as prebiotics. Adopting circular economy ideas on a global scale entail setting industry norms that incorporate waste products into cycles of production. Global collaboration and information sharing can result in uniform methods for handling food waste throughout borders. It is possible to put into place policies that encourage sustainable production and waste management methods. Industries can support a more linked and sustainable global food system by rethinking the value of by-products.

5. Conflicts of Interest

The authors declare no conflict of interest.

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Makroalgal Karotenoidlerin Bazı Biyoaktif Özellikleri

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Özet: Sürdürülebilir, ucuz ve alternatif gıda kaynaklarının bulunması, gıda alanındaki temel ve acil araştırma konularından birini oluşturmaktadır. Bu noktada makroalgler, dünya nüfusuna paralel olarak artan gıda talebinin karşılanması için önemli bir gıda kaynağı olarak değerlendirilmektedir. Makroalgler, ekilebilir araziye ihtiyaç duymamaları ve minimun besin maddesi ile büyüyebilmelerinin yanı sıra içerdikleri polifenol, sterol, tanen, flavonoid, protein, karbonhidrat, çoklu doymamış yağ asitleri, mineral, alkoloid, tokoferol gibi çeşitli biyoaktif bileşikler açısından zengindir. Macroalgler ayrıca pigment üreten organizmalar olarak kabul edilir ve sahip oldukları fotosentetik pigmentasyon yapılarına göre kırmızı makroalg (Rhodophyta), kahverengi makroalg (Phaeophyta) ve yeşil makroalg (Chlorophyta) olmak üzere üç grup altında toplanırlar. Yeşil makroalglerde baskın olarak bulunan karotenoidler; β-karoten, lutein, violaksantin, neoksantin ve zeaksantindir. Kırmızı makrolaglerde lutein ve zeaksantinin yanı sıra α- ve β-karotenler bulunurken, kahverengi makroalgler pek çok karotenoide ilave olarak fukoksantin, perinidin, diatoksantin, heteroksantin ve siphonaksantin karotenoidleri ve astaksantin ve kantaksantin gibi karotenoid türevlerinden olan karotenoproteinleri içerirler. Karotenoidler hücrelerin korunmasında rol oynayan pigmentlerdir ve başta antioksidan aktivite olmak üzere, antikanser, antidiyabetik ve antienflamatuar aktivitelere ilave olarak kardiyovasküler hastalıklara karşı olumlu etkilerinin bulunduğu rapor edilmiştir. Örneğin, ksantofil sınıfına ait bir karotenoid olan fukoksantin kahverengi makroalglerde yaygın olarak bulunan bir karotenoiddir ve güçlü antioksidan aktivitesi ile oksidatif strese karşı koruyucu etki gösterdiği tespit edilmiştir. Benzer şekilde, makroalglerden izole edilen diğer karotenoidler olan β-karoten, zeaksantin ve violaksantinin antikanser, antidiyabetik ve antienflamatuar aktivite sergilediği rapor edilmiştir. Sunulan bu çalışmada, makroalglerin sınıflandırılması, kimyasal kompozisyonu, karotenoidlerin stabilitesi, makroalglerin karotenoid içerikleri ve algal karotenoidlerin antioksidan, antikanser, antidiyabetik, antienflamatuar ve antihipertansif aktivite gibi biyoaktif özellikleri hakkındaki bazı bilgiler derlenmiştir.

Anahtar Kelimeler: Makroalg, karotenoid, pigment, antioksidan aktivite, biyoaktif özellik.

Some Bioactive Properties of Macroalgal Carotenoids

Abstract: Finding sustainable, cheap and alternative food sources is one of the fundamental and urgent research matter in the field of food. In this point, macroalgae are considered an important food source satisfying the increasing food demand in parallel with the growing world population. Macroalgae do not require arable land and are able to grow on minimal nutrients, they are rich in various bioactive compounds such as polyphenols, sterols, tannins, flavonoids, proteins, carbonhydrates, polyunsaturated fatty acids, minerals, alkoloids, tocopherols. It is known that these secondary metabolites have properties such as antioxidantive, antimicrobial, antiviral, anticarcinogenic, antidiabetic, anti-inflammatory and antiobesity. Moreover, macroalgae are considered pigment-producing organisms and are classified into three groups: red (Rhodophyta), brown (Phaeophyta) and green (Chlorophyta) macroalgae, according to their photosynthetic pigmentation structure. Carotenoids predominantly found in green macroalgae are β-carotene, lutein, violaxanthin, neoxanthin and zeaxanthin. Red macroalgae include in addition to lutein and zeaxanthin, α- and β-carotenes, and brown macroalgae have in addition to many carotenoids, fucoxanthin, perinidine, diatoxanthin, heteroxanthin and siphonaxanthin, and also carotenoproteins being carotenoid derivatives such as astaxanthin and canthaxanthin. Carotenoids are be divided into two main groups: carotens and xanthophylls. Carotenoids are pigments that play a role in protection of cells and have been reported to have beneficial effects against cardiovascular diseases, in addition to antioxidant activity, anticancer, antidiabetic and anti-inflammatory activities. For instance, fucoxanthin, a carotenoid belonging to the xanthophyll group, commonly found in brown macroalgae and has been found to have a protective effect against oxidative stress with its strong antioxidant activity. Similarly, β-carotene, zeaxanthin, and violaxanthin carotenoids isolated from macroalgae have been reported to exhibit anticancer, antidiabetic, and anti-inflammatory activities, as well. In this study, some information about classification of macroalgae, their chemical composition, stability of carotenoids, carotenoid content of macroalgae and some bioactive properties of algal carotenoids such as antioxidant, anticancer, antidiabetic, anti-inflammatory and antihypertensive activity were reviewed.

Keywords: Makroalgae, carotenoid, pigment, antioxidant activity, bioactive property.

Review

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1. Giriş

Dünya nüfusunun 2050 yılında 9,8 milyardan fazla olacağı ve bu hızlı artışın insanlığın ve gezegendeki çoğu organizmanın varlığını riske atacak gıda krizine, küresel ısınmaya, zehirli gaz emisyonuna ve şiddetli iklim değişikliğine neden olabileceği öngörülmektedir (Zhu ve diğ., 2016; Rebello ve diğ., 2010). Ayrıca, Uluslararası Tarım ve Kalkınma Fonu (IFAD), Birleşmiş Milletler Gıda ve Tarım Örgütü (FAO), Dünya Sağlık Örgütü (WHO) ve Dünya Gıda Programı (WFP) tarafından hazırlanan "Dünyada Gıda Güvenliği ve Beslenmenin Durumu Raporu", 2019 yılında neredeyse 690 milyon insanın, yani dünya nüfusunun yaklaşık yüzde 8,9'unun yetersiz beslendiğini göstermektedir (Bongaarts, 2021). Bu noktada, alternatif ve sürdürülebilir gıda kaynaklarının bulunması büyük önem arz etmektedir. Alglerin ekilebilir araziye ihtiyaç duymamaları ve minumun besin maddesi ile büyüyebilmeleri nedeni ile geleneksel gıdalara iyi bir alternetif olabileceği düşünülmektedir (Norsker ve diğ., 2011).

Algler taksonomik olarak mikroalgler ve makroalgler olarak iki grup altında toplanırlar (El Gamal, 2010). Makroalgler polifenoller, flavonoidler, proteinler ve çoklu doymamış yağ asitleri (PUFA) gibi çeşitli biyoaktif bileşikler açısından zengindir (Pal ve diğ., 2014). Makroalglerin ayrıca karotenoidler açısından da zengin olduğu rapor edilmiştir (Ben-Amotz ve Fishler, 1998; Gouveia ve Empis, 2003). Makroalglerde bulunan diğer pigmentler ise klorofiller ve fikobiliproteinlerdir. Kahverengi makroalglerin bir karotenoid olan fukoksantin miktarı yüksek iken, kırmızı makroalglerin fikobiliprotein ve yeşil makroalglerin klorofil içerikleri yüksektir (Cikoš ve diğ., 2022). Bu pigmentler çevresel stresin bir göstergesidir ve stress koşullarında daha yüksek miktarda üretilirler. Diğer taraftan, makroalgin yetiştiği deniz suyunun sıcaklığı ve tuzluluğu gibi faktörlere ilave olarak algin türü pigment miktarını etkilemektedir.

Karotenoidler, makro ve mikroalgler dışında, fotosentetik bitkiler ve mantarlar tarafından sentezlenen ve çeşitli meyve ve sebzelerin turuncu, sarı ve kırmızı renklerinden sorumlu olan lipofilik bileşiklerdir. Karotenoidler, sentetik pigmentlere yönelik tüketicilerin artan sağlık endişeleri nedeni ile doğal pigmentler olarak çeşitli alanlarda kullanım potansiyeline sahiptir (Mattea ve diğ., 2009). Örneğin gıda, yem ve kozmetik endüstrilerinde β-karoten, lutein, likopen, astaksantin gibi karotenoidler yaygın olarak kullanılmaktadır (Jaswir ve diğ., 2011). Deepika ve diğ. (2022)'ne göre, β-karoten, astaksantin, lutein, zeaksantin ve likopenin de aralarında bulunduğu toplam 40 karotenoid ticari olarak üretilmektedir. Temel algal pigmentlerden olan *Dunaliella salina*'dan β-karoten (kuru maddede %14), *Haematococcus pluvialis*'dan astaksantin (kuru maddede %3) ticari olarak ilgi uyandıran karotenoidlerdir (Deepika ve diğ., 2022).

Bu çalışmada, geleneksel gıda kaynaklarına alternatif olarak değerlendirilen makroalglerin sınıflandırılması, kimyasal kompozisyonu, karotenoidlerin stabilitesi, makroalglerin karotenoid içerikleri ve algal karotenoidlerin antioksidan, antikanser, antidiyabetik, antienflamatuar ve antihipertansif aktivite gibi biyoaktif özellikleri hakkındaki bazı bilgiler derlenmiştir.

2.Makroalgler

Deniz yosunları olarak da adlandırılan makroalgler, suda yaşayan besin zincirindeki diğer canlılar için besin kaynağı olan, fotosentez yapan ve okyanuslardaki devasa ormanları oluşturan "thallus" adı verilen yapıları geliştiren ototrofik organizmalardır (Figueroa ve diğ., 2021). Makroalgler, deniz kıyılarında ve nehir ve göl gibi tatlı su ekosistemlerinde bulunurlar (Panzella ve Napolitano, 2017). Yüksek besin içerikleri ve düşük kalori değerlerinden dolayı birçok makroalg türü Çin, Japonya ve Kore gibi pek çok Asya ülkesinde antik zamanlardan beri gıda olarak tüketilmektedir. Diğer taraftan makroalgler, gıda, kimya, tarım, kimya, kozmetik, farmosötik ve tıp gibi alanlarda geniş bir uygulama alanına sahip pek çok değerli bileşenin önemli bir kaynağıdır. Örneğin gıda endüstrisinde, temel olarak karregenan, aljinat, fikokolloit agar gibi teknofonksiyonel polisakkaritlerin kaynağı olarak kullanılmaktadırlar (Pangestuti ve Kim, 2011; Konda ve diğ., 2015; Figueroa ve diğ., 2021; Ślusarczyk ve diğ., 2021).

Makroalglerin bilinen yaklaşık 10.500 türü bulunmaktadır ve bu türler arasında yaklaşık 500 tür yüzlerce yıldır insanlar tarafından ya gıda olarak doğrudan ya da agar ve karragenan gibi bazı bileşenlerinin ekstrakte edilmesi yolu ile dolaylı olarak tüketilmektedir (Chopin ve Tacon, 2021). Makroalglerden 220'den fazla türün yetiştiriciliği yapılmakla birlikte, bunların sadece 20'si FAO'nun FISHSTAT veri tabanında listelenmiştir. FAO'nun 2018 yılı verilerine göre 8 tür tüm yetiştiriciliği yapılan makroalg üretiminin %96,8'ini oluşturmaktadır. Bu türler; kombu olarak da bilinen *Saccharina japonica* (%35,3), *Eucheuma* spp. (%29), *Gracilaria* spp. (%10,7), *Porphyra* spp. ve nori olarak da bilinen *Pyropia* spp. (%8,9), wakame olarak da bilinen *Undaria pinnatifida* (%7,2), *Kappaphycus* spp. (%4,9) ve *Sargassum* spp. (%0,8)'dir (FAO, 2020). Yetiştiriciliği yapılan makroalglerin üretimi; 2000 yılından (10,6 milyon ton) 2005 yılına (14,8 milyon ton) %40,0, 2010 yılına (20,2 milyon ton) %36,0 ve 2015 yılına (31,1 milyon ton) %53,4 artmıştır. Diğer taraftan, 2018 yılında yetiştiriciliği yapılan (31,5 milyon ton) ve toplanan (0,9 milyon ton) makroalg miktarı ise toplam 32,4 milyon tondur (Chopin ve Tacon, 2021). Ayrıca, FAO'ya göre yetiştiriciliği yapılarak üretilen makroalglerin 13,3 milyar dolarlık bir ekonomik değeri bulunmaktadır (FAO, 2020).

2.1 Makroalglerin sınıflandırılması

Makroalgler sahip oldukları fotosentetik pigmentasyona göre kırmızı makroalg (Rhodophyta), kahverengi makroalg (Phaeophyta) ve yeşil makroalg (Chlorophyta) olmak üzere üç grup altında toplanmaktadır (Wang ve diğ., 2015). Kırmızı makroalglerin fotosentetik hücrelerinde genellikle fazla miktarda fikoeritrin kırmızı pigmentleri bulunmaktadır. Bu kırmızı pigment, diğer çeşitli pigmentlerle kombinasyon halinde, yarı saydam soluk pembe, lavanta, mor, kestane ve bordodan yanardöner maviye kadar geniş bir renkten sorumludur. Kahverengi makroalgler ise fazla miktarda bir kahverengi pigment olan fukoksantin içermektedir. Aljinik asit ve fukoidin gibi önemli bileşenleri içeren selüloz duvarları vardır. Neredeyse tüm kahverengi alglerin yüzeylerinde, besin alımı için yüzey alanını artırmaya hizmet edebilecek ince (mikroskobik) tüyler bulunmaktadır (Litter ve Litter, 2013). Diğer taraftan, yeşil makroalgler baskın pigment olarak klorofil içerirler (Dawes, 1998). Yeşil algler aynı zamanda ikinci düzeyde karotenoid pigmentlerini ihtiva ederler (Hoek ve diğ., 1995).

2.2 Makroalglerin kimyasal kompozisyonu

Alglerin yüksek besin ve biyoaktif madde içeriği; karbonhidratlar, proteinler, peptitler, lipitler ve mineraller ile fenoller, alkoloidler, terpenler ve pigmentler gibi çok sayıda molekülü içermesinden kaynaklanmaktadır (Barkia ve diğ., 2019; Kosanić ve diğ., 2015). Makroalgler kuru maddede %7-31 protein, %2-13 lipit ve %32-60 karbonhidrat içeriğine sahiptir (Kazir ve diğ., 2019). Diğer taraftan, türe bağlı olarak,

A, B_1, B_2, B_9, B_{12}, C, D, E ve K vitaminlerini ve kalsiyum, demir, iyot, fosfor, potasyum, bakır, magnezyum, selenyum, çinko, bakır ve florür gibi mineralleri içermektedir (Panzella ve Napolitano, 2017).

Makroalglerin kimyasal bileşimi ışık yoğunluğu, deniz suyunun tuzluluğu ve sıcaklığı gibi çevresel koşullara ve türler arasındaki genetik farklılıklara bağlı olarak değişmektedir (Mæhre ve diğ., 2014). Örneğin sıcaklık, makroalglerin büyümesi, gelişmesi ve bileşenlerin biyosentezi üzerinde önemli bir etkiye sahiptir. Bazı araştırmacılar su sıcaklığındaki artışın lipit ve protein birikimi üzerinde olumlu bir etkiye sahip olduğunu ve aynı zamanda karotenoid birikimini de teşvik ettiğini belirtmiştir (Boëchat ve Giani, 2000; Roleda ve Hurd, 2019). Örneğin, haziran ve eylül ayları arasında yetişen makroalglerin kimyasal kompozisyonundaki değişimlerin araştırıldığı bir çalışmada, polifenol içeriğinde (kuru maddede %5,35-6,02'den %14,66-16,80'e), pigment seviyesinde (klorofil içeriğinde kuru maddede %0,13, karotenoid miktarında kuru maddede %0,04) ve doymuş yağ asitlerinde (kuru maddede %3,21) artış meydana gelmiştir. Bu değişimlerin güneşteki fotosentetik aktiviteden ve deniz suyu sıcaklığından kaynaklandığı rapor edilmiştir (Konstantin ve diğ., 2023). Diğer taraftan, aşırı güneş radyasyonu, fotoinhibisyona yol açarak kloroplast lamellerinin tahrip olmasına ve enzim sistemlerinde işlev bozukluğuna neden olabilir. Bu nedenle, makroalgler başta polifenoller olmak üzere foto koruyucu metabolitler üretmektedir (Roleda ve diğ., 2019).

2.1.1 Protein içeriği

Makroalglerin protein içeriği türe göre farklılık göstermektedir. Kahverengi makroalglerin protein düzeyi genel olarak düşük (%3-15), yeşil makroalglerin orta düzeyde (%9-26) olmasına rağmen, kırmızı makroalglerin protein içeriği yaklaşık %47'ye ulaşabilmektedir (Fleurence ve diğ., 2018). Bundan dolayı makroalgler, yüksek protein içeriği nedeniyle sürdürülebilir ve alternatif protein kaynağı olarak değerlendirilmektedir (Biris-Dorhoi ve diğ., 2020). Diğer taraftan, makroalglerin protein içeriği deniz suyu sıcaklığına ve tuzluluğuna, okyanus akıntılarına, dalga koşullarına ve alg türüne bağlı olarak farklılışabilmektedir (Yucetepe ve diğ., 2023). Örneğin Atlantik kıyılarından toplanan *Palmaria palmata* makroalginin protein miktarı deniz suyunun sıcaklığına bağlı olarak %9-25 arasında değişmiştir ve en yüksek değerler kış ve ilk bahar mevsimlerinde elde edilmiştir (Fleurence ve diğ., 2004; Gordalina ve diğ., 2021).

2.1.2 Lipit içeriği

Makroalgler %0,60-4,15 oranında lipit içermektedir (Shannon ve Abu-Ghannam, 2019). Makroalglerin lipit miktarı ve yağ asidi profilindeki farklılıklar çevresel koşullardan ve tür farklılığından etkilenmektedir. Genel olarak kahverengi türlerin yeşil makroalglere göre daha yüksek lipit içeriğine sahip olduğu ifade edilmiştir (Biancarosa ve diğ., 2018; Jeon ve diğ., 2010). Makrolagler genel olarak düşük lipit içeriğine sahip olmakla birlikte, çoklu doymamış yağ asitleri bakımından zengindir (Pereira, 2018). Makroalglerde bulunan lipitlerin neredeyse yarısı, eikosapentaenoik asit (EPA) ve araşidonik asit (AA) gibi çoklu doymamış yağ asitlerinden oluşmaktadır. Makroalg lipidleri arasında glikolipitler ve fosfolipitler de bulunmaktadır. Glikolipitler, hücresel membranın temel bileşeni olan bir lipide glikozidik bağ yoluyla bağlanan karbonhidratlardır (Biancarosa ve diğ., 2018; Ma ve diğ., 2014). Fosfolipitler ise tüm biyolojik membran sistemlerinin temel yapıtaşıdır ve yalnızca yapısal moleküller olarak değil, aynı zamanda hücrelerin dinamik ve işlevsel açıdan önemli bileşenleri olarak da tanımlanırlar (Hanahan ve Nelson, 1984).

2.1.3 Polisakkarit içeriği

Makroalgler yapısal ve enerji depolama dahil olmak üzere önemli işlevlere sahip polisakkaritleri yüksek miktarlarda içerebilmektedir ve genel olarak değişken bir polisakkarit içeriğine sahiptirler. Makroalglerdeki toplam polisakkarit içeriği kuru maddede %4 ile %76 arasında değişmekte olup en yüksek polisakkarit içeriğine *Ascophyllum, Porphyra ve Palmaria* spp. makroalgleri sahiptir (Holdt ve Kraan, 2011). Kumar ve diğ. (2021)'ne göre, yeşil makrolagler %17,0-83,2, kahverengi makroalgler %12,5-47,43 ve kırmızı makrolagler %2,7-65,0 oranında karbonhidrat içeriğine sahiptir.

Makroalglerde bulunan polisakkarit fraksiyonlardan biri olan karragenan, kırmızı makroalglerin hücre duvarının ana bileşenlerinden biridir ve kuru alg ağırlığının %30 ile %75'ini oluşturur. Örneğin, *Gracilaria gracilis*'ten elde edilen bir polisakkarit olan agar; gıda, farmosötik ve biyoteknolojik uygulamalarda kullanılmaktadır (Ferreira ve diğ., 2021). Diğer taraftan, başka bir polisakkarit olan aljinatlar ise kuru alg ağırlığının %17 ile %45'ini oluşturur ve kahverengi makroalglerde hücre duvarının temel bileşenidir. *Ulva* spp. yeşil makroalginin hücre duvarının bir bileşeni olan ulvan polisakkaritleri ise kuru alg kuru ağırlığının %8 ile %29'unu oluşturmaktadır ve çeşitli biyoaktif özelliklerine (antioksidan, anticoagulant, antiviral, antikanser aktivite gibi) rağmen potansiyeli yeterince değerlendirilememektedir (Pradhan ve diğ., 2023; Vera ve diğ., 2011; Ferreira ve diğ., 2021).

2.1.4 Pigment içeriği

Pigmentler, gıdaların renklerinin oluşmasında önemli unsurlardır (Ghosh ve diğ., 2022). Son yıllarda artış gösteren sağlıklı beslenmeye yönelik tüketici endişeleri, hem sentetik renklendiricilere alternatif hem de sağlığı teşvik eden bileşenler olarak kabul edilen doğal pigmentleri kullanma eğilimini arttırmaktadır (Ghosh ve diğ., 2022; Hosseinkhani ve diğ., 2022).

Makroalglerde bulunun pigmentler karotenoid, klorofil ve fikobilin (fikobiliprotein)'lerdir. Karotenoidler apolar pigmentlerdir. Yapısal olarak terpenoid pigment sınıfına aittir ve sahip oldukları mor, kırmızı, turuncu ve sarı gibi farklı renkler, yüksek oranda konjuge olan polien zincirinden kaynaklanmaktadır (Poojary ve diğ., 2016). Klorofil; klorofil a, klorofil b ve klorofil c içeren yağ pigmentleri ailesidir. Klorofil pigmenti, yeşil ışığı iyi yansıtabildiğinden genel olarak yeşil görünüm sağlar ve fotosentezin ışık emilimi sürecinde merkezi bir rol oynamaktadır (Bednarczyk ve diğ., 2021). Fikobilin, yalnızca alglerde bulunan bir fotosentetik pigmenttir. Fikobilin

yapısal olarak klorofile benzer ve metilenle bağlanan dört pirol halkasından oluşur, ancak klorofilden farklı olarak düz zincirli bir moleküldür ve magnezyum atomu içermez (Mysliwa-Kurdziel ve Solymosi, 2017). Tablo 1'de bazı makroalglerde bulunan karotenoidler ve miktarları verilmiştir.

Tablo 1. Bazı makroalglerdeki karotenoid/klorofil a ve karotenoid/klorofil b oranı ile fukoksantin ve lutein miktarları (mg/kg).
Table 1. Carotenoid/ chlorophyll a and Carotenoid/ chlorophyll b ratios and fucoxanthin and lutein contents in some macroalgae (mg/kg).

Makroalg Sınıfı	Makroalg Türü	Karotenoid/ Klorofil a oranı	Karotenoid/ Klorofil b oranı	Fukoksantin	Lutein	Referanslar
Yeşil makroalgler (Chlorophyta)	*Caulerpa racemosa*	0,02	0,13			(Kumar ve diğ., 2009)
	Cladophora fascicularis	0,003	0,02			
	Ulva lactuca linn	0,04	0,15			
	Fragilaria crotonensis			33,8		(Deventer ve Heckman, 1996)
Kahverengi makroalgler (Phaeophyta)	*Dictyota bartayresiana*	0,04	0,13			(Kumar ve diğ., 2009)
	Padina gymnospora	0,23	0,75			
	Sargassum ilicifolium	0,05	0,21			
	Padina australis			0,27		(Zailanie ve Purnomo, 2011)
	Turbinaria conoides			0,21		
Kırmızı makroalgler (Rhodophyta)	*Champia compressa*	0,07	0,16			(Kumar ve diğ., 2009)
	Liogora erecta	0,07	0,15			
	Scinaia farcellata	0,03	0,12			
	Soliera robusta	0,04	0,14			
	Amphiroa rigida				5,83	(Cikoš ve diğ., 2021)

3. Karotenoidler

Sekonder metabolitler olarak bilinen karotenoidlerin doğada 750'den fazla türü bulunmaktadır (Shahidi ve Brown, 1998; Ribeiro ve diğ., 2010; Koizumi ve diğ., 2018). Karotenoidler sekiz izoprenoid biriminden oluşan hidrokarbonlardır ve güçlü antioksidatif aktivite sergileyen, konjuge çift bağa sahip, yağda çözünebilen pigmentlerdir (Nakano ve Wiegertjes, 2020). En yaygın karotenoidler, 11 konjuge çift bağ içeren 40 karbonlu izoprenoidlerdir. Çift bağlar, cis ya da trans izomeri oluşturabilmelerine rağmen doğada genellikle trans izomeri formunda bulunmaktadır (Denizci, 1990). Karotenoidlerin renklerini, yapılarında bulunan çok sayıdaki konjuge (C=C) çift bağlar belirler. Bu konjuge çift bağların sayısı arttıkça karotenoidlerin renkleri koyulaşmaktadır. Yapısında 9 tane konjuge çift bağ içeren β-karotenin rengi sarı-turuncu iken, yapısında 11 tane konjuge çift bağ içeren likopenin rengi kırmızıdır (Özkan ve Cemeroğlu, 1997).

Karotenoidler karoten (likopen, α-karoten, β-karoten gibi) ve ksantofil (astaksantin, fukoksantin, lutein gibi) olmak üzere iki gruba ayrılırlar. Bilinen ilk sınıf olan karotenler kimyasal yapılarında bulundurdukları karbon ve hidrojen atomlarına ek olarak likopen, α-karoten, β-karoten ve torulen içerir. Ksantofiller ise karbon ve hidrojen atomuna ek olarak yapılarında oksijen atomunu da bulundururlar. Lutein, astaksantin, zeaksantin, violaksantin ve β-kriptoksantin ksantofil sınıfında yer almaktadır (Mussagy ve diğ., 2019).

Karotenoidler bulundukları kaynakta ya kristal formda ya da yağ asitleri ve proteinler dahil olmak üzere diğer moleküllerle kompleks oluşturmuş olarak bulunurlar (Ribeiro vd., 2010). Örneğin, ksantofiller tipik olarak birçok meyve, çiçek, yumru sebzeler gibi bitki organlarında yağ asitleriyle ester oluşturmuş halde bulunur (Mínguez-Mosquera ve Hornero-Méndez, 1994). Öte yandan bazı karotenoidler, suda çözünen ve karotenoidleri stabilize eden proteinlerle kompleks oluşturabilirler (Bhosale ve Bernstein, 2007). Örneğin likopen; α-karoten, β-karoten ve diğer karotenoidler gibi kloroplastlarda karotenoid-protein kompleksi halinde veya kromoplastlar içinde kristal formda bulunmaktadır (Shi ve Le Maguer, 2000; Parada ve Aguilera, 2007). İnsanlarda bağırsaktan emilen karotenoidlerin %80'den fazlası lipoproteinler tarafından taşınarak yağ dokularında birikir (Parker, 1996).

4. Karotenoidlerin Stabiliteleri

Gıdaların depolanması sırasında, sıcaklık, ışık ve oksijen maruziyeti ile depolama süresi, su aktivitesi ve kullanılan ambalaj malzemesi gibi çeşitli faktörler karotenoidlerde kayıplara neden olabilmektedir (Meléndez-Martínez ve diğ., 2022). Karotenoid miktarlarının, izomerizasyon ve oksidasyondan kaynaklı olarak gıda ürünlerinin ambalajlanması, taşınması, depolanması ve diğer gıda işleme prosesleri sırasında azalabileceği ileri sürülmektedir (Anguelova ve Warthesen, 2000). Örneğin kurutma, meyve suyuna işleme ve soyma işlemleri karotenoidlerde önemli kayıplara neden olabilmektedir. Benzer olarak, García-Alonso ve diğ. (2009) yaptıkları bir çalışmada, domates suyunu Tetrapak® ve cam şişelerde 12 ay boyunca 8, 22 ve 37 °C'de depolamışlar ve likopen, askorbik asit, toplam fenolik ve toplam flavonoid miktarlarını ölçmüşlerdir. Genel olarak, likopen, toplam fenolik ve toplam flavonoid miktarları, kullanılan ambalaj malzemesine bakılmaksızın, 12 ay boyunca depolama sırasında neredeyse sabit kalmıştır. Bu durum, domates sularının raf ömrü boyunca antioksidan aktivitesini korunduğunu göstermektedir. Diğer taraftan, 37 ºC'de depolanan domates sularında toplam antioksidan seviyesinde %10-16 oranında düşüş, cis-izomerlerinde ise artış görülmüştür. Başka bir çalışmada, farklı depolama sıcaklığı (4, 25 ve 40 ºC) ve paketleme (N_2 ve atmosferik hava) koşullarının kurutulmuş kabaklardaki karotenoidlerin parçalanması üzerine etkisi araştırılmıştır (Song ve diğ., 2018). Çalışmanın sonuçlarına göre, depolama süresince luteinde, α-karoten ve β-karotene göre daha düşük düzeyde parçalanma gerçekleşmiştir. N_2 ile paketlenmiş örneklerde β-karotenin, Z-izomerlerinin özellikle 40 ºC depolama sıcaklığında daha stabil olduğu rapor edilmiştir. Depolama sırasında oluşan β-karotenin bu Z-izomerlerinin oksidatif

reaksiyonlara katıldığı varsayılmaktadır. Diğer taraftan, atmosferik hava ile paketlenen kabak örneklerinde, oksijen varlığı ve sıcaklık ile katalizlenen oksidasyon reaksiyonlarından dolayı daha yüksek oranda bozunma meydana geldiği ifade edilmiştir (Song ve diğ., 2018).

Dondurma işlemi ve antioksidan ilavesi genellikle karotenoidleri korumaktadır (Dugave ve Demange, 2003; Liu ve diğ., 2021). Diğer taraftan, dondurarak depolamada karotenoid stabilitesi, bitki dokularının ve incelenen karotenoidlerin türlerine bağlı olarak değişkenlik göstermektedir. Yapılan bir çalışmada, polietilen ambalajlardaki sebzelerin (mısır, havuç, brokoli, ıspanak, bezelye, yeşil fasulye, çilek ve yaban mersini) -27,5 °C'de 90 gün boyunca depolanmaları sırasında β-karoten miktarı, yeşil fasulye ve brokolide değişmeden kalırken, bezelye (%70), ıspanak (%45) ve havuçta (%41) önemli düzeyde düşmüştür. Araştırmacılar, bu azalmaların dondurak saklama sırasında gerçekleşen oksidasyon reaksiyonlarından kaynaklandığını belirtmişlerdir (Bouzari ve diğ., 2015). Başka bir çalışmada ise dilimlenmiş havuçları vakum altında poliamid torbalarda, farklı donma sıcaklarında (-15, -18, -30 ve -50 °C) 2 yıl boyunca depolanmışlardır (Behsnilian ve Mayer-Miebach, 2017). Dilimlenmiş havuçların α-karoten, β-karoten ve lutein içerikleri, tüm depolama sıcaklarında 2 yıl boyunca sabit kalırken, yaklaşık 3 ay sonunda ürünlerde %57 seviyelerinde likopen kaybı tespit edilmiştir. Araştırmacılar, likopenin daha düşük stabilitesini, muhtemelen havuçların donmamış fazındaki çözünmüş oksijenin varlığından ve ayrıca likopenin β-karoten ile karşılaştırıldığında oksidasyona ve otooksidasyona girmeye daha yatkın olmasından kaynaklanabileceğini belirtmişlerdir (Behsnilian ve Mayer-Miebach, 2017). Yapılan bir diğer çalışmada ise pişirme koşullarının depolanan kabaklardaki α-karoten, β-karoten ve toplam karotenoid içeriği üzerindeki etkisi araştırılmıştır (Carvalho ve diğ., 2014). Bu çalışmada, çiğ kabak numunelerinin karotenoid içeriği kaynamış suda pişirilmiş, buharda pişirilmiş ve şeker ilavesiyle pişirilmiş örnekler ile karşılaştırılmıştır ve buharda pişirilen kabaklarda, diğerlerine göre belirgin şekilde daha yüksek α-karoten, β-karoten ve toplam karotenoid içeriği tespit edilmiştir (Carvalho ve diğ., 2014).

5. Makroalgal Karotenoidler

Makroalglerde bulunan karotenoidler alglerin pigmentasyon yapılarına göre farklılaşmaktadır. Örneğin yeşil makroalglerde β-karoten, lutein, violaksantin, neoksantin ve zeaksantin en yaygın olarak bulunan karotenoidlerdir. Özellikle zeaksantin alglerde karasal bitkilere kıyasla daha yaygın olarak bulunmaktadır. Kırmızı makroalglerde bulunan baskın karotenoidler ise lutein ve zeaksantinin yanı sıra α- ve β-karotenlerdir. Diğer taraftan, kahverengi makroalgler pek çok karotenoidi bol miktarda içermekle birlikte ayrıca fukoksantin, perinidin, diatoksantin, heteroksantin ve siphonaksantin karotenoidlerini ve astaksantin ve kantaksantin gibi karotenoid türevlerinden olan karotenoproteinleri içermektedir (Shahidi ve Brown, 1998; Koizumi ve diğ., 2018). Christaki ve diğ. (2013) genel olarak yeşil makroalglerde α-karoten, neoksantin, violaksantin, siphonoksantin; kırmızı makroalglerde β-karoten, lutein, zeaksantin ve kahverengi alglerde fukoksantin, β-karoten ve violaksantin karotenoidlerinin bulunduğunu bildirmiştir. Bianchi ve diğ. (1997)'nın çalışmasında ise Baltık Denizi'nden toplanan bazı makroalglerde baskın olan karotenoidler araştırılmıştır. Bu çalışmanın sonuçlarına göre; yeşil makroalgler *Cladophora glomerata* ve *Enteromorpha intestinalis*'de; fukoksantin ve zeaksantin belirlenememişken, β-karoten, violaksantin, lutein tespit edilebilmiştir. Kahverengi makroalgler; *Chorda filum*, *Dictyosiphon foeniculaceus*, *Fucus vesiculosus* ve *Pilayella littoralis*'da lutein ve zeaksantin mevcut değilken; fukoksantin, β-karoten, violaksantin belirlenebilmiştir. Kırmızı makroalgler; *Ceramium tenuicorne*, *Furcellaria lumbricalis*, *Phyllophora* spp, *Polysiphonia nigrescens* ve *Rhodomela corifervoides*'de fukoksantin ile violaksantin tespit edilememişken, β-karoten, lutein ve zeaksantin tespit edilebilmiştir. Xie ve diğ. (2020)'nin çalışmasında, kırmızı makroalg *Pyropia yezoensis*'de β-karoten ve zeaksantin konsantrasyonu üzerine ışığın etkisi araştırılmış ve yüksek ışık miktarının, β-karoten ve zeaksantin konsantrasyonlarını arttırdığı rapor edilmiştir. Kanda ve diğ. (2020)'in çalışmasında ise yeşil makroalg, *Monostroma nitidum*'da ana karotenoidin lutein olduğu ifade edilmiştir. Hu ve diğ. (2008)'nin çalışmasında *Dunaliella salina*'nın karotenoid profili kromatografik teknikler kullanılarak araştırılmıştır. Analiz sonuçlarına göre; bütün-trans-lutein, bütün-trans-zeaksantin, 13- ya da 13'-cis- β-karoten, bütün-trans-α-karoten, 9- ya da 9'-cis-α-karoten, bütün-trans- β-karoten, 9- ya da 9'-cis- β-karoten olmak üzere toplam 290,77 mg/g algal karotenoid tespit edilmiştir.

6. Makroalgal Karotenoidlerin Biyoaktif Özellikleri

6.1 Antioksidan aktivite

Hücresel düzeyde, oksidantlar ve antioksidantlar arasında bir denge mevcuttur. Hücreler, serbest radikaller ve reaktif oksijen türlerden (ROS) kaynaklanan dejeneratif etkilere karşı hücresel antioksidan kapasitesi ile korunurlar. Bir şekilde, homeostasisde dengesizlik olursa, hücrelerde serbest radikallerin ve ROS'un birikmesi söz konusu olabilir ve oksidasyon sırasında hücresel bileşenler olan protein, yağ ve nükleik asitler zarar görmeye başlar. Oksidatif stres olarak tanımlanan, protein, nükleik asit ve hücre zarının, süperoksit anyonu ($O_2^{\cdot-}$), hidrojen peroksit (H_2O_2) ve hidroksil radikali ($HO^{\cdot}$) gibi reaktif oksijen türlerine maruz kalmaları sonucu kanser, inme, kalp krizi, diyabet gibi pek çok dejeneratif hastalık ortaya çıkabilmektedir (Yucetepe, 2022). Bu nedenle, yaşam boyunca oksidatif stresi yönetebilmek önemlidir.

Diğer taraftan, oksidasyon gıda sistemlerinde de meydana gelebilmektedir. Özellikle, gıda lipitleri, oksidasyona karşı çok hassastır. Bu noktada, lipit oksidasyonu gıdaların üretimi, depolanması ve dağıtımı sırasında meydana gelen bozunmanın ana sebeplerinden birini oluşturmaktadır. Gıdalarda meydana gelen lipit oksidasyonu ile tat kaybı veya kötü tatların oluşması ile birlikte renk kaybı, besin değeri kaybı ve tüketicilerin sağlığına zararlı olabilecek yan ürünler oluşmaktadır (Maher ve Yamamoto, 2010).

Gıdalarda oksidasyonu geciktirmenin en etkili yollarından biri gıda ürünlerine antioksidanların ilave edilmesidir (Wasowicz ve diğ., 2004). Antioksidan bileşikler, reaktif oksijen türlerinden kaynaklanan zararlı oksidasyon proseslerini önleyebilir, geciktirebilir ya da erteleyebilirler. Böylece, gıdaların raf ömrünü, besin değerini ve kalitesini etkileyerek gıda ürünlerinin depolanmaları sırasında bozunmasını yavaşlatabilirler (Christodouleas ve diğ., 2015).

Karotenoidler biyolojik sistemlerde çeşitli reaktif radikal türlerin etkisiz hale getirilmesinde önemli rol oynarlar (El-

Agamey ve diğ., 2004). Koruma mekanizmaları tekli oksijeni söndürme ve serbest radikalleri temizlemeyi içerir ve düşük oksijen ve kısmi basınç altında lipit peroksidasyonunu engellerler (Burton, 1989). Yapılan bir çalışmada, genç sağlıklı yetişkin kadın deneklerde astaksantinin olası bağışıklık arttırıcı, antioksidan ve antienflamatuar aktivitesi araştırılmış ve astaksantinin inflamasyonu azaltabildiği ve bağışıklılığı artırabileceği belirtilmiştir (Park ve diğ., 2010).

Macroalgal karotenoidlerden fukoksantin, ksantofil sınıfına aittir ve kahverengi makroalglerde yaygın olarak bulunan bir karotenoiddir. Kahverengi makroalg *Sargassum siliquastrum*'dan ekstrakte edilen fukoksantinin *in vitro* olarak güçlü bir antioksidan olduğu ve oksidatif strese karşı koruyucu etki gösterdiği tespit edilmiştir (Heo ve diğ., 2008). Başka bir çalışmada, böbrek epitel hücreleri artan konsantrasyonlarda fukoksantin (5, 50, 100 ve 200 µM) ile muamele edilmiş ve ardından 24 saat boyunca 50 µM H_2O_2 uygulanmıştır. Karotenoid ile ön muamelenin, oksidatif maddenin sitotoksik etkisini doza bağlı bir şekilde önleyebildiği ve hücre canlılığını sırasıyla %63,6, %69,4, %78,5 ve %89,2 oranlarında koruyabildiği rapor edilmiştir (Heo ve Jeon, 2009). Tablo 2'de bazı makroalgal karotenoidlerin biyoaktif özellikleri verilmiştir.

6.2 Antikanser aktivite

Kanser günümüzde, dünyadaki en önemli sağlık sorunlarından birini oluşturmaktadır. Kanserin tedavisinde kullanılan kemoterapi ve radyoterapi kanser hücrelerinin hızlı bir şekilde bölünmesini ve gelişmesini engelleyerek kanserin ölümcül etkisini azaltmak amacıyla uygulanmaktadır (Yucetepe ve diğ., 2022). Fakat kullanılan bu yöntemler normal hücrelere de zarar verebilmekte ve toksik etkiler gösterebilmektedir (Çetik ve diğ., 2015). Diğer taraftan, birçok çalışmada, doğal antioksidanların kemoterapiye bağlı toksik etkileri azaltabildiği rapor edilmiştir (Christen ve diğ., 2000).

Lutein, α-karoten, β-karoten, astaksantin, fukoksantin gibi bazı karotenoidler, kanserin önlenmesini ve ilerlemesini yavaşlatabilmektedir (Rock ve diğ., 2009). Bu nedenle, son yıllarda, kanserin neden olduğu hasarın ortadan kaldırılmasında anahtar bir faktör olan bağışıklık sistemi mekanizmasının güçlendirilmesini sağlayan antioksidan aktivitedeki karotenoidler ve bunların makroalg gibi doğal kaynaklarına olan ilgi artmaktadır (Das ve diğ., 2007).

Makroalgler, tümör ve farklı kanser türlerinin (meme, kolon, lösemi, vb.) tedavisinde etkili olan önemli biyoaktif bileşenleri içermektedir. Fukoksantin doğada, özellikle deniz ortamında en bol bulunan karotenoidlerden birini temsil etmektedir ve toplam doğal karotenoid üretiminin yaklaşık %10'una oluşturmaktadır (Dembitsky ve Maoka, 2007). Fukuksantin, hücre çoğalmasını önleyici etkisi nedeniyle, araştırılan ilk karotenoid kaynaklarından biridir. Hosokawa ve diğ., (1999)'nın çalışmasında, fukoksantin, lösemi hücreleri (HD-60, HL-60) ve epitelyal kolorektal adenokarsinom hücreleri (Caco-2, DLD-1, HT-29) gibi çeşitli hücre dizileri üzerinde *in vitro* olarak test edilmiştir ve HL-60 hücreleri 11,3 ve 46,2 µM fukoksantin ile muamele edildiğinde hücre canlılığı doza bağlı olarak güçlü bir şekilde azalmıştır (Hosokawa ve diğ., 1999). Zhang ve diğ., (2008)'nın çalışmasında ise kahverengi makroalg *Laminaria japonica*'den ekstrakte edilen fukoksantinin (20 µM), insan idrar kesesi kanseri hücrelerinin çoğalma oranını büyük ölçüde azalttığı rapor edilmiştir. Başka bir çalışmada, makroalgal fukoksantinin, insan mide adenokarsinomunda hücre canlılığını veya çoğalmasını azalttığı gösterilmiştir (Yu ve diğ., 2018).

6.3 Antidiyabetik aktivite

Basitçe diyabet olarak adlanadırılan diabetes mellitus, insülin salgılanmasındaki kusurlardan kaynaklanan hiperglisemi ile karakterize bir grup metabolik değişikliktir. Tip I ve Tip II olmak üzere iki tipten oluşmaktadır. Genellikle juvenile diabetes olarak adlandırılan Tip I diyabet, insüline bağımlıdır ve diyabetik popülasyonun yaklaşık %5'ini etkilediği bilinmektedir. İnsüline bağımlı olmayan Tip II diyabet ise, genellikle 40 yaş üstü yetişkinlerde gelişebilmektedir. Kronik hipergliseminin, özellikle gözler, böbrekler, sinirler, kalp ve kan damarları olmak üzere organlarda uzun süreli hasar ve işlev bozukluğu ile ilişkili olduğu bilinmektedir (Mamun-or-Rashid ve diğ., 2014).

Diyabet tadavisinde kullanılan ilaçlar, insülin duyarlılığını iyileştirerek ve üretimini artırarak ve kandaki glikoz miktarını azaltarak diyabeti tedavi etmeyi amaçlamaktadır. Diğer taraftan, bazı bitkilerin de farmakolojik olarak aktif bileşenlerinin hipoglisemik etki ile diyabet gelişiminden sorumlu farklı kan parametrelerinin doğrudan ve dolaylı etkilerini azaltarak antidiyabetik aktiviteye sahip oldukları bilinmektedir (Mamun-or-Rashid ve diğ., 2014).

Karotenoidlerin antidiyabetik aktivitesi üzerine yapılan bazı çalışmalarda, Tip 2 diyabetin önlenmesi ve tedavisinde karotenoidlerin diyet alımındaki önemi bildirilmiştir (Stahl ve Sies, 2005). Astaksantinin, diyabetik nefropatinin ilermesi üzerine etkisinin araştırıldığı bir çalışmada, astaksantinin Tip 2 diyabetin kemirgen modelinde renal hücreler üzerinde yararlı etki gösterebileceği ve diyabetik nefropatinin ilerlemesini iyileştirebileceği bildirilmiştir (Naito ve diğ., 2004). Makroalgden izole edilen fukoksantin üzerine yapılan bir çalışmada bu karotenoid uygulaması ile farelerde kan şekeri ve insülin seviyelerinin normale döndürebildiği rapor edilmiştir (Maeda ve diğ., 2009). Jung ve diğ. (2012)'nin çalışmasında, *Eisenia bicyclis* ve *Undaria pinnatifida*'dan izole edilen fikoksantinin diyabetin yanı sıra diyabetle ilişkili komplikasyonların yönetimi için terapötik bir ajan olarak potansiyeli ortaya konulmuştur.

6.4 Antienflamatuar aktivite

Enflamasyon, vücudun bağışıklık sisteminin doku hasarına karşı verdiği olağan bir tepkidir. Küçük polipeptitler olan ve iltihap bölgesinde salınan sitokinler ve diğer aracılar tarafından gerçekleştirilir. Ancak sitokinlerin kontrolsüz ve aşırı üretimi dokulara zarar vererek kalp-damar hastalıkları, romatoid artrit, bronşit ve kanser gibi kronik inflamasyon kaynaklı hastalıklara yol açabilmektedir. Bu nedenle antienflamatuar aktiviteye sahip bileşenler ile enflamasyon aracılarının baskılanması aşırı ve kontrolsüz enflamasyonla ilişkili arterit, hepatit, gastrit, periodantal hastalık, kolit, zatürre ve nöroenflamatuar gibi hastalıkların tedavisinde önemli bir stratejidir (Yucetepe, 2022; Vaughan ve diğ., 2013; Pangestuti ve Kim, 2011).

Son yıllarda astaksantin, antienflamataur aktivitesi nedeniyle araştırmacıların ilgisini çekmektedir. Astaksantinin önleyici etkilerinin, yaygın antienflamatuar ilaç olan prednizol ile karşılaştırıldığı bir çalışmada, astaksantinin 100 mg/kg konsantrasyonda antienflamatuar etkisinin, 10 mg/kg prednizol ilacından daha yüksek olduğu görülmüştür (Ohgami

ve diğ., 2003). Başka bir çalışmada, *Haematococcus pluvialis* alginden ekstrakte edilen astaksantin ile beslenen farelerin, mide iltihabı seviyelerinde bir azalma gözlemlenmiştir ve 10 gün boyunca 200 mg/kg algal ekstrakt ile beslenen fareler, astaksantin ile muamemle edilmeyen farelere kıyasla midelerinde önemli ölçüde daha düşük düzeyde iltihaplanma ve mukoza-bakteriyel yük göstermiştir (Bennedsen ve diğ., 2000).

Tablo 2. Bazı makroalglerde bulunan karotenoidler ve biyoaktivitesi.
Table 2. Carotenoids and their bioactivities found in some macroalgae.

Makroalg	Karotenoid	Biyoaktivite	Referanslar
Iridaea cordata	β-karoten, zeaksantin	Antikanser aktivite	
Cystosphaera jacquinotii	β-karoten, zeaksantin, fukoksantin ve violaksantin	Antikanser aktivite	(Frassini ve diğ., 2019)
Desmarestia anceps	β-karoten, fukoksantin ve violaksantin	Antikanser aktivite	
Ulva lactuca, *Ulva fasciata*	Karotenoid içeren ekstrakt	Antioksidan, antikanser ve antimikrobiyal aktivite	(Saeed ve diğ., 2020)
Eucheuma denticulatum, *Gracilaria tikvahiae*, *Kappaphycus striatum*, *Caulerpa lentillifera* ve *Padina pavonica*	Lutein, zeaksantin, beta-karoten ve violaksantin	Antioksidan ve antimikrobiyal aktivite	(Othman ve diğ., 2018)
Scytosiphon lomentaria, *Cystoseira barbata* ve *Padina pavonica*	Karotenoid içeren etanolik ekstrakt	Antioksidan aktivite	(İlknur ve Turker, 2018)
Caulerpa racemos	Fukoksantin, lutein, astaksantin, kantaksantin, zeaksantin, β-karoten ve β-kriptoksantin	Antidiyabetik, antiobesize, antienflamatuar ve antioksidan aktivite	(Kurniawan ve diğ., 2023)
Sargassum siliquastrum	Fukoksantin	Antikanser aktivite	(Heo ve diğ., 2008)
Conticribra weissflogii	Fukoksantin	Antienflamatuar aktivite	(Su ve diğ., 2019)
Codium adhaerens	Fukoksantin içeren ekstrakt	Antioksidan aktivite	(Radman ve diğ., 2021)

6.5 Antihipertansif Aktivite

Hipertansiyon, ciddi komplikasyonlara neden olması ve toplumda sık görülmesi nedeniyle önemli bir halk sağlığı sorunudur. Son yıllarda, ilaç tedavisinde sağlanan önemli gelişmelere rağmen hipertansiyon, gelişmiş ve gelişmekte olan ülkelerin en önemli sağlık sorunlarından biri olmaya devam etmektedir (Lewington ve diğ., 2003). Sistolik kan basıncını 140 mmHg ve diyastolik kan basıncını 90 mmHg'nin altında olacak biçimde düzenlemek ve gerekli yaşam biçim değişikliklerini yapmak, kardiyovasküler komplikasyonların ve ölümlerin önlenmesi açısından önemlidir (Smith ve diğ., 1990).

Kan basıncını düşüren ilaçlar, özellikle anjiyotensin-I-dönüştürücü-enzim (ADE) inhibitorler, renin-anjiyotensin sistemde kan basıncını düzenlemek amacıyla kullanılırlar. ADE, aktif olmayan prohormon anjiyotensin I'den aktif hipertansif hormon anjiyotensin II'nin üretiminde bir katalizördür ve kan damarlarının genişlemesini sağlayan bir vazodilatör olan bradikininin parçalanmasında rol oynar. ADE'nin kan basıncı üzerindeki etkisi nedeniyle, bu enzimin inhibisyonu hipertaniyonun tedavisinde önemlidir (Daskaya-Dikmen ve diğ., 2017).

Makroagler, önemli düzeyde antihipertansif etki gösteren aktif maddeler içermektedir. Makrolag tüketiminin, diyet lifinin hipotansif etkilerine ve zengin nitrat içeriğine bağlı olabilecek kan basıncının düşmesine yardımcı olduğu görülmüştür (Mendis ve diğ., 2011). Makroalglerin sekonder metabolitleri, hipoglisemik ajanlar olarak görev yapmakta, kan basıncını düşürmekte ve kolestrol seviyelerini düzenlemektedir (Saito ve diğ., 2002).

Likopenin güçlü bir antioksidan olarak tanınması ve oksidatif strese bağlı kronik hastalıklardaki önleyici rolünün anlaşılmasından bu yana, araştırmacılar onun diğer hastalıklardaki rolünü araştırmaya başlamışlardır. Oksidatif stres ile hipertansiyon görülme sıklığı arasında nedensel bir ilişki olduğu kabul edilmektedir (Paran ve Engelhard, 2001). Yapılan bir çalışmada, 8 hafta boyunca günde 15 mg oranında likopen takviyesinin, hafif hipertansiyonlu kişilerde sistolik kan basıncının 144 mmHg'lik başlangıç değerinden 134 mmHg'ye önemli ölçüde düşürdüğünü göstermiştir (Paran, 2006). Raji ve diğ. (2023)'nın çalışmasında kahverengi alg *Sargassum wightii*'den saflaştırılan fikoksantinin hipertansiyonlu sıçanlarda kan basıncını ve ADE aktiviteyi önemli düzeyde azalttığı gösterilmiştir.

7. Sonuç

İlerleyen yıllarda, insanları ve dünyada yaşayan birçok organizmanın yaşamını tehlikeye atacak olan gıda krizi, iklim değişikliği, küresel ısınma gibi birçok olumsuz durumla karşılaşılabileceği tahmin edilmektedir. Bu durum, araştırmacıları farklı gıda kaynaklarını araştırmaya yöneltmektedir. Bu açıdan, makroalgler içerdikleri biyoaktif bileşikler nedeniyle dikkat çekmektedir. Makroalgal karotenoidler sadece doğal renk maddesi olarak değil, aynı zamanda insan sağlığı üzerinde olumlu etkileri olan biyoaktif maddelerdir. Karotenoidlerin koruyucu etkisinin antioksidan aktivitesinden kaynaklandığı düşünülmektedir. Karotenoidler üzerine yapılan çalışmaların çoğu, esas olarak bu moleküllerin diyabet, kanser, hipertansiyon, kardiyovasküler hastalıklar gibi çeşitli kronik hastalıklardaki önleyici ve koruyucu etkilerine odaklanmıştır. Bu derleme çalışmasında, makroalglerin kimyasal özellikleri, karotenoidlerin stabilitesi ve algal karotenoidlerin bazı biyokaktif özellikleri ortaya konmuştur. Gelecek çalışmalarda, makroalglerden elde edilen karotenoidlerin bu çalışmada değinilmeyen antiviral, antiobezite, antiaging gibi diğer biyoaktif özellikleri ve gıda uygulamaları ile biyoerişilebilirlikleri araştırılabilir.

8. Teşekkür ve Bilgi

Bu derleme makale, 221O673 numaralı TÜBİTAK projesi ve Neşe Balkesen'in yüksek lisans tezi kapsamında yazılmıştır.

9. Çıkar Çatışması

Yazarlar çıkar çatışması beyan etmemektedir.

10. Kaynaklar

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Havalı Güneş Kollektör Destekli Sera Gıda Kurutucu Sisteminin Performansının İncelenmesi

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Özet: Kurutma katı maddelerden ısıl yöntemlerle su veya uçucu maddelerin giderilmesi işlemini tanımlamaktadır. Güneş enerjisi ile tarım ürünlerini kurutma, en eski gıda saklama yöntemlerinden birisi olarak bilinmektedir. Güneşte kurutmada çevresel faktörler nedeniyle gıdanın kalitesi ciddi olarak azalmaktadır. Bu nedenle kurutma işleminin özel amaçlı yapay kurutucular ile yapılması hem kuruma süresini kısaltmakta hem de uzun raf ömrüne sahip daha kaliteli ve temiz ürün elde edilmesini sağlamaktadır. Bu çalışmada gıda kurutma kalitesini ve performansını arttırmak için havalı güneş kolektör (HGK) destekli sera tipi bir kurutucu tasarlanmıştır. Deneyler, açık güneşli ortam şartlarında, Mayıs 2023 tarihinde, Elazığ ili iklim şartlarında gerçekleştirilmiştir. Kurutma deneyleri sonrası kurutulacak ürünün ısı ve kütle transferi analizleri yapılmıştır. Deneylerde nem içeriği, nem oranı, konvektif ısı transfer katsayısı parametreleri hesaplanmıştır. Deneyler süresince, sera ve HGK giriş ve çıkış sıcaklıkları, güneş ışınımı ve ürün ağırlık değerleri 15 dakikalık periyotlarla ölçülmüştür. HGK desteği ile sera kurutucunun ürün kurutma süresi %24 oranında azalmıştır. Böylelikle daha hızlı bir kurutma süreci elde edilmiştir. Ayrıca kurutma işlemlerinde önemli bir parametre olan konvektif ısı transfer katsayısı hesaplanmış ve bu parametre için makine öğrenmesi (MÖ) algoritmaları ile tahminsel modeller elde edilmiştir. Bu çalışmanın amacı, sera tipi gıda kurutucuların performansını arttırmak için havalı güneş kollektörü kullanmak ve konventif ısı transferi için MÖ algoritmaları kullanılarak faydalı modellerin üretilmesidir. Kısaca hem yapay zekâ hem de deneysel uygulamaların yapılacağı termodinamik bir sistem elde edilmiştir. Makine öğrenmesi algoritmaları olarak yapay sinir ağı (YSA) ve karar ağacı (KA) algoritmaları seçilmiştir. MÖ algoritmaları ile elde edilen model sonuçları ile deneysel sonuçlar karşılaştırılmıştır. Deneysel sonuçları ile YSA sonuçları arasındaki hata %1 iken, KA sonuçları arasındaki hata %7'dir.

Anahtar Kelimeler: Güneş kollektörü, konvektif ısı transfer katsayısı, makine öğrenmesi, sera kurutucu.

Investigation of Performance of Air Solar Collector Assisted Greenhouse Food Dryer System

Abstract: Drying refers to the process of removing water or volatile substances from solids by thermal methods. Drying agricultural products with solar energy is known as one of the oldest food storage methods. In solar drying, the quality of food is seriously reduced due to environmental factors. For this reason, drying with special purpose artificial dryers shortens the drying time and provides a better quality and cleaner product with a long shelf life. In this study, an air solar collector (ASC) supported greenhouse type dryer was designed to improve the quality and performance of food drying. The experiments were carried out under open sunny conditions in May 2023 in the climatic conditions of Elazığ province. After the drying experiments, heat and mass transfer analysis of the product to be dried were carried out. Moisture content, moisture ratio, convective heat transfer coefficient parameters were calculated in the experiments. During the experiments, greenhouse and ASC inlet and outlet temperatures, solar radiation and product weight values were measured at 15-minute intervals. With the support of the ASC, the product drying time of the greenhouse dryer was reduced by 24%. Thus, a faster drying process was achieved. In addition, convective heat transfer coefficient, which is an important parameter in drying processes, was calculated and predictive models were obtained with machine learning (ML) algorithms for this parameter. The aim of this study is to use air solar collectors to improve the performance of greenhouse type food dryers and to produce useful models for convective heat transfer using ML algorithms. In short, a thermodynamic system in which both artificial intelligence and experimental applications will be performed has been obtained. Artificial neural network (ANN) and decision tree (DTA) algorithms were selected as machine learning algorithms. Model results obtained with ML algorithms are compared with experimental results. The error rate between the experimental results and ANN results is 1%, while the error rate between KA results is 7%.

Keywords: Greenhouse dryer, solar collector, convective heat transfer coefficient, machine learning.

Araştırma makalesi

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1.Giriş

Türkiye coğrafi konumu nedeniyle sahip olduğu güneş enerjisi potansiyeli açısından diğer birçok ülkeye göre nispeten şanslı durumdadır. Ülkemiz güneş kuşağı adı verilen ve güneş enerjisince zengin bir bölgede yer almasına karşın güneş enerjisinden sıcak hava elde etmek için yeteri kadar faydalanılamamaktadır.

Dünyadaki enerji kaynaklarının sürekli azalması ve gün geçtikçe pahalanmasından dolayı, güneş enerjisi gibi yeni ve yenilebilir enerji kaynaklarına yönelim gerekmektedir. Güneş enerjisi ucuz yolla elde edilebilen, tükenmeyen, çevre kirliliğine yol açmayan bir enerji olduğundan çok fazla avantajlıdır. Güneş enerjisinden faydalanarak yapılan güneş enerjili kurutma tesisleri ne yazık ki Türkiye gibi tarım yönünden çok güçlü olan bir ülkede çok fazla ilgi görmemektedir. Oysa bu tesislerde kurutulan ürünler hem daha temiz bir ortamda daha çabuk kurutulmakta hem de hava şartlarından gelebilecek zararlardan korunmaktadır. Gıda kurutma sistemlerinin biri de sera kurutma sistemleridir. Sera tipi kurutucular direkt güneş enerjisiyle kurutulan sistemler kategorisinde ve bazen de kombine tipte (indirekt + direkt tipte) kurutma sistemleri kategorisinde değerlendirilmektedirler (Belessiotis ve Delyannis, 2011).

Sera tipi kurutucular basit bir tasarıma, kolay imalata ve düşük maliyete sahiptirler (Selimefendigil ve diğ., 2022). Sera tipi kurutucular ile alakalı birçok değerli çalışmalar mevcuttur. Kumar ve Tiwari (Jain ve Tiwari, 2004) ürünün kütlesinin ve hacminin konvektif kütle transfer katsayısı üzerine etkisini incelemek amacıyla sera tipi kurutucuda zorlanmış ve doğal taşınım içerisinde kurutma yapmışlardır. Bu çalışmada, araştırmacılar üç farklı boyuttaki tepsilere toplam 0,75 kg ve 2,0 kg ağırlıktaki ürünü kurutmuşlardır. Elde ettikleri veriler yardımıyla da konvektif kütle transfer katsayılarını bulmuşlardır. Anwar ve Tiwari (2001) tarafından sera tipi kurutucu içerisinde kaju ürünü farklı ağırlıklarda kurutularak, kurutulan ürünün kütlesi için önemli bir işlev olan kütle transfer katsayısı hesapları yapılmıştır. Kumar ve Tiwari (2007) soğan dilimlerinde konvektif ısı transfer katsayısının kütle üzerindeki etkisini çalışmak için doğal taşınımda direkt güneş altında ve serada, zorlanmış taşınımda serada kurutma çalışmaları yapmışlardır. Araştırmacılar, soğanı 300 gr, 600 gr ve 900 gr olmak üzere üç farklı ağırlıkta kurutmuşlardır. Kurutma işlemi güneşte ve serada 33 saat sürmüştür. Güneş altında ve seradaki deneylerden elde edilen verilerle regresyon analizi yaparak konvektif ısı transfer katsayısını hesaplamışlardır.

Yapay zekâ yöntemleri çözümlemesi zor olan birçok veriyi daha kolay işlemek için kullanılan popüler bir yöntemdir. Birçok alanda yapay zekâ uygulamaları olduğu gibi sera kurutma da bu yöntemler kullanılmıştır. De Jesús Rubio ve diğ. (2019) çalışmalarında portakal kurutmak için sera güneş kurutucusu kullanmışlardır. Elde ettikleri kurutma verilerini makine öğrenmesi olan Kalman filtresi ile modellemişlerdir. Kurutma sıcaklık değerleri ve ürün nem değerleri için makine öğrenmesi modellemesi gerçekleştirmişlerdir. Değerleri ortalama %5 hata ile modellemişlerdir. Janjai ve diğ (2018) bir sera güneş kurutucusunda et ürünü kurutmuş ve deneysel kurutma verilerini yapay sinir ağı ile modellemişlerdir. Litchi etinin kurutulması için kurutucunun performansını modellemek için bir yapay sinir ağı (YSA) yaklaşımı kullanmışlardır. YSA modeli için geri yayılım algoritmasını kullanmışlardır. Deneysel veriler ile YSA verilerinin birbirleri ile uyumlu olduğunu göstermişlerdir (Janjai ve diğ., 2018). Chauhan ve diğ. (2018) acı kabak pullarını kurutmak için güneş enerjisi hava ısıtma sistemi eklenmiş bir sera kurutucuları kullanmışlardır. Doğrusal olmayan regresyon analizi ile ürünün sıcaklık ve nem değerlerini modellemişlerdir. Ortalama %3 hata ile deneysel verileri modellemişlerdir.

Kushwah ve diğ. (2022) Hindistan'ın Gwalior kentindeki MITS kampüsünün çatısında vakum tüplü güneş enerjili gıda kurutucuda mantar kurutmuş ve kurutma süreci boyunca konvektif ısı transferi katsayısını hesaplamışlardır. Yapay sinir ağını, konvektif ısı transfer katsaysını tahmin etmek için geliştirmişlerdir. Geliştirilen yapay sinir ağı modeli, güneş ışını, bağıl nem, çevresel sıcaklık ve zaman gibi girdi faktörleri kullanılarak eğitildikten sonra ısı transfer katsayısını tahmin etmeye yardımcı olmuştur. Geliştirilen yapay sinir ağı modelinin R^2 değeri 0,99'dur, bu da modelin hesaplanan ısı transfer katsayılarına çok yakın değerler tahmin ettiğini göstermişlerdir (Kushwah ve diğ., 2022). Rasooli ve diğ. (2021) konvektif bir kurutucuda elma dilimlerini 50, 60 ve 70 °C'de 1,0 m/s hava hızında kurutmuşlardır. Elma dilimlerinin nem oranı (MR) üzerindeki performansını YSA ile modellemişlerdir. YSA sonuçlarına göre, MR tahmininde R^2 değerinin 0,9991 olduğunu hesaplamışlardır (Rasooli ve diğ., 2021). Zadhossein (2022) kavun dilimlerinin kurutulması için bir hibrit kızılötesi-konvektif kurutucunun enerji ve ekserji analizini sunmuştur. Deneyleri üç sıcaklık seviyesinde (40, 55 ve 70°C), bir hava hızı seviyesinde (0,5 m/s) gerçekleştirmiştir. Giriş işlem parametreleri (IR gücü, giriş hava sıcaklığı ve kuruma süresi) ile kurutulmuş ürünün termodinamik özellikleri arasındaki ilişkileri, yapay sinir ağı (YSA) ve ANFIS uygulanarak modellemiştir. YSA ve ANFIS kullanılarak geliştirilen modellerin, ANFIS modelinin nem oranı, enerji verimliliği ve ekserji kaybını YSA modelinden daha iyi tahmin ettiğini gözlemlemiştir. Ayrıca kuruma hızı ve ekserji verimliliğini tahmin etme doğruluğu YSA modeli için ANFIS'ten daha iyi olduğunu saptamıştır (Zadhossein, 2022).

Bu çalışmada; zorlanmış taşınımlı güneş enerjisi destekli sera tipi bir kurutma için HGK tasarımı yapılarak bütünleşik bir gıda kurutma sistemi imal edilmiş, imal edilen kurutucuda Bayramiç beyazı (şeftali) ürünü kurutulmuş, kurutma parametreleri ve kurutulan ürünlerin konvektif ısı transfer katsayıları hesaplanmıştır. Elde edilen veri setleri kullanılarak makine öğrenmesi algoritmaları (yapay sinir ağı ve karar ağacı gibi) ile ürünün ısı ve kütle transferi modellemeleri yapılmış ve yapay zekâ yöntemleri ile sera kurutucuda kurutulacak ürün için kullanılacak faydalı kurutma modelleri elde edilmiştir.

2. Materyal ve Metot

2.1. Deneysel kurulum

Çalışma hem deneysel hem de yapay zekâ yöntemsel uygulama ağırlıklıdır. Deneyde kullanılan kurutma ürünü 10 mm kalınlığında oval olarak kesilmiş şeftali ürünüdür. Kurutma deneylerinde 200 g şeftali ürünü kullanılmıştır. Şeftali ürünlerinin kurutma işleminde, sera tipi güneş enerjisi destekli bir kurutucu tasarlanıp imal edilmiştir. Kurutma sisteminde kullanılacak sıcak hava 1000x600 mm ebatlarındaki havalı kolektörden sağlanmıştır. Kolektörün en önemli elemanı olan güneş ışınımını absorbe eden yutucu yüzey siyaha boyanmış galvanizli çelikten yapılmıştır. Yutucu yüzey üzerinde havanın uzun süre dolaşmasını sağlamak ve dolayısıyla ısı transfer yüzeyini artırmak için farklı tip, konum ve açılarda kanatçıklar yerleştirilmiştir. Havalı kollektörden elde edilen sıcak hava sera etkisine sahip olan kurutma odasına gönderilmiştir. Güneş enerjili destekli bir kurutma sisteminin enerji ve ekserji analizinin yapıldığı ve kurutma parametrelerinin belirlendiği bu çalışmada öncelikle farklı hızlarda toplayıcıya ve sera etkili kurutma odasına havanın giriş ve çıkış sıcaklığı, çevre sıcaklığı, anlık ve istenen zaman aralığında toplam güneş ışıması ve rüzgar hızı ölçümleri periyodik aralıklarla yapılmıştır. Güneş ışıması ölçümlerinde anlık ve belirli zaman aralıkları ile ölçüm yapabilen bir piranometre ve onunla bağlantılı hale getirilecek solar integratör kullanılmıştır.

Sıcaklık ölçümleri demir-constantan ısıl çiftlerle ve dijital sıcaklık okuyucuyla, rüzgâr hızı ve kurutucudan çıkan havanın hızı anemometreyle, kurutulan ürünlerin kütle kaybı değerleri dijital hassas teraziyle, kurutma odası içinin ve çevrenin bağıl nem değerleri higrotermometreyle tespit edilmiştir.

Yapılan deneyler sonucunda belirli periyotlarda okunan sıcaklık, bağıl nem değerleri, kurutulan ürünün nem içeriği ve güneş ışınım şiddeti değerleri kullanılarak güneş enerjisi destekli kurutma sisteminde kurutma parametreleri belirlenmiş, kurutulan ürünlerin ısı transfer katsayıları hesaplanmıştır. Tasarlanmış ve imal edilmiş olan deney setinin görseli Şekil 1'de verilmiştir.

Şekil 1. HGK destekli sera kurutma sistemi.
Figure 1. ASC assisted greenhouse drying system.

Deneylerde kullanılan ölçüm cihazları ve ölçüm belirsizlik değerleri Tablo 1'de verilmiştir.

Tablo 1. Ölçüm cihazları ve hata değerleri.
Table 1. Measuring instruments and error values.

Cihaz	Model	Hata değeri
Işınım Ölçer	Mastech SM206	±10 W/m^2
Isıl Çift	AEC-TECH	±0,1℃
Anemometre	Unit UT362	±(3%+0,5)
Hassas Terazi	Üniversal	±0,1g

2.2. Teorik hesaplamalar

Kütle değişimi nemlilik ölçüsü

Gıda ürünlerinin ihtiva ettiği nem oranı, kendi bünyelerinde yer alan su miktarı olarak değerlendirilir. Gıda içerisindeki bu su miktarını ifade etmek için yüzdelik parametreler kullanılır. Nem miktarları belirlenirken yaş ve kuru baz esaslı tanımlar kullanılmaktadır. Gıda ürününün yaş baz (y.b.) ve kuru baz (k.b.) nem içeriğini hesaplamak için aşağıda sırasıyla Eşitlik (1) ve Eşitlik (2) kullanılmıştır (Akpinar ve Toraman, 2016).

$$MC_{y.b} = \frac{W_s}{W_s + W_k}.100 \quad (1)$$

$$\%MC_{k.b} = \frac{W_s}{W_k} \quad (2)$$

Eşitlik (1) ve (2) 'de; Ws yaş ağırlık ve Wk kuru ağırlıktır. Boyutsuz nem oranı (MR) değerleri Eşitlik (3) kullanılarak hesaplanmıştır (Daş ve diğ., 2021).

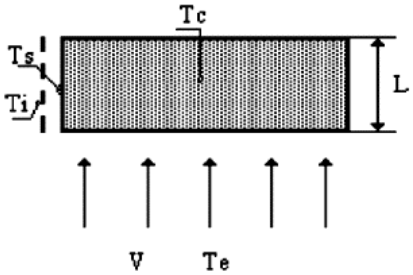
$$MR = \frac{M - M_e}{M_o - M_e} \quad (3)$$

Eşitlik 3'te Me, kurutulan ürünün denge bağıl nem değeridir. Kurutulacak şeftali dilimlerinin denge nem içeriği 14,9 g su/g katı madde olarak Shimadzu MOC63u nem tayin cihazı ile belirlenmiştir.

Isı transfer katsayısı

Gıda kurutma süreçlerinde ortaya çıkan ürün ile sıcak akışkan arasındaki ısı transferini ifade etmek için konvektif ısı transferi katsayısı kullanılır. Ürün yüzeyi düz plaka olarak tanımlanırsa, bu çalışmada ürün yüzeyi üzerindeki zorlanmış akışta oluşabilecek konvektif ısı transferi katsayısı üzerinde durulmuştur. Plaka üzerindeki akışkanın viskozitesi sayesinde plaka yüzeyinde akışkan hızının sıfır hızına sahip olması gerektiğinden, yani bir sınır tabaka mevcut olduğundan dolayı, plaka yüzeyindeki akış ile olarak laminer akış şeklinde değerlendirilecektir. Fakat akış rejiminden dolayı Reynolds sayısı yeterli miktarda yüksek olduğunda, akış türbülansa da dönüşebilir (Khanlari ve diğ., 2020).

Konvektif ısı transfer katsayısı hesaplamalarında, ürün yüzeyi olarak kabul ettiğimiz plakanın, sabit bir iç sıcaklığa (Ti) sahip olduğu ve plakanın mevcut uzunluğunun (L) kurutma bölgesinde bir türbülans akışı oluşturmayacak veya tetiklemeyecek kadar kısa olduğu hesaplama öncesi kabul edilmiştir. Ürün yüzeyi olarak belirtilen plaka üzerindeki zorlanmış akış şartlarında meydana gelebilecek olan konvektif ısı transferi Şekil 2'de sunulmuştur.



Şekil 2. Düz bir plaka üzerinde zorlanmış akış için konvektif ısı transferi.
Figure 2. Convective heat transfer for forced flow over a flat plate.

Ortalama ısı transfer katsayısı, laminer akış için Pohllhausen Eşitliği (Eşitlik 4) ve aşağıda verilen (Daş ve diğ., 2021) diğer Eşitlikler (Eşitlik 5-7) kullanarak hesaplanmıştır.

$$Nu_{lam} = 0.664.Re^{1/2}.Pr^{1/3}$$

(Re<2x105) (4)

$$Nu = \frac{h_c.L}{K_v} \quad (5)$$

$$\mathrm{Re} = \frac{L.V.\rho_v}{\mu_v} \quad (6)$$

$$\mathrm{Pr} = \frac{\mu_v.C_v}{K_v} \quad (7)$$

Eşitliklerde belirtilen parametreler için, Re; Reynolds sayısını ve Pr; Prandtl sayısını hesaplamak için nemli havanın termofiziksel özellikleri kullanılmıştır. Bu Eşitlikler (Eşitlik 8-12) aşağıda verilmiştir. Eşitliklerde yer alan havanın yoğunluğu (ρv), özgül ısı değeri (Cv), ısıl iletkenlik değeri (Kv) ve viskozite değeri (μv) kullanılmıştır.(S. I. Anwar ve Tiwari, 2001).

$$\rho_v = \frac{353.44}{(T_i + 273.15)} \quad (8)$$

$$K_v = 0.0244 + 0.6773 \times 10^{-4} T_i \quad (9)$$

$$C_v = 999.2 + 0.1434 T_i + 1.101 \times 10^{-4} Ti^2 - 6.7581 \times 10^{-8} Ti^3 \quad (10)$$

$$\mu_v = 1.718 \times 10^{-5} + 4.620 \times 10^{-8} T_i \quad (11)$$

$$T_i = \frac{(Ts + Te)}{2} \quad (12)$$

Yukarıda verilen eşitliklerde ifade edilen havanın termofiziksel özellikleri hesaplamaları için kullanılan Ti sıcaklık değeri, kurutma ortamı sıcaklık değerini ifade eden Te sıcaklığı ile ürün yüzey sıcaklığını ifade eden Ts sıcaklık değerlerinin ortalaması ile belirlenmiştir.

Belirsizlik analizi

Yapılacak olan sıcaklık, hava hızı, nem ve ışınım parametrelerinin ölçülmesi sırasında ortaya çıkacak belirsizlikler için imalat hatalarının, sabit hataların ve rastgele oluşabilecek hataların etkili olduğu düşünülecektir. Belirsizlik oluşturabilecek bu etkenlerin toplam ölçüm belirsizliğine olan etkilerini belirlemek gerekir. Ölçülen değerleri dikkate alarak bu etkilerden kaynaklanan toplam hataların hesabı için Eşitlik 13 kullanılacaktır (Akpinar ve Koçyiğit, 2010).

$$W_R = \left[\left(\frac{\delta R}{\delta x_1} w_1 \right)^2 + \left(\frac{\delta R}{\delta x_2} w_2 \right)^2 + \ldots\ldots\ldots + \left(\frac{\delta R}{\delta x_n} w_n \right)^2 \right]^{1/2} \quad (13)$$

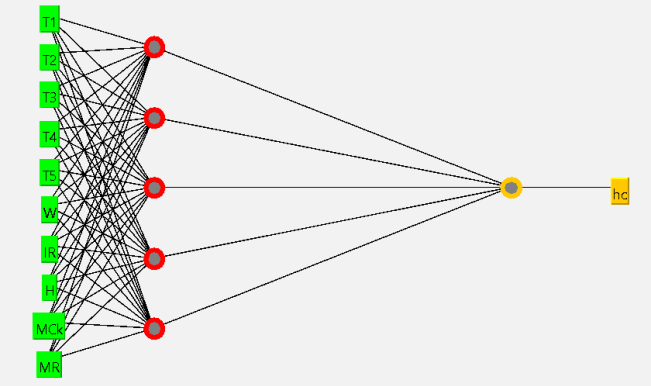
2.3. Hesapsal zekâ yöntemleri

Makine öğrenmesi yöntemleri, yapay zeka metotları arasında en çok kullanılan yöntemlerdir. Makine öğrenmesi algoritmalarını tanımlamak gerekirse, veriler üzerinde yapısal öğrenme yapabilen, sınıflandırma kuralları üretebilen ve belirtilen parametreyi tahmin edebilen metotlar olarak ifade edilebilir. Makine öğrenmesinde örnek veri setleri kullanılarak tahminsel modeller elde edilir. Bazı bilimsel çalışmalar sonucu ortaya çıkan büyük miktardaki verilerin işlenmesi oldukça zahmetli bir süreçtir. Bu sebeple çalışmadaki ana amaca ulaşmak için çözülmesi gereken problem için bu probleme ait verilerden elde edilen setler, makine öğrenmesi ile modellenerek çözüme ulaşmak hedeflenmektedir (Das ve Akpinar, 2018).

Konvektif ısı transferi değerleri, birer yapay zeka yöntemi olan yapay sinir ağı ve karar ağacı makine öğrenmesi algoritmaları kullanılarak modellenmiştir. Yapay zeka ile model oluşturmak için MATLAB 2021a yazılımı kullanılmıştır. Kurutma deneyi sonucunda elde edilen veri setinden, 250 adet giriş ve 25 adet çıkış verisi kullanılmıştır. Toplam 275 verinin 190 tanesi eğitim işleminde kullanılmıştır. 85 tanesi ise test işleminde kullanılmıştır. Öğrenme algoritması olarak Feed Forward Back Propagation (ileri doğru beslemeli ve geriye yayılım) algoritması kullanılmıştır. Eğitim için Levenberg Marquardt algoritması kullanılmıştır.

Yapay sinir ağları

İnsan beyni çalışma prensibini benimseyen bir yöntem olan yapay sinir ağları, herhangi bir veri seti içerisindeki istenilen parametreyi, aynı veri seti içerisindeki diğer parametrelere bağlı olarak tahmin edebilen bir makine öğrenmesi algoritmasıdır. Modellemeyi gerçekleştirirken adaptasyon, öğrenme, transfer ve test fonksiyonlarını kullanır. Yapay sinir ağları ile görüntü işleme, veri tahmin etme, veri kümelere veya sınıflandırma yapılabilir. Yapay sinir ağları tıpkı insanlardaki gibi öğrenme dürtüsüne ihtiyaç duyarlar. Bu yüzden modellenecek olan veri seti kümesini, eğitim ve test kümesi olarak iki farklı guruba ayırır (Ghritlahre ve diğ., 2020). Sunulan bu çalışmada kullanılan yapay sinir ağı model yapısı Şekil 3'te verilmiştir.

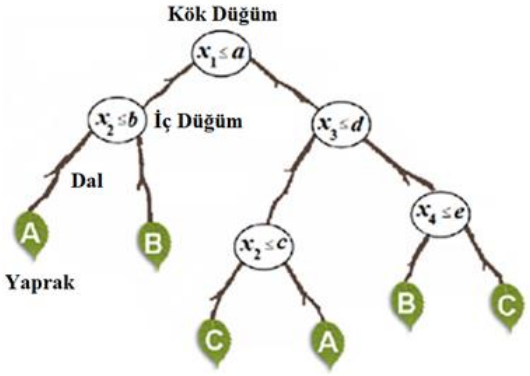


Şekil 3. YSA ağ yapısı.
Figure 3. ANN network structure.

Şekil 3'te T1, T2 kollektör giriş çıkış sıcaklığı, T3,T4, sera giriş çıkış sıcaklığı, T5 sera iç ortam sıcaklığı, W ürün ağırlık değeri, IR güneş ışınım değeri, H nem, MCk kuru baz nem içeriğini ve MR nem oranı değerini ifade etmektedir.

Karar ağacı

Literatürde son zamanlarda popülerliği giderek artan bir makine öğrenmesi yöntemi de karar ağaçları algoritmalarıdır. Karar ağaçlarının yaygın olarak kullanılmasındaki nedenlerden en önemli olanı, veri setlerini farklı kurallara göre ayırarak bir ağaç yapısına benzer bir yapı oluşturmasıdır. Tıpkı bir ağaç gibi kök, gövde ve dalları ifade eden veri kuralları ve eşitlikler üreterek yaprak olarak ifade edilen model parametreye ulaşırlar. Karar ağacının yapısal anlatımı Şekil 4'de verilmiştir. Şekilde verilen ağaçta her bir nitelik (nem, sıcaklık, hız v.b.) bir kök düğüm tarafından ifade edilir. Ağaç yapısından hedef, en üstte bulunan ve kök olarak ifade edilen parametreyi gövde ve dallardaki kurallara göre modelleyebilmektir (Çerçi ve Daş, 2019). Ağaç yapısını daha detaylı anlatacak olursak, veri setlerini içeren kök düğümü, diğer iç düğümler olarak ifade edilen dallar ve uç düğüm olarak ifade edilen yapraklar oluşturur. Düğümler ve dallar oluşturulurken karar ağacı algoritması sınıflandırma kurallarını rastgele belirler. Daha sonra bu kuralara göre veri setleri içerisinden aldığı cevaplara göre en kısa sürece cevaba gidecek olan modeli ifade eden ağaç yapısını oluştururlar. Karar ağaçları algoritmaları içerisinden yaygın olarak kullanılan algoritma M5P algoritmasıdır. (Alic ve diğ., 2019).



Şekil 4. Karar ağacını temsilen üç sınıftan oluşan karar ağacı yapısı (Alic ve diğ., 2019).
Figure 4. Decision tree structure with three classes representing the decision tree (Alic et al., 2019).

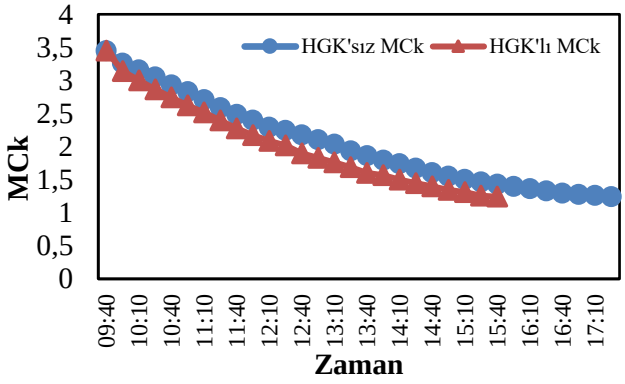
Deney sonuçları ile makine öğrenmesi sonuçlarını karşılaştırmak için Tablo 2'de ki hata analiz yöntemleri kullanılmıştır. Ortalama bağıl hata (MAE) ve kök ortalama karesel hata (RMSE) istatistiksel hata analizleri kullanılmıştır.

Tablo 2. Hata analizleri.
Table 2. Error analysis.

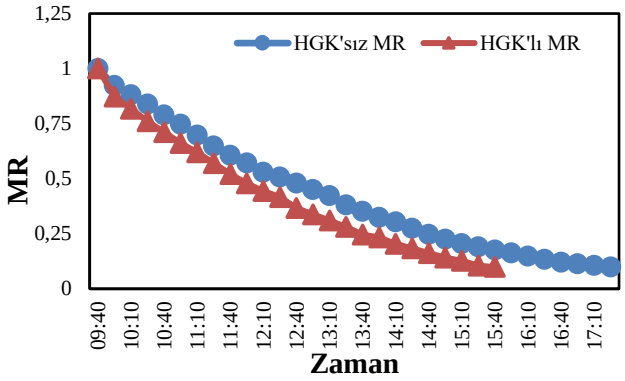
Hata analizi	Formülü	Parametreler
MAE	$\frac{\lvert P_1 - A_1\rvert + ... + \lvert P_n - A_n\rvert}{n}$	P: Tahmin Değeri A: Gerçek Değer n: Toplam Hata Değeri
R^2	$1 - \frac{\sum (A-P)^2}{\sum (P-A)^2}$	P: Tahmin Değeri A: Gerçek Değer
RMSE	$\sqrt{\frac{(P_1 - A_1)^2 + ... + (P_n - A_n)^2}{n}}$	P: Tahmin Değeri A: Gerçek Değer n: Toplam Hata Değeri

3. Bulgular ve Tartışma

Yapılan deneyler sonucu HGK'lı ve HGK'sız yapılan kurutma işlemlerinde ürün kuru baz nem içeriği (MCk) değerleri ve nem oranı (MR) değerleri sırası ile Şekil 5 ve 6 da verilmiştir.



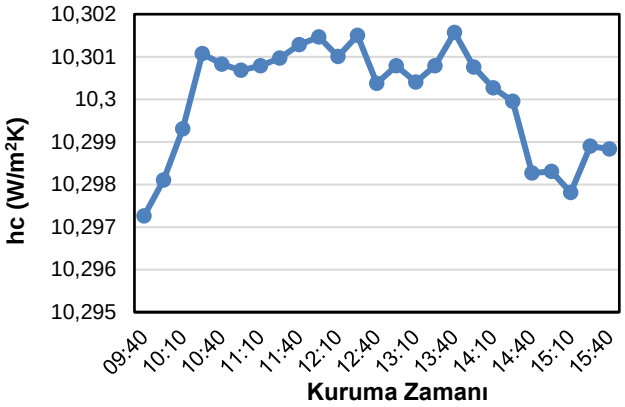
Şekil 5. Kuru baz nem içeriği değerlerinin kuruma zamanına göre değişimi.
Figure 5. Variation of dry base moisture content values according to drying time.



Şekil 6. Nem oranı değerlerinin kuruma zamanına göre değişimi.
Figure 6. Variation of moisture content values according to drying time.

Şekil 5 ve Şekil 6'da açıkça görüldüğü gibi, HGK'lı sera kurutmada ürün kuruma davranışları daha kısa sürmüş ve denge ağırlığına daha erken sürede ulaşılmıştır.

HGK'lı serada sabit fan devrinde kurutma işlemi gerçekleştirilmiştir. Sera çıkışından ölçülen hava hızı 2,59 m/s'dir. Ürün yüzeyinden ölçülen hava hızı 1,02 m/s'dir. Konvektif ısı transferi katsayısı hesaplamaları için ürün yüzeyindeki hız önemli olduğu için Reynolds hesaplamalarında ürün yüzey hızı kullanılmıştır. HGK'lı serada Eşitlik 6 ile ortalama Re sayısı 476,5 olarak belirlenmiştir. HGK'lı kurutma işleminde ürünün yüzeyi ile sera ortamındaki hava arasında meydana gelen konvektif ısı transfer katsayısı değerlerinin kuruma süresince değişimi Şekil 7'de verilmiştir. hc değerleri 10,29 ile 10,302 W/m^{2}K arasında değişiklik göstermiştir.



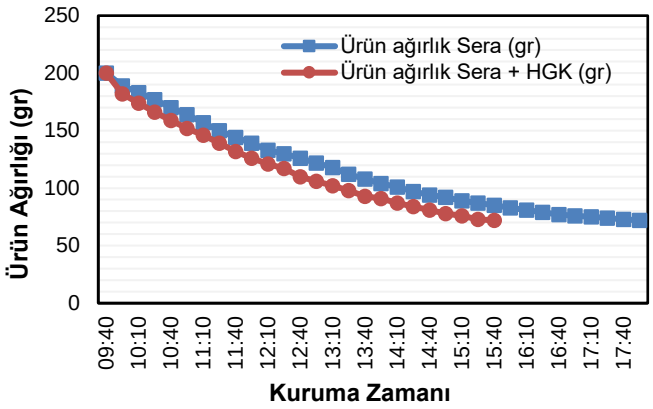
Şekil 7. Konvektif ısı transferi değerlerinin kuruma zamanına göre değişimi.
Figure 7. Variation of convective heat transfer values according to drying time.

Yapılan deneylerde ürün kurutmak için HGK destekli bir sera kurutucu kullanılmıştır. Kullanılan HGK nın kurutma performansına etkisini göstermek için Şekil 8 eklenmiştir. Şekil 8'de aynı ağırlıktaki ürünler için HGK lı ve HGK sız serada ürünlerin kuruma zamanı gösterilmiştir. HGK lı serada aynı ürün 120 dakika daha erken kurumuştur.

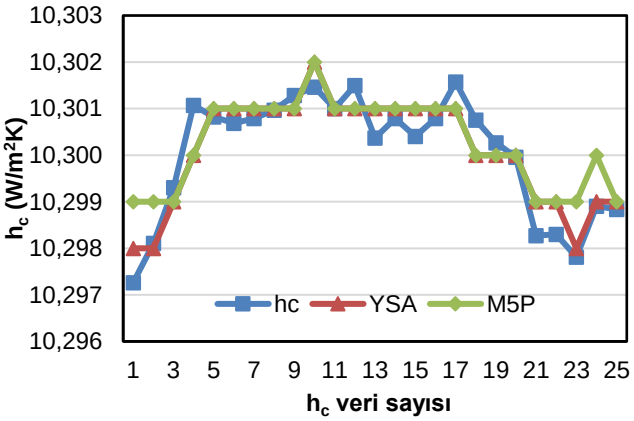
Yapay sinir ağı ve karar ağacı (M5P) ile konvektif ısı transferi katsayısı tahminsel modellerinin değerleri ile deneysel değerler arasındaki benzeşim Şekil 9'da gösterilmiştir. YSA ile modellenen konvektif ısı transferi değerleri ile deneysel değerler birbirlerine daha yakındır.

Makine öğrenmesi algoritmaları ile modellenen konvektif ısı transferi değerleri ile deneysel değerler arasındaki hata

analizleri sonuçları Tablo 3'te gösterilmiştir. Tabloya göre en az hata değeri her iki analize göre YSA modellerindedir.



Şekil 8. HGK destekli sera kurutucuda ve HGK sız sera kurutucuda ürün ağırlık değişimi.
Figure 8. Product weight change in greenhouse dryer with HGK and greenhouse dryer without HGK.



Şekil 9. HGK'lı sera kurutucu için deneysel ve tahminsel h_c verileri karşılaştırılması.
Figure 9. Comparison of experimental and predicted hc data for greenhouse dryer with HGK.

Tablo 3. İstatistiksel hata değerleri.

Table 3. Statistical error values.

Hata analizi	YSA	M5P
MAE	0,01	0,07
R^2	0,971	0,957
RMSE	0,09	0,011

Literatürde farklı kurutma proseslerindeki kurutma parametreleri için yapılan yapay zekâ çalışmaları incelendiğinde, elde edilen hata değerleri literatürde yapılan çalışmalara yakın olduğu gözlemlenmiştir. Bu durumu ifade etmek için Tablo 4 eklenmiştir. Tablo 4'de mevcut çalışma ile literatürde yapılan çalışmalardaki yapay zekâ modellerinin hata oranları karşılaştırılmıştır.

Tablo 4. Literatürde kurutma parametreleri için yapılan yapay zekâ çalışmalarının hata değerleri.

Table 4. Error values of artificial intelligence studies for drying parameters in the literature.

İncelenen parametre	Yapay zeka yöntemi	Hata değeri	Referans
Isı transfer katsayısı	YSA	0,972 R^2	Kushwah ve diğ., 2022
MR	YSA	0,04 MAE	Rassoli ve diğ., 2021
Enerji verimi	ANFIS	0,98 R^2	Zadhossein ve diğ., 2022
Kurutma sıcaklığı	Karar Ağacı	0,021 RMSE	Abdelkader ve diğ., 2024
Ürün ağırlığı	YSA	0,97 R^2	Daliran ve diğ., 2023
Isı transfer katsayısı	YSA	0,971 R^2 0,01 MAE 0,9 RMSE	Mevcut çalışma

4. Sonuç

Yapılan çalışma sonucunda bir sera kurutucusunun performansını arttırmak için kullanılan HGK nın etkilerinin ve konvektif ısı transfer katsayısı modellerinin sonuçları aşağıda maddeler halinde verilmiştir.

1- Sera ile kurutulan ürün 495 dakikada kuru ağırlığa ulaşırken, benzer deneysel şartlarda ve aynı ürün ağırlığında HGK destekli kurutucuda 360 dakikada kuruma işlemi gerçekleştirilmiştir. Bu durumda HGK desteği ile aynı ürün, HGK desteği olmadan yapılan sera kurutmada %28 daha hızlı sürede kurumuştur.
2- HGK destekli sera kurutucusunda hesaplanan konvektif ısı transferi değerleri 10,29 ile 10,302 W/m^{2}K arasında değişiklik göstermiştir.
3- Konvektif ısı transferi değeri makine öğrenmesi algoritmaları ile modellenmiş, en iyi model sonucu %1 hata ile YSA tarafından gerçekleştirilmiştir.

Çalışmada farklı ürünler kurutularak, daha fazla veri setleri elde edilebilir ve farklı makine öğrenmesi algoritmaları ile daha az hata değerine sahip modeller ortaya sunulabilir. Bu çalışmanın gelecekteki planlamasında sera içerisindeki ısı ve sıcaklık dağılımını ifade edebilen sayısal modeller elde etmek ve en uygun sera geometri tasarımı ortaya koymak vardır.

5. Çıkar Çatışması

Yazarlar çıkar çatışması beyan etmemektedir

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Effects of Ultraviolet – C Treatment on Postharvest Physiologies and Decay of Berries: A Review

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Abstract: Berries have a short shelf-life due to their high metabolic activities and susceptibility to weight loss, mechanical damage, softening, and microbial decay. Ultraviolet-C light (UVC) treatment, a non-thermal and non-chemical method, has improved the microbiological, physiological, and nutritional quality of postharvest fruit and vegetables. This review examines postharvest berry physiology such as ethylene production, respiration rate, texture (firmness, weight loss, and cell wall), phenolic compounds, antioxidant capacity, color, flavor, and microbial decay during storage as affected by UVC treatment. Studies have shown that UVC treatment has a beneficial effect on increasing phenolic compounds, antioxidant capacity, and maintaining the firmness of berries. Besides, softening and weight loss can be inhibited in UVC-treated berries during postharvest. However, UVC treatment can increase ethylene production and respiration rate, causing flavor degradation and early senescence. The effectiveness of UVC treatment depends on berry cultivars, UVC doses, and other processing parameters. Moreover, combining physical and chemical treatments with UVC in a hurdle approach may enhance berry physiology compared to UVC treatment alone.

Keywords: Berry, UVC, respiration, ethylene, texture, bioactive compounds.

Ultraviyole-C Uygulamasının Hasat Sonrası Dutsu Meyvelerin Fizyolojisine ve Bozulmasına Etkisi: Derleme

Özet: Dutsu meyveler yüksek metabolik aktivite, ağırlık kaybı, yumuşama ve mikrobiyal çürümeye yatkınlıkları nedeniyle kısa raf ömrüne sahiptir. Isıl ve kimyasal olmayan bir yöntem olan ultraviyole-C ışık (UVC) uygulaması, hasat sonrası meyve ve sebzelerin mikrobiyolojik, fizyolojik ve besinsel kalitesini iyileştirmek için kullanılmaktadır. Bu derlemede, hasat sonrası UVC uygulamasının dutsu meyvelerde etilen üretimi, solunum hızı, doku (sertlik, ağırlık kaybı ve hücre duvarı), fenolik bileşikler, antioksidan kapasite, renk, lezzet ve mikrobiyal çürüme gibi kalite özellikleri üzerine etkileri incelenmiştir. Çalışmalar, UVC uygulamasının dutsu meyvelerde fenolik bileşikleri ve antioksidan kapasiteyi arttırmada ve meyvelerin sertliğini korumada yararlı bir etkiye sahip olduğunu göstermiştir. Ayrıca, hasat sonrasında UVC ile muamele edilen meyvelerde yumuşama ve ağırlık kaybı engellenebilmektedir. Bununla birlikte, UVC uygulaması etilen üretimini ve solunum hızını artırarak aromanın bozulmasına ve erken yaşlanmaya neden olabilmektedir. UVC uygulamasının etkinliği meyve çeşitlerine, UVC dozuna ve diğer uygulama parametrelerine bağlıdır. Ayrıca, engel teknolojisi kullanılarak fiziksel ve kimyasal uygulamaların UVC ile kombinasyonu, tek başına UVC işlemine kıyasla dutsu meyvelerin fizyolojisinde daha olumlu etkilere neden olabilir.

Anahtar Kelimeler: Dutsu meyveler, UVC, solunum, etilen, tekstür, biyoaktif bileşikler.

Review

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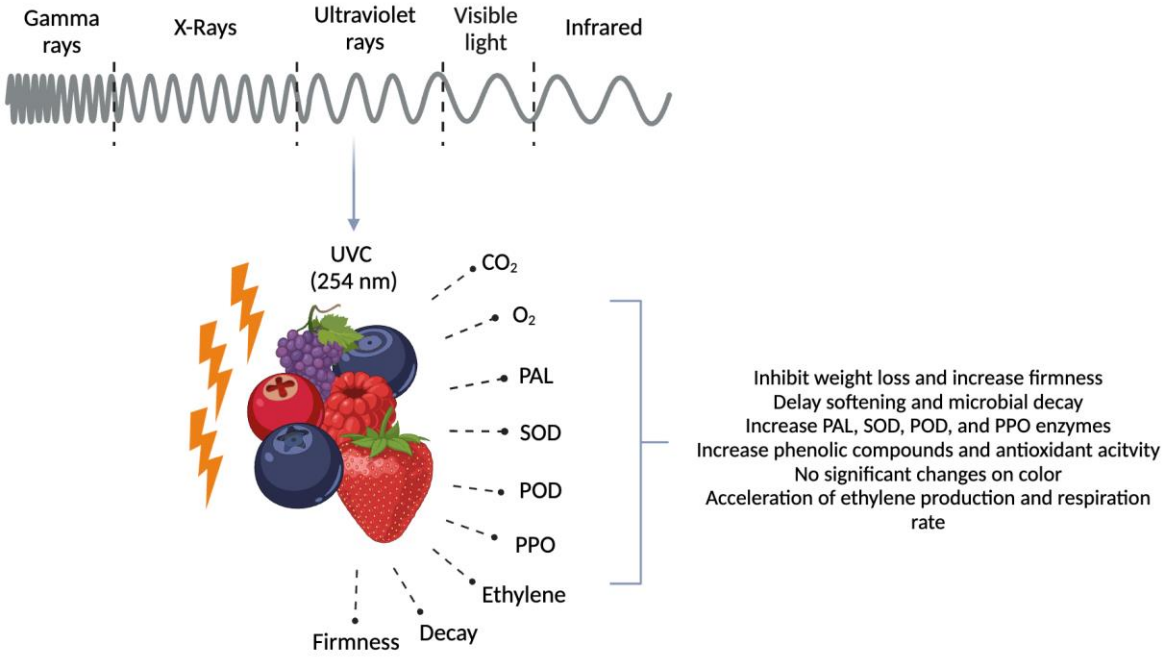
Graphical Abstract



1. Introduction

Berries are small, soft-fleshed fruits that ripen from the ovary wall's outer layer into an edible pericarp (Dickenson, 2020). Berries, including blueberries, cranberries, blackberries, raspberries, strawberries, black currant, chokeberry, mulberry, and acai, are commonly used in culinary customs due to their visual appearance and high secondary metabolite content such as flavonoids (anthocyanins, flavanols, and flavonols), phenolic acids, tannins, ascorbic acid, and carotenoids (Horvitz, 2017; Skrovankova et al., 2015; Szajdek & Borowska, 2008). In addition to being high in fiber, natural vitamins and antioxidants are contained in berries (Basu et al., 2010). They are beneficial for human health due to their antioxidant and anti-inflammatory properties, which can lower the risk of cancer, cardiovascular disease, diabetes, and cataracts (Padmanabhan et al., 2016; Vidovic, 2018). Additionally, consuming berries can delay cognitive aging (Devore et al., 2012).

Berries are highly perishable and have short storage life due to their high metabolic activities and susceptibilities to pathogen attack, mechanical damage, and moisture loss (Liu et al., 2019; Van der Steen et al., 2002). To ensure high quality and extended shelf life, it is crucial to harvest and handle berries at optimum ripening stages with precise methods (Baietto & Wilson, 2015). After harvesting, berries' quality and nutritional value decrease readily due to mechanical damage, improper handling, and being highly perishable and susceptible to spoilage (Liu et al., 2019; Skrovankova et al., 2015). They are consumed as fresh, frozen, or processed into pulp, purees, jams, jellies, and juices (EFSA, 2014; Pritts, 2017). Nevertheless, a significant fraction of them become waste after postharvest processing (Kaur et al., 2022). The commercial preservation of the whole berries is carried out using non-destructive and non-thermal methods such as cold storage (Liu et al., 2019). Alternatively, controlled atmosphere storage (Gunes et al., 2002), modified atmosphere packaging (Gimeno et al., 2021), gamma-irradiation (Basaran and Kepenek, 2011; C. Wang et al., 2017), ozone (Piechowiak, 2021; Piechowiak et al., 2021), edible coating (Ascencio-Arteaga et al., 2022; Falcó et al., 2019), high-pressure processing (Lou et al., 2022), cold plasma (Ji et al., 2020; Lacombe et al., 2015), and their combination (Pinto et al., 2020; Rodriguez and Zoffoli, 2016) have been searched to prolong berries' shelf-life, inhibit microbial decay, and ensure food safety against microorganisms such as *Salmonella, Botrytis cinerea* and norovirus.

In recent decades, ultraviolet-C (UVC) light has been used as a non-thermal and non-chemical technique to ensure the quality and safety of postharvest berries. Numerous research studies have extensively examined the postharvest physiology of UVC-treated berries, such as strawberries (Jin et al., 2017; Li et al., 2019), blueberries (Jaramillo Sánchez et al., 2021), raspberries (Gimeno et al., 2021). Nevertheless, there is no recent review on UVC effects on the post-harvest physiology of berries. Therefore, this study aims to discuss the effects of UVC treatments on various physiological aspects such as respiration rate, ethylene biosynthesis, texture (including cell wall degradation, firmness, and weight loss), phenolic compounds (flavonoids, non-flavonoids), antioxidant capacity, flavor, texture, color, and microbial decay in berries such as strawberry (*Fragaria ananassa*), raspberry (*Rubus idaeus*), blueberry (*Vaccinium* spp.), and boysenberry (*Rubus ursinus* × *Rubus idaeus*) during postharvest storage. The effects of combined treatments with UVC are also discussed.

2. Ultraviolet-C treatments: principles and application parameters

Ultraviolet (UV) region (100 – 400 nm) is placed between X-ray and visible light in the electromagnetic spectrum (Lewis, 2023). UVC refers to 200 – 280 nm and has germicidal effects on microorganisms, especially at 254 nm. Sun is a natural source of UVC light, but the ozone layer in the atmosphere absorbs it so that the Earth is protected from its harmful effects (Koutchma, 2019; Urban et al., 2016). Artificial UVC sources used in research and industry have included low- and medium-pressure mercury lamps and xenon lamps. Mercury-based lighting is widely used due to its prevalence in the market and the FDA's approval of low-pressure (LP) mercury lamps (253.7 nm) (Darré et al., 2022; FDA, 2013). FDA has

approved the use of xenon lamps and LP mercury lamps emitting a 253.7 nm wavelength for reducing pathogens and microorganisms on juice products, sterilizing potable water used in food production, and controlling surface microorganisms on food and food products in food processing and treatments under regulation 21CFR179.39 since 2000. However, as LP mercury lamps contain toxic mercury, the transition to mercury-free lighting is planned with the Minimata Convention on Mercury for Climate Action (Minamata Convention on Mercury, 2023). As an alternative, light-emitting diodes (LEDs) are suggested being mercury-free. LEDs have also been utilized due to their compactness, small size, low cost, and non-fragile structure during the last couple of decades (Cassar et al., 2020).

UVC light can be absorbed, reflected, or scattered on materials or food matrices. The UVC treatment dose expressed as kJ/m^2, is related to intensity and exposure time (Koutchma, 2014). UVC treatment effectiveness may be reduced due to its absorption by soluble materials and suspended particles in the food matrix and it may not reach all parts of the food matrix (Delorme et al., 2020). Therefore, it cannot penetrate turbid liquid and solid food although it can easily penetrate through pure water (Choudhary and Bandla, 2012; Koutchma, 2008). Thus, UVC treatment is generally considered as a surface treatment. Various studies have been conducted to examine inactivation effects of UVC light on surface microorganisms in foods including fruits and vegetables such as lettuce, strawberries, and tomatoes (Cho et al., 2022), cherries (Kutlu et al., 2022), fresh-cut pitaya (Zhai et al., 2021), strawberries (Janisiewicz et al., 2021; Ortiz-Solà et al., 2021), apple (Rios de Souza et al., 2020), lettuce (Green et al., 2020), oranges (Gündüz et al., 2015), pear (Sun et al., 2022), apricot (Hakguder Taze and Unluturk, 2018). Dose, exposure times, wavelength, light sources, the distance of sample and lamps, the number of UVC lamps and their position, uniform distribution of light on all surfaces (effective exposure), temperature, type and characteristics of foods, type of microorganism on food surfaces, are vital parameters to determine the efficiency of the UVC treatment. UVC can inhibit DNA replication and transcription by forming DNA photoproducts like pyrimidine 6-4 pyrimidone and cyclobutene pyrimidine dimers, leading to mutagenesis and cell death (Artés and Allende, 2015; Harm, 1980).

3. Effects of UVC on Postharvest Berries Physiology

Postharvest storage of fruit and vegetables is accompanied by cellular respiration which involves the breakdown of macromolecules (i.e., carbohydrates, lipids, etc.) to produce ATP/energy through glycolysis, tricarboxylic acid cycle (TCA) cycle, and electron transport chain. Respiration rate is affected by the physiological conditions of the fresh produce and storage atmosphere conditions such as temperature, relative humidity, and modified atmospheres (MA). Most berries are non-climacteric fruits; they do not ripen after harvesting. So, they must be harvested at horticultural maturity. Their quality and nutritional content can be significantly reduced during storage (Liu et al., 2019). They are highly perishable and susceptible to weight loss, softening, microbial spoilage, and decaying (Horvitz, 2017; Paniagua et al., 2013). Moreover, their postharvest shelf-life barely exceeds 2 – 6 weeks under typical refrigeration conditions during storage (Gimeno et al., 2021; Khanizadeh et al., 2009; Xu and Liu, 2017).

3.1. Ethylene biosynthesis and respiration rate

Ethylene biosynthesis

Ethylene, a natural plant hormone, plays a vital role in the ripening of fruits. Berries are mainly non-climacteric fruits. However, blackberries can be both climacteric (Walsh et al., 1983) and non-climacteric (Lipe, 1978), depending on their cultivars. Similarly, blueberries also vary in ethylene production depending on their cultivars (Farneti et al., 2022). However, ethylene production is mainly low in berries such as cranberry, blackberry, and raspberry, with production rates ranging from < 0.10, 0.32 – 0.40, and 0.29 – 0.49 µL/kg.h, irrespective of cultivars during postharvest storage (Gunes et al., 2002; Shah et al., 2023). Furthermore, strawberries, blackcurrants, mulberries, acai, bilberries, and gooseberries have relatively low ethylene production and respiration rates after harvesting (Fan et al., 2022). Only a few studies have established the effects of postharvest UVC treatment on ethylene production in berries (Table 1). For instance, Xu and Liu (2017) showed that although blueberries' ethylene production increased during 8-d storage at 4 °C, untreated and UVC-treated samples showed no significant difference in ethylene production (3.2 – 3.4 µL/kg.h). In contrast, Li et al. (2014) found that UVC treatment increased ethylene production of strawberries initially, and its level remained 4.4 and 11.7 times higher than that in untreated fruits after 1 and 4 d, respectively. Similarly, 4 kJ/m^2 UVC treatment increased ethylene production in strawberries in the first 6 h (Nigro et al., 2000). The increase in ethylene might be induced due to the activation of strawberry defense system against stress (Li et al., 2014; Nigro et al., 2000). However, ethylene production decreased in treated strawberries at the end of the storage (48 h) (Li et al., 2014; Nigro et al., 2000). However, the decrease of samples at the end of the 48 h was higher than the control (Nigro et al., 2000). Therefore, UVC can stimulate ethylene production immediately after the treatment, but it can be decreased substantially at the end of the storage in some berries.

Respiration rate

During the postharvest term, berries have mainly high respiration rates (41 – 245 mg CO_2/kg.h at 20 °C) (Huynh et al., 2019). For instance, ripe blackberry and raspberry have 41.4 – 53.28 and 45 – 76.32 mg CO_2/kg.h respiration rate at 20 °C, respectively (Shah et al., 2023). Transpiration and respiration are the primary causes of nutrient and water loss during storage, leading to weight loss in postharvest berries. The higher the respiration rate the higher the metabolic activity leading to shorter storage life (Bovi et al., 2019). Contrasting findings have been reported on UVC treatment effects on berries' respiration rate (Table 1). Postharvest blueberries' respiration rate increased by UVC treatment at 4 kJ/m^2 during 8-d of storage at 4 °C (Xu et al., 2016; Xu & Liu, 2017). UVC treatment at 4 kJ/m^2 suppressed the respiration rate to ~2.1 mg CO_2/kg compared to the untreated samples (2.43 mg CO_2 kg.h) at 4 °C in blueberries (Xu et al., 2016). Similarly, red raspberries' respiration rates were slightly increased from 10.03 to 14.67 mL CO_2/kg.h by UVC treatments at 2 and 4 kJ/m^2 during 12 d storage at 6°C (Gimeno et al., 2021). UVC treatment (1 - 15 kJ/m^2 dose) did not affect the respiration rate of strawberries at the end of the 6-d storage at 2°C (Allende et al., 2007). However, another study showed that the UVC treatment at 4 kJ/m^2 decreased the respiration rate of strawberries after 5 d of storage (Cote et al., 2013). Similarly, UVC treatment at 9.2 kJ/m^2 inhibited the respiration rate of boysenberry by 24.22 % and 7.92 % during storage for 1 d at 20 °C and 4 d at 4 °C, respectively (Vicente et al., 2004). The inhibitory effects of UVC treatment on the respiration rate of berries might be due to delaying microbial decay and cell wall degradation, as discussed in other sections in this review. The impact of the treatment depends on the applied dose, temperature, type, and physiological conditions of the berries. Few studies have been conducted on UVC treatment effects on berries' respiration rate (Table 1). Therefore, further research is required to get more precise results.

Table 1. Effect of postharvest ultraviolet-C treatment on berries' ethylene biosynthesis and respiration rate.
Tablo 1. Hasat sonrası ultraviyole-C uygulamasının dutsu meyvelerin etilen biyosentezi ve solunum oranı üzerindeki etkisi.

Berries	Dose (kJ/m^2)	Ethylene biosynthesis (µL/kg.h)	Respiration rate (CO_2 /kg.h)	References
Blueberry (*Vaccinium* spp. Berkeley)	4	NA	Decreased from 2.43 to ~2.1 mg	(Xu et al., 2016)
Blueberry (*Vaccinium* spp. Berkeley)	4	Unaffected (3.2 – 3.4)	Increased from 2.43 to > 2.5 mg	(Xu and Liu, 2017)
Boysenberry	9.2	NA	Decreased from 116.92 to 107.65 mL	(Vicente et al., 2004)
Red raspberry (*Rubus idaeus* L.)	2 and 4	NA	Increased from 10.03 ± 1.02 to 14.67 ± 1.60 mL	(Gimeno et al., 2021)
Strawberries (*Fragaria* × *ananassa* Duch., cv. *Camarosa*)	4	NA	Decreased	(Cote et al., 2013)
Strawberry (*Fragaria ananassa* Duch. cv. Akihime)	4.1	Increased 4.4 and 11.7-fold on 1 and 4 d, respectively	NA	(Li et al., 2014)

NA: not assessed

3.2. Texture (firmness, weight loss, and cell wall)

Maintenance of textural quality of berries is crucial for consumer acceptance and shelf-life. Key parameters for textural evaluation include firmness, weight loss, and cell wall enzymes and components in berries as reported by various researchers (Table 2).

Firmness

Firmness of berries decreases at refrigerated and room temperature during postharvest storage. Most studies have demonstrated that UVC treatment significantly prevented the loss of firmness in berries. For instance, Amiri et al. (2021) reported that UVC treatment at 0.5 kJ/m^2 resulted in higher firmness (2.47 N) in strawberries compared to the control samples (2.15 N) after 12 d storage at 5 °C. Also, UVC treatment with 4 kJ/m^2 doses ranging from single to multi-step increased strawberry hardness and compression resistance after 13 d of storage at 0 °C (Ortiz Araque et al., 2019). Similarly, UVC treatment at 6 kJ/m^2 resulted in better retention of firmness in blueberry compared to control samples after 28-d storage at 0°C (Nguyen et al., 2014). Nevertheless, Perkins-Veazie et al. (2008) reported that blueberries' firmness remained unaffected by treatments at 1 – 4 kJ/m^2 doses. Jaramillo Sánchez et al. (2021) evaluated the epicarp and mesocarp of blueberries post-UVC treatment and found that the treatment did not significantly impact the rupture force and deformation. Gimeno et al. (2021) found that UVC treatment at 4 kJ/m^2 caused a 12.5 % reduction in the firmness of red raspberry compared to the control while treatment at a lower dose (2 kJ/m^2) resulted in a 7.5 % increase in the firmness compared to the control after 12 d storage at 6 °C. Overall, several studies have shown that UVC treatment maintained the flesh firmness of berries such as strawberries (Amiri et al., 2021; Li et al., 2014; Severo et al., 2015), blueberries (Jaramillo Sánchez et al., 2021; Nguyen et al., 2014; Perkins-Veazie et al., 2008; Xu et al., 2016), and red raspberries (Gimeno et al., 2021), as shown in Table 2.

Weight loss

Water vapor released during transpiration and respiration causes weight loss in berries due to increased membrane permeability and decreased cell strength (Lu et al., 2016; Xu et al., 2016). In addition to inhibiting loss of firmness, UVC treatment can reduce weight loss. Several studies demonstrated that UVC treatment decreased the accelerated weight loss in berries such as blueberry (Nguyen et al., 2014; Xu et al., 2016), red raspberry (Gimeno et al., 2021), and strawberry (Amiri et al., 2021), as shown in Table 2. For instance, the weight loss of strawberries exposed to UVC at 0.5 kJ/m^2 was 1 %, while the control group was 1.95 % after 12 d of storage at 5 °C and 90 % relative humidity (Amiri et al., 2021). Besides, the weight loss of UVC-treated blueberries declined by ~1.3 % compared to untreated ones after 21 d of storage (Nguyen et al., 2014). Moreover, the weight loss of UVC-treated blueberries (~1.8 %) was lower than that of the control samples (2.6 %) after 8-d of storage at 4 °C (Xu et al., 2016). However, other studies reported no effect of UVC treatment on the weight loss of blueberries (Jaramillo Sánchez et al., 2021; Perkins-Veazie et al., 2008). The mechanism of UVC treatment for the reduction of weight loss is unclear. Reduced respiration rate may be associated with reduced weight loss. (Xu et al., 2016) showed that respiration rate and weight loss had a strong correlation (R = 0.869). Besides, UVC treatment may minimize weight loss by forming a thin dry layer on the surface of the commodity, which may inhibit the release of water vapor (Abdipour et al., 2020).

Cell wall metabolism

Cell walls in fruit and vegetables, particularly berries with thinner-skinned fruit, affect their textural quality and softening. Changes in the primary cell wall constituents such as cellulose (CEL), hemicelluloses (HCEL), and water-soluble pectin (WSP), the strength of adhesion in middle lamella, and the cell turgor can be associated with the loss of firmness and flavor (Chen et al., 2015). Besides, enzyme activities, such as cellulase (CL), polygalacturonases (PG), pectin methylesterase (PME), pectin lyase (PL), and rhamnogalacturonan lyase (RGL), can also cause deformation of cell wall structure and softening (Pombo et al., 2009; Priya Sethu et al., 1996; Sheng et al., 2018). Moreover, β-glycanases and β-glucosidases (β-gal) cleave xyloglucan, a common HCEL polymer (Ortiz Araque et al., 2019), while β-galactosidase promotes flesh softening by eliminating galactose from cell wall components (Trainotti et al., 2001).

Ortiz Araque et al. (2019) reported that UVC treatment inhibited the activity of β-glucanase, PG, PME, β-gal, and Xylase in strawberries after 13-d storage at 0 °C in darkness and preserved firmness. Severo et al. (2015) found that UVC treatment of strawberries may enhance firmness, inhibit cell wall degradation, and delay surface deterioration due to decreasing PL transcription genes despite increasing β-gal, PG, and PME genes compared to the control. Besides, Pombo et al. (2009) found that PG, PME, and endoglucanase were decreased or unaffected in strawberries by UVC treatment compared to control. In addition, they concluded that UVC treatment at 4.1 kJ/m^2 delayed strawberry softening, possibly due to decreased gene transcription involved in cell wall degradation (Pombo et al., 2009). Thus, UVC treatment can delay softening and maintain strawberry texture by reducing weight loss, pectin solubilization, and inhibiting cell

wall degrading enzyme activity. The evaluations on the effects of UVC treatment on strawberries' cell wall metabolism were reported (Ortiz Araque et al., 2019; Pombo et al., 2009; Severo et al., 2015) but, further research on other berries is needed.

Table 2. Effect of postharvest ultraviolet-C treatment on berries' texture (firmness, weight loss, and cell wall).
Tablo 2. Hasat sonrası ultraviyole-C uygulamasının dutsu meyvelerin tekstür (sıkılık, ağırlık kaybı ve hücre duvarı) üzerindeki etkisi.

Berries	Dose (kJ/m^2)	Firmness	Weight loss (%)	Cell wall	References
Blueberry	1 – 4	Unaffected	Unaffected	NA	(Perkins-Veazie et al., 2008)
Blueberry	<11.4	NA	Unaffected	NA	(Jaramillo Sánchez et al., 2021)
Blueberry	4	Inhibited loss of firmness	Decreased from 2.6 to ~1.8	NA	(Xu et al., 2016)
Blueberry	6	Increased ~0.3 N	Decreased by ~1.3	NA	(Nguyen et al., 2014)
Red raspberry	2 and 4	Increased by 7.5 % at 2 kJ/m^2 but decreased by 12.5 % at 4 kJ/m^2	Decreased from 8.1 to 6.9	NA	(Gimeno et al., 2021)
Strawberry	4.1	Increased from ~2.9 to ~3.2 N	NA	Decreased or unaffected PG, endoglucanases, and PME	(Pombo et al., 2009)
Strawberry	4.35	Increased by ~0.5 N	NA	Decreased pectate lyases transcript accumulation	(Severo et al., 2015)
Strawberry	4.1	Increased	NA	NA	(Li et al., 2014)
Strawberry	0.5	Increased from 2.47 to 2.15 N	Decreased from 1.95 to 1	NA	(Amiri et al., 2021)
Strawberry	Single-Step: 4 Two-Step: 2 x 2 Multi-Step: 5 x 0.8	Increased	NA	Decreased β-glucanase, PG, and PME activity and WSP	(Ortiz Araque et al., 2019)

NA: not assessed, ND: not determined, PG: polygalacturonases, PME: pectin methyl esterase, WSP: water-soluble pectin

3.3. Phenolic compounds

Berries contain phenolic compounds, including phenolic acids, flavonoids, and tannins, which contribute to their color and antioxidant capacity (Horvitz, 2017; Szajdek & Borowska, 2008). These compounds are formed in the epidermis and tissue and can be found in water-soluble or water-insoluble forms (Skrovankova et al., 2015). Berries contain phenolic compounds such as resveratrol, anthocyanins, and chlorogenic acid in high concentrations (Häkkinen, 2000; Rodriguez-Mateos et al., 2012; Spinardi et al., 2019; H. Wang et al., 2017).

Total phenolic content

Postharvest UVC treatment increased total phenolic contents (TPC) in berries such as blueberries (González-Villagra et al., 2020; Nguyen et al., 2014), red raspberries (Gimeno et al., 2021), and strawberries (Amiri et al., 2021; Jin et al., 2017; Severo et al., 2015) during storage as shown in Table 3. For instance, UVC at 0.5 kJ/m^2 increased TPC by 47.75 % in strawberries (198.21 mg GAE/g fresh weight) compared to the control samples (103.97 mg GAE/g fresh weight) at the end of 12 d-storage (Amiri et al., 2021). Likewise, TPC in blueberries was increased by ~15 mg GAE/100 g fresh weight after UVC treatment at 6 kJ/m^2 compared to control samples (Nguyen et al., 2014).

Flavonoids (flavanols, flavonols, anthocyanins)

Berries have high levels of flavonoids, including anthocyanins, isoflavones, chalcones, flavonols, and flavones. These compounds are responsible for the biological activities, color, and aroma of fruit and have several effects on health (Del Rio et al., 2010; Devore et al., 2012). UVC treatment at 2 and 4 kJ/m^2 increased total flavonoid content (TFC) by 86.9 – 72 % in red raspberries during 12-d storage at 6 °C (Gimeno et al., 2021). Flavonol accumulation in blueberries was not affected by a UVC treatment at 2.76 kJ/m^2 (Yang et al., 2019).

Anthocyanins are a crucial group of flavonoids and are known as fruit colorants (Skrovankova et al., 2015). The flavylium cation (AH+) structure of anthocyanins makes them acidic pigments that give strawberry fruit its reddish color (pelargonidin-3-glycoside and cyanidin-3-glycoside) (Crecente-Campo et al., 2012; Wang and Zheng, 2001). The main characteristic of these substances is their capacity to scavenge free radicals (Tena et al., 2020). Amiri et al. (2021) showed that untreated strawberries' TAC was ~10 mg/100 g FW higher than the UVC-treated (0.5 kJ/m^2) ones during 12 d of storage. However, UVC treatment at 4 kJ/m^2 accelerated the increase of major anthocyanin compounds (pelargonidin-3-glucoside, cyanidin-3-glucoside-succinate, and cyanidin-3-glucoside at ranging from 232.8–302.3 mg kg^{-1}, 10.5–13.6 and 68.3–79.6, respectively) in fresh-cut strawberries during storage at 4 °C for 7 d (Li et al., 2019). Also, UVC treatment at range 2 and 4.35 kj/m^2 dose increased TAC in strawberries (M. Li et al., 2019; Severo et al., 2015) and red raspberries (Gimeno et al., 2021). (Xu and Liu, 2017) have reported that UVC treatment at 4 kJ/m^2 increased TAC from ~250 to 300 mg/100 g in blueberries stored at 4 °C for 8 d. Similarly, González-Villagra et al. (2020) have reported that UVC treatment at 4.6 kJ/m^2 increased TAC by 80 %, 50 %, and 20 % in 'Bluegold', 'Brigitta', and 'Legacy' blueberry cultivars, respectively, compared to control samples after 5-d storage. 'Bluecrop' blueberry cultivars' TAC levels were increased by 10 % after UVC treatments at 2 – 4 kJ/m^2, while the 'Collins' cultivars' TAC remained unaffected at the same doses and storage condition (7 d, 5 °C) (Perkins-Veazie et al. 2008). Furthermore, as reported by Wang et al. (2009), individual

anthocyanins (delphinidin-3-galactoside, cyanidin-3-galactoside, delphinidin-3-arabinoside, petunidin-3-galactoside, petunidin-3-glucoside, petunidin-3-arabinoside, malvidin-3-galactoside, malvidin-3-arabinoside) and flavonols (myricetin-3-arabinoside, quercetin-3-galactoside, quercetin-3-glucoside, kaempferol-3-glucuronide, kaempferol-3-glucoside) in UVC-treated (2.15, 4.30, or 6.45 kJ/m^2) blueberries were increased by up to 150 % compared to the untreated ones. Moreover, UVC treatment at 2.76 kJ/m^2 increased delphinidin, petunidin, cyanidin, peonidin, and malvidin in blueberries and activated anthocyanin biosynthesis during the postharvest term (Yang et al., 2018). Also, UVC treatment at the same dose (2.76 kJ/m^2) increased anthocyanin accumulation in immature (turning from green to purple and pink) and mature blueberries by 261.8 and 23.1 %, respectively, compared to control samples (Yang et al., 2019). Besides, UVC treatment activated genes responsible for flavonoid biosynthesis-related gene expression, including *Vaccinium corymbosum* leucoanthocyanidin reductase (*VcLAR)*, anthocyanidin reductase (*VcANR)*, and myeloblastosis proto-oncogene proanthocyanidin (*VcMYBPA1*) (Yang et al., 2019). These genes exhibit a high positive correlation with flavonol and proanthocyanins biosynthesis. Therefore, total flavonoids, flavonoid subgroups such as flavonols and anthocyanins, and related gene expressions were affected positively by UVC treatment (Table 3). The effects of UVC on the TAC depend on the dose and types of berries and their cultivars (Perkins-Veazie et al., 2008; Wang et al., 2009).

Table 3. Effect of postharvest ultraviolet C treatment on berries' phenolic compounds.
Tablo 3. Hasat sonrası ultraviyole-C uygulamasının dutsu meyvelerin fenolik bileşikler üzerindeki etkisi.

Berries	Wavelength	Dose (kJ/m^2)	Effects	References
Blueberry	254 nm	4	Increased TAC from ~250 to 300 mg/100 g and PAL activity	(Xu and Liu, 2017)
Blueberry	254 nm	2.76	Increased anthocyanin biosynthesis and associated genes, TAC by 196.4 and 40.8 % in turning (from green to purple and pink) and mature blueberries	(Yang et al., 2018)
Blueberry	254 nm	2.76	Unaffected flavonols (~ 0.5 g/kg FW) Increased anthocyanins by 261.8 and 23.1 % in turning (from green to purple and pink) and mature blueberries and proanthocyanidins (by 56 %)	(Yang et al., 2019)
Blueberry	254 nm	6	Increased TPC (by ~15 mg GAE/100 g FW), TAC (by ~88 mg/100 g), and individual anthocyanins	(Nguyen et al., 2014)
Blueberry	254 nm	2.3 and 4.6	Increased TPC (up to ~ 150%), TAC (by 80 %, 50 %, and 20 % depending on cultivars)	(González-Villagra et al., 2020)
Blueberry	254 nm	4	Increased PAL activity, TAC (by ~ 50 mg/100 g)	(Xu et al., 2016)
Blueberry	254 nm	2.15, 4.30, and 6.45	Increased chlorogenic acid from 40.6 ± 4.8 to 55.3 ± 6.8, 45.1 ± 6.1, and 46.0 ± 5.3 µg/g FW at 2.15, 4.30, and 6.45 kJ/m^2, respectively, and increased resveratrol (from 13.0 ± 0.7 to 17.4 ± 0.2 µg/g fresh weight)	(Wang et al., 2009)
Blueberry	254 nm	2 - 4	Increased 'Bluecrop' blueberry cultivars' TAC by 10 %, unaffected 'Collins' blueberry cultivars' TAC	(Perkins-Veazie et al. 2008)
Red raspberry	254 nm	2 and 4	Increased TPC at 4 d of storage, decreased TPC at 12 d of storage, increased TAC during 12 d of storage, and TFC (by ~87 – 72 %)	(Gimeno et al., 2021)
Strawberry	ND	4.1	Increased PAL activity	(Pombo et al., 2011)
Strawberry	254 nm	4.35	Increased TPC, TAC, individual phenolics (gallic acid, hydroxybenzoic acid, ρ-coumaric acid, quercetin and (+)-catechin), PAL and ANS activity	(Severo et al., 2015)
Strawberry	ND	2	Increased TPC, PAL activity	(Jin et al., 2017)
Strawberry	ND	0.5	Increased TPC from 103.97 to 198.21 mg GAE/g FW, decreased TAC	(Amiri et al., 2021)
Strawberry (Fresh-cut)	ND	4.0	Increased TPC (by ~ 0.12 g/kg), TAC (from 0.41 to 0.51 g/kg), individual phenolic compounds (*P*-coumaroyl glucose, kaempferol-3-glucoside, ellagic acid, ellagic acid glucoside), and anthocyanin compounds (pelargonidin-3-glucoside, cyanidin-3-glucoside-succinate and cyanidin-3-glucoside at ranging from 232.8 – 302.3 mg/kg, 10.5 – 13.6 and 68.3 – 79.6, respectively)	(Li et al., 2019)

ANS: anthocyanidin synthase, FW: fresh weight, ND: not determined, PAL: phenylalanine ammonia-lyase, TAC: total anthocyanin content, TFC: total flavonoid content, TPC: total phenolic content.

Non-flavonoids

Berries have non-flavonoids such as phenolic acids (benzoic acid and cinnamic acid derivates) and others (resveratrol, lignans, etc.) (Del Rio et al., 2010; Kaur et al., 2022; Smeds et al., 2012). UVC treatment at 4 kJ/m^2 increased *p*-coumaroyl glucose, ellagic acid, and ellagic acid glucoside in fresh-cut strawberries during 7-d storage at 4 °C (Li et al., 2019). Similarly, UVC treatment at 4 kJ/m^2 increased gallic acid, hydroxybenzoic acid, and *p*-coumaric acid in strawberries at 7 d storage at 20 °C (Severo et al., 2015). UVC treatments at 2.15, 4.30, or 6.45 kJ/m^2 increased chlorogenic acid in blueberries (55.3, 45.1, and 46.0 µg/g fresh weight, respectively), compared to the untreated ones (40.6 ± 4.8 µg/g fresh weight) (Wang et al., 2009). However, a lower UVC dose (0.43 kJ/m^2) did not affect the chlorogenic acid content (Wang et al., 2009). Besides, proanthocyanidins were increased by 56 % after UVC treatment at 2.76 kJ/m^2 in blueberries during postharvest storage (Yang et al., 2019). Non-flavonoids like hydroxybenzoic acid, *p*-coumaroyl glucose, ellagic acid, ellagic acid glucoside, and *p*-coumaric acid have anticarcinogenic, antibacterial, antiviral, antimutagenic, and anti-inflammatory properties (Mattila et al., 2006). Thus, UVC treatment can increase non-flavonoid contents and thus increase the biological activities of berries.

Enzymes involved in phenolic biosynthesis

Phenylalanine ammonia-lyase (PAL) is a key enzyme for increasing the biosynthesis of phenolic compounds such as flavonoids and anthocyanins (Deshi et al., 2020; Gimeno et al., 2021; Wen et al., 2008). PAL, as well as other enzymes such as chalcone synthase (CHS), flavanone 3-hydroxylase (F3H), leucoanthocyanidin reductase (LAR), anthocyanidin synthase (ANS), cinnamate-4-hydroxylase (C4H), 4-coumaroyl coenzyme A ligase (4CL), and stilbene synthase (STS), are responsible for biosynthesis of phenolic compounds and accumulating gene transcripts related to the pathways of phenolics (anthocyanins, resveratrol, etc.) (Gimeno et al., 2021; Sheng et al., 2018; J.-F. Wang et al., 2015). UVC treatment at 4 kJ/m^2 increased blueberry PAL activity by 2.7% compared to control after 8-d storage at 4°C (Xu and Liu, 2017). Similarly, UVC increased PAL activities in strawberries (Jin et al., 2017; Pombo et al., 2011; Severo et al., 2015). Li et al. (2019) also reported that UVC treatment activated PAL, 4CL, and C4H enzymes and their gene expression of *FaPAL*, *FaC4H*, and *Fa4CL* in strawberries compared to control. Moreover, PAL activities were promoted by a UVC treatment at 4.1 kJ/m^2 in strawberries, although anthocyanin accumulation was suppressed (Li et al. 2014). The authors argued that the suppression of anthocyanins might be due to the inhibition of 4CL and DFR enzymes by the UVC treatment. On the other hand, PAL, ANS, C4H, dihydro flavonol 4-reductase (DFR), chalcone isomerase (CHI), flavonoid 3-O-glucosyltransferase (UFGT) which are responsible for anthocyanin biosynthesis were not induced by UVC treatment in berries (Yang et al., 2018).

Overall, the studies show that UVC treatment increased phenolic compounds such as flavonoid and non-flavonoids, enzyme activities, and their relevant gene expression in berries (Table 3). The existing literature collectively shows that postharvest UVC treatment can increase phenolic compounds mainly anthocyanins by increasing PAL, ANS, and other biosynthesis enzymes in berries.

3.4. Antioxidant capacity

UVC treatment enhanced total antioxidant capacity in berries such as blueberries (Nguyen et al., 2014; Wang et al., 2009; Yang et al., 2019) and strawberries (Severo et al., 2015), possibly due to increased phenolic compounds, as shown in Table 4.

For instance, Amiri et al. (2021) showed that UVC at 0.5 kJ/m^2 caused a 29.9 % increase in the total antioxidant content of strawberries during storage. Besides, UVC treatment at 2 kJ/m^2 increased antioxidant enzymes such as catalase (CAT), ascorbate peroxidase (APX), and superoxide dismutase (SOD) in strawberries in comparison to the control group, during 12-d storage at 5 °C (Jin et al., 2017). Similarly, SOD activity in UVC-treated (at 4 kJ/m^2) fresh-cut strawberries increased by 39.5 % during storage (Li et al., 2019). Moreover, UVC treatment increased the activities of SOD, CAT, and APX enzymes involved in reactive oxygen species (ROS) metabolism in fresh-cut strawberries during 7-d storage at 4 °C (Li et al., 2019). However, SOD activity in blueberries was not affected by UVC treatment during 8-d storage at 4 °C (Xu et al., 2016). Accumulations of superoxide, hydroxyl, and hydrogen peroxide lead to oxidative stress in fruits. Increasing antioxidant enzymes (SOD, APX, and CAT) is important for reducing oxidative stress and tissue damage, and promoting cell survival (Jiang et al., 2010). As a result, postharvest UVC treatment at 0.5 – 6 kJ/m^2 maintained the total antioxidant capacity and their relative enzyme activities in berries.

3.5. Color

Berries' visual appeal is primarily due to their color formed through chlorophyll degradation and pigment synthesis. Berries are rich in anthocyanins commonly known as fruit colorants (red-blue-purple) (Skrovankova et al., 2015). Enzymes such as peroxidase (POD) and polyphenol oxidase (PPO) are responsible for enzymatic browning reactions in fruit during the postharvest storage period, causing color changes (Costa et al., 2021). Jin et al. (2017) demonstrated that UVC treatment at 2 kJ/m^2 increased the POD and PPO activities in strawberries by ~60 and 38.7 %, respectively, after 12-d storage at 5 °C and 90–95 % relative humidity. Increasing PPO and POD with UVC treatment might cause enzymatic browning and discoloration. However, UVC treatment at 4 kJ/m^2 inhibited the POD activity of blueberries by 10.2 % end of 8-d storage at 4 °C (Xu and Liu, 2017).

The International Commission on Illumination (CIE) – L*a*b* color space system is used in determining the color characteristics of berries quantitatively using L* (lightness), a* (greenness to redness), and b* (blueness to yellowness) values (Markovic et al., 2013). These parameters are determined by the chemical and physical changes in the product and show the visual color quality important for the sensory perception of products. UVC treatment at 4 kJ/m^2 caused no systematic changes in the a* and b* values of strawberries (Li et al., 2014), blueberries (Xu et al., 2016; Xu and Liu, 2017), and red raspberries (Gimeno et al., 2021) during postharvest storage. For instance, the L* value of blueberries was unaffected by a UVC treatment at 4 kJ/m^2 (Xu and Liu, 2017), while the UVC treatment at the same dose caused an increase in the L* value of strawberries (Li et al., 2014). Besides, as mentioned earlier, numerous studies showed that anthocyanin, which is responsible for red, purple, and blue colors, was increased by UVC treatment. Overall results might be concluded that exposure to UVC treatment could preserve berries' color and increase anthocyanin levels and PAL activity, although PPO and POD activities were also increased.

Table 4. Effect of postharvest ultraviolet-C treatment on berries' antioxidant capacity.
Tablo 4. Hasat sonrası ultraviyole-C uygulamasının dutsu meyvelerin antioksidan kapasitesi üzerindeki etkisi.

Berries	Wavelength	Dose (kJ/m^2)	Effects	References
Blueberry	254 nm	4	Unaffected antioxidant enzymes activities (SOD; ~190 U/g FW)	(Xu et al., 2016)
Blueberry	254 nm	6	Increased antioxidant activities	(Nguyen et al., 2014)
Blueberry	254 nm	4.6	Increased antioxidant properties and radical scavenging activity	(González-Villagra et al., 2020)
Blueberry	254 nm	2.76	Increased antioxidant capacity and their enzyme activity (SOD) (39.5 %)	(Yang et al., 2019)
Red raspberry	254 nm	2 and 4	Increased antioxidant activity	(Gimeno et al., 2021)
Strawberry	ND	0.5	Increased antioxidant activity (29.91 %) and L-ascorbic acid content	(Amiri et al., 2021)
Strawberry	ND	2	Increased antioxidant enzymes activities (SOD, CAT, APX)	(Jin et al., 2017)
Strawberry	254 nm	4.35	Increased antioxidant activity	(Severo et al., 2015)
Strawberry (Fresh-cut)	ND	4.0	Increased antioxidant capacity	(Li et al., 2019)

APX: ascorbate peroxidase, CAT: catalase, FW: fresh weight, ND: not determined, SOD: superoxide dismutase

3.6. Flavor

The free sugar content, total soluble solids (TSS), and titratable acidity (TA) are crucial parameters for flavors and sensory properties of berries. The effect of UVC treatment on flavor in berries is shown in Table 5**.** UVC treatment decreased sugar (fructose and glucose) (Yang et al., 2018) and total soluble sugar (González-Villagra et al., 2020) in berries, while unaffecting TSS and TA in berries such as strawberries (Amiri et al., 2021) and blueberries (González-Villagra et al., 2020). During storage, strawberries' TSS content increased from 6.73 % to 7.93 % but UVC inhibited this increase in the first 3 d (Li et al., 2014). Furthermore, UVC treatment at 4.35 kJ/m^2 increased the synthesis of the aroma-producing ester volatiles such as alcohol dehydrogenase (*ADH*) and alcohol acetyltransferase (*AAT*) transcript accumulation in strawberries (Severo et al., 2015). Li et al. (2019) reported that UVC exposure at 4 kJ/m^2 suppressed the increase of sour, bitter, and astringent tastes of fresh-cut strawberries. On the other hand, PPO and POD are responsible for off-flavor and off-odor in fruit during the postharvest storage period (Costa et al., 2021). Jin et al. (2017) found that UVC treatment at 2 kJ/m^2 increased POD and PPO activities in strawberries by ~60 % and 38.7 %, respectively, however, it inhibited blueberries' POD activity by 10.2 % at 4 kJ/m^2 during 12-d storage at 5°C.

Table 5. Effect of postharvest ultraviolet-C treatment on berries' flavor.
Tablo 5. Hasat sonrası ultraviyole-C uygulamasının dutsu meyvelerin lezzeti üzerindeki etkisi.

Berries	Wavelength	Dose (kJ/m^2)	Effects	References
Blueberry	254 nm	4	Inhibited soluble solid content	(Xu et al., 2016)
Blueberry	254 nm	2.76	Decreased sugar (fructose and glucose) and total soluble sugar	(Yang et al., 2018)
Blueberry	254 nm	4.6	Unaffected TSS and TA	(González-Villagra et al., 2020)
Strawberry (Fresh-cut)	ND	4.0	Decrease of increase of sourness, bitterness, and astringency tastes Increased volatile compounds	(Li et al., 2019)
Strawberry	ND	0.5	Unaffected TA	(Amiri et al., 2021)
Strawberry	ND	4.1	Decreased TSS	(Li et al., 2014)
Strawberry	254 nm	4.35	Increased aroma-producing ester volatiles and *ADH* and *AAT* transcript accumulation	(Severo et al., 2015)
Blueberry	254 nm	4	Inhibited soluble solid content	(Xu et al., 2016)
Blueberry	254 nm	2.76	Decreased sugar (fructose and glucose) and total soluble sugar	(Yang et al., 2018)

AAT: alcohol acetyl transferases, ADH: alcohol dehydrogenase, ND: not determined, TA: titratable acidity, TSS: total soluble solids

3.7. Microbial Decay

Postharvest microbial decay, primarily caused by *Botrytis cinerea, Rzihopus,* and *Colletotrichum*, significantly affects the shelf life of berries, making them highly susceptible to spoilage (Kumar et al., 2018). Several studies have been conducted on the effects of UVC treatment on microbial decay in berries, as shown in Table 6. Xu and Liu (2017) showed that blueberry decay incidence was suppressed by UVC treatment at 6 kJ/m^2 compared to control, during 8-d storage at 4 °C. Zhou et al. (2019) indicated that decay incidence in UVC-treated blueberries at 2.67 kJ/m^2 (~17.69 %) was lower than that in the control (~35.49 %) after 8-d storage. Similarly, the incidence of rot in red raspberries was inhibited by 15 – 20 % upon UVC treatment at 2 and 4 kJ/m^2 after 12-d storage at 6 °C (Gimeno et al., 2021). Besides, UVC at 0.5 – 2 kJ/m^2 effectively inhibited gray mold decay in strawberries inoculated with *B. cinerea* (Amiri et al., 2021; Jin et al., 2017). Jin et al. (2017) also found that strawberries treated by UVC at 2 kJ/m^2 had a 36.1 % and 24.2 % reduction in *B. cinerea* lesion diameter after 9- and 12-d storage, respectively, at 5 °C. Adhikari et al. (2015) found that higher UVC-induced inactivation rates were observed in fruits with smoother and less hydrophobic surfaces (apples and pears) compared to the ones in fruits with rougher surfaces such as strawberries and raspberries. Thus, surface characteristic is a critical factor for UVC efficiency.

Table 6. Effect of postharvest ultraviolet-C treatment on berries' microbial decay.
Tablo 6. Hasat sonrası ultraviyole-C uygulamasının dutsu meyvelerin mikrobiyal çürüme üzerindeki etkisi.

Berries	Wavelength	Dose (kJ m^{-2})	Effects	References
Blueberries	254 nm	4	Decreased decay incidence	(Xu and Liu, 2017)
Blueberries	254 nm	6	Reduced decay	(Nguyen et al., 2014)
Blueberry	275 nm (UVLED)	0.16	0.91–0.95 log reduction *Escherichia coli*	(Haley et al., 2023)
Blueberry	ND	2.67	Reduced decay incidence and *Botrytis cinerea,* total aerobic mesophilic bacteria, total mold and yeast	(Zhou et al., 2019)
Blueberry	254 nm	<11.4	Delaying and reducing *B. cinerea* and fungal infection	(Jaramillo Sánchez et al., 2021)
Blueberry	254 nm	4	Inhibited decay incidence	(Xu et al., 2016)
Blueberry (in water)	254 nm	9.5 – 47.4	< 5.2 log reduction *E. coli* O157:H7	(C. Liu et al., 2015)
Raspberry	254 nm	10.5	Reduction of 1.1 log *E. coli* and 1 log *Listeria monocytogenes*	(Adhikari et al., 2015)
Red raspberries	254 nm	2 and 4	Reduced rot incidence and total aerobic mesophilic bacteria, total mold and yeast	(Gimeno et al., 2021)
Strawberry	ND	2	Inhibited gray mold decay by *B. cinerea*	(Jin et al., 2017)
Strawberry	ND	0.5	Decreased decay *B. cinerea*	(Amiri et al., 2021)
Strawberry	254 nm	7.2 and 11.9	Reduction of 2 log *E. coli and* 1 log *L. monocytogenes*	(Adhikari et al., 2015)
Strawberry (Fresh-cut)	ND	4.0	Reduced microbial growth and total aerobic bacterial count	(M. Li et al., 2019)

ND: not determined

UVC treatment also had a significant impact on the safety of berries through inactivating human pathogens. Berries are highly susceptible to microbial contamination. Thus, several studies have evaluated UVC's effectiveness in inactivating human pathogens in berries. For instance, Haley et al. (2023) showed that UVC treatment at 0.16 kJ/m^2 and 275 nm caused up to 0.95-log inactivation in *E. coli* on blueberries. UVC treatment at higher doses (9.5 – 47.4 kJ/m^2) and 254 nm decreased *E. coli* O157:H7 count by 5.2-log in another study (Liu et al., 2015). A 2-log reduction in *E. coli* and a 1-log reduction in *L. monocytogenes* were obtained by UVC treatment at 7.2 and 11.9 kJ/m^2 in strawberries (Adhikari et al., 2015).

The inhibition of pathogens and microbial decay can also be related to increasing phenolic compounds and antioxidants (Amiri et al., 2021; Jin et al., 2016; Nigro et al., 2000). In addition, PAL, chitinases, and β-1,3-glucanases are also known as defense-related enzymes against pathogens (Abd El-Rahman et al., 2012; Nigro et al., 2000; Pombo et al., 2009). Chitinase hydrolyzes fungal cell wall chitin and β-1,3-glucanase releases the oligosaccharides pathogen microorganism cell walls. After UVC treatment, their activity and relevant gene (*CCR-1 allele, CAT, CHI2, PPO,* and *PLA6*) expression were induced (Jin et al., 2017; Sheng et al., 2018). Thus, these enzymes, phenolic compounds, and antioxidants that are induced by UVC treatment might also be associated with inhibition of microbial decay and relevant microorganisms in berries. Therefore, overall results indicate that UVC treatment effectively inhibits microbial decay, inactivates pathogens, and enhances disease resistance against gray mold in berries.

4. Effects of UVC combined with other applications on berries' physiology

Cold storage (Amiri et al., 2021; Nguyen et al., 2014; Ortiz Araque et al., 2019), edible coating (Mannozzi et al., 2017), *Aloe vera* gel (Sempere-Ferre et al., 2022), and active packaging (Chiabrando et al., 2019) have been studied to extend shelf life of berries. UVC treatment has been studied as a non-thermal and non-chemical treatment for extending shelf life (Green et al., 2020; Rabelo et al., 2020; Zhai et al., 2021). Although postharvest UVC treatment has several advantages like decreasing weight loss, inhibiting microbial decay, and increasing phenolic compounds, antioxidant capacity, and firmness, it can cause increased respiration rate and ethylene production, and insufficient surface decontamination in berries. Consequently, other physical or chemical treatments combined with UVC treatments have been studied, as shown in Table 7**.** For instance, Xu and Liu (2017) conducted UVC treatment combined with 1-methylcyclopropene (1-MCP), which is commercially used to inhibit the ethylene action in climacteric fruits. The combination of the two treatments showed better results in maintaining the quality and extending the shelf life of blueberries compared to using 1-MCP or UVC treatments alone. Also, the combined treatment decreased ethylene production in blueberries by 5.9 % and exhibited higher TAC values than the control and individual treatments during 8-d storage at 4 °C (Xu and Liu, 2017). Gimeno et al. (2021) reported that a combination of passive modified atmosphere packaging (MAP) and UVC (254 nm at 4 kJ/m^2) treatment in raspberries effectively delayed senescence, prolonged shelf life, and increased bioactive compounds. Aqueous chlorine dioxide (ClO_2) and UVC combination inhibited microorganism growth, delayed maturity and senescence, and extended the shelf-life of blueberries (Xu et al., 2016). The efficiency of combined treatment was higher than individual treatments (UVC or ClO_2). Li et al. (2014) indicated that 1 mM abscisic acid (ABA) combined with UVC treatment (4.1 kJ/m^2) significantly enhanced antioxidant capacity in strawberries. The strawberries treated with the combined ABA and UVC produced less ethylene than those treated with UVC alone. Mild heat treatment (45 °C, 3 h in air) and UVC combination had higher effects on delaying spore germination of *B. cinerea* in *in-vitro* assays compared to each treatment alone (Pan et al., 2004). Also, Marquenie et al. (2003) showed that pulsed white light (30 µs pulses, 15 Hz, 40 to 250 s) combined with UVC at 1 kJ/m^2 increased the inactivation *Monilia fructigena* and *B. cinerea* in strawberries, compared to UVC alone. As a result, combining UVC with additional treatments (mild heat treatment, pulsed white light, ClO_2, ABA, MAP, 1-MCP) may lead to improved postharvest preservation of berries.

Table 7. Effect of postharvest ultraviolet-C treatment and other combined applications on berries' physiology.
Tablo 7. Hasat sonrası ultraviyole-C uygulamasının ve diğer kombine uygulamaların dutsu meyvelerin fizyolojisi üzerindeki etkisi.

Berries	Treatments with combination UVC	Combination effects	References
Blueberry	Aqueous chlorine dioxide (ClO_2)	-inhibited microbial growth, respiration rate, weight loss, decay incidence -delayed maturity, senescence, and decline of firmness, color -maintained shelf-life quality, anthocyanin content	(Xu et al., 2016)
Blueberry	1-methylcyclopropene (1-MCP)	-maintained quality -extended shelf life -inhibited respiration rate, ethylene production, decay incidence, POD activity -delayed softening -increased total anthocyanin content	(Xu and Liu, 2017)
Raspberry	Modified atmosphere packaging (MAP) film	-delayed senescence -prolonged shelf life -maintained bioactive compounds	(Gimeno et al., 2021)
Strawberry	Abscisic acid (ABA)	-enhanced antioxidant capacity -decreased ethylene production compared to UVC alone	(Li et al., 2014)
Strawberry	Heat treatment	- decreased total sugar content (slightly) -delayed spore germination of *B. cinerea*	(Pan et al., 2004)
Strawberry	Pulsed white light	-increased inactivation of *Monilia fructigena* and *B. cinerea*	(Marquenie et al., 2003)

5. Conclusion

UVC treatment is an effective, simple, and eco-friendly method for preserving berry physiologies including reducing weight loss, inhibiting cell wall metabolism, enhancing antioxidant capacity and biosynthesis enzymes such as SOD, APX, and CAT, and increasing phenolic compounds (flavonoids, non-flavonoids, and their synthesis enzymes such as PAL, ANS, etc.). Besides, it has great potential to inhibit microbial decay, inactivate pathogens, and enhance disease resistance against gray mold in berries caused by *B. cinerea*. However, UVC treatment can cause increasing PPO and POD, thereby causing enzymatic browning and degradation of flavor. In addition, UVC treatment might increase respiration rate and ethylene production, causing rapid senescence and flavor degradation. On the other hand, UVC doses, berry types, and other processing parameters are all important parameters for UVC efficiency. Furthermore, combining UVC treatment with other chemical and physical techniques like cold storage, 1-MCP, MAP, ABA, and pulsed white light has increased the efficiency of the control of berries' postharvest physiology compared to UVC treatment alone. Thus, the hurdle approach could be more effective for berries postharvest term. Future research may focus on the commercial applicability of the UVC technology alone or in combination with other treatments for controlling berries' physiology. In addition, more research needs to be conducted to understand the effects of UVC exposure on berries' sensory evaluation, ethylene production, and respiration rates.

6. Conflicts of Interest

The authors declare no conflict of interest.

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Simulated gastric digestion survivability and bioprocess compatibility of a novel *Pichia kudriavzevii* FOL-27

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Abstract: The objective of this study was to investigate *Pichia kudriavzevii* FOL-27's: i) survivability in simulated gastric juice (SGJ) and simulated bile juice (SBJ), ii) growth kinetics under batch bioreactor trials (BT) and fed-batch bioreactor trials (FBT). Viability of FOL-27 as determined by calculating relative cell density ratio (RCDR) under SGJ and SBJ conditions was conducted at pH=3, pH=2, pH=1.5, and control and ox-bile levels of 0.2%, 1%, 2%, and control (0%), respectively. Microbial growth kinetics was obtained by measuring absorbances at OD_{600} in BT or in FBT where pH, dissolved oxygen (DO) and temperature were controlled at 5.5, 25%, and 30°C, respectively. In addition, effect of DO at 12.5% or 25% were evaluated to determine the growth and performance of FOL-27 in FBT by utilizing exponential feed. The doubling-time, maximum specific growth rate, and final cell densities achieved for BT were 101.8 min, 8.202 h^{-1} and 28.7, respectively. FBT at 25% O_2 or 12.5% O_2 level yielded a doubling-time, maximum specific growth rate, and final cell density of 90.18 min, 3.95 h^{-1}, 22.51 and 88.8 min, 2.83 h^{-1}, 26.6, respectively. RCDRs achieved were similar for pH=3 and control vs both were significantly higher ($p<0.05$) than pH=1.5 and pH=2 with the latter two pH-levels were significantly different ($p<0.05$) from each other. RCDRs were not significantly different across control, 0.2%, 1%, and 2% ox-bile levels ($p>0.05$). *P. kudriavzevii* FOL-27 exerts probiotic characteristics via tolerating SGJ and SBJ conditions similar to that of human gastrointestinal conditions. An observable elevation in biomass when grown under FBT conditions reveals that *P. kudriavzevii* FOL-27 is compatible to bioprocess development.

Keywords: *P. kudriavzevii* FOL-27, probiotics, fed-batch, bioprocess, dissolved-oxygen.

Yeni bir *Pichia kudriavzevii* FOL-27'nin simüle edilmiş mide sindiriminde hayatta kalma yeteneği ve biyoproses uyumluluğu

Özet: Bu çalışmanın amacı, *Pichia kudriavzevii* FOL-27'nin: i) simüle edilmiş mide suyu (SGJ) ve simüle edilmiş safra suyunda (SBJ) hayatta kalma kabiliyetini, ii) kesikli biyoreaktör denemeleri (BT) ve beslemeli kesikli biyoreaktör (FBT) denemeleri altında büyüme kinetiğini araştırmaktır. SGJ ve SBJ koşulları altında bağıl hücre yoğunluğu oranının (RCDR) hesaplanmasıyla belirlenen FOL-27'nin canlılığı, pH=3, pH=2, pH=1,5'te ve %0,2, %1, %2 ve kontrol (%0) sığır-safrası seviyelerinde gerçekleştirildi. Mikrobiyal büyüme kinetiği, pH'nın çözünmüş oksijenin (DO) ve sıcaklığın sırasıyla 5,5, %25 ve 30°C'de kontrol edildiği BT veya FBT'de OD_{600}'de absorbansların ölçülmesiyle elde edildi. Ek olarak, logaritmik besleme kullanılarak FOL-27'nin FBT'deki büyümesini ve performansını belirlemek için DO'nun %12,5 veya %25'teki etkisi değerlendirildi. BT için elde edilen iki katına çıkma süresi, maksimum spesifik büyüme oranı ve nihai hücre yoğunlukları sırasıyla 101,8 dakika, 8,202 h^{-1} ve 28,7 olarak tespit edildi. %25 O_2 veya %12,5 O_2 seviyesindeki FBT, sırasıyla 90,18 dakika, 3,95 h^{-1}, 22,51 ve 88,8 dakika, 2,83 h^{-1}, 26,6'lık bir ikiye katlama süresi, maksimum spesifik büyüme oranı ve nihai hücre yoğunluğu sağladı. Elde edilen RCDR'ler pH=3 için benzer ve kontrole karşı her ikisi de pH=1.5 ve pH=2'den önemli ölçüde daha yüksek olarak bulundu ($p<0.05$). Son iki pH seviyesi ise birbirinden önemli ölçüde farklı RCDR ile sonuçlandı ($p<0.05$). RCDR değerleri kontrole karşı %0,2, %1 ve %2 sığır-safra seviyeleri arasında önemli ölçüde farklı olarak bulunmadı ($p>0,05$). *P. kudriavzevii* FOL-27, insan gastrointestinal durumlarına benzer SGJ ve SBJ koşullarını tolere ederek probiyotik özellikler sergileyebilmektedir. FBT koşulları altında büyütüldüğünde biyokütlede gözlemlenebilir bir artış, P. kudriavzevii FOL-27'nin biyoproses geliştirme konusunda uyumlu olduğunu ortaya koymaktadır.

Anahtar Kelimeler: *P. kudriavzevii* FOL-27, probiyotikler, kesikli besleme, biyoproses, çözünmüş oksijen.

Research article

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1. Introduction

Fermentation technique is one of the oldest technics used to preserve food and enhances the aroma and flavour of the food systems. In addition, products such as enzymes, vitamins or antimicrobials can be produced by fermentation. Therefore, fermentation technology is still being considered as one of the critical applications in fermented food industry. The most common microorganisms used for production of fermented beverages and foods are bacteria and yeast (Alakeji and Oloke, 2020). Yeasts are being classified under eucaryotic organisms and are unicellular. The history of the yeasts goes back to ancient times, and it is still one of the most important organisms used by human beings (Liti, 2015). For example, yeasts have been used to produce bread, wine, and beer for thousands of years. When selecting a yeast organism for manufacturing an alcoholic beverage, the major criteria would be ethanol production rate during fermentation and its contribution to the sensorial profile of the resulting product (Walker, G.M, 2016). Yeasts can exist in variety of different ecological niches such as plant-based or even animal-derived systems which they could impact the organoleptic properties of foods (Chelliah et al., 2016).

Yeasts could rapidly form colonies when grown in nutrient-rich environments and co-exist with other microorganisms such as probiotic bacteria (Yetiman et.al 2022). Yeasts are extremely influential in the realms of biotechnology, food, everyday chemicals, and pharmaceuticals, and they have played a crucial role in human development throughout history. They were domesticated to power industrial fermentations and have been genetically altered for the manufacturing of pharmaceuticals and industrial chemicals. Normally, they predominate in spontaneously fermented foods, adding to desired tastes. Of these, *Saccharomyces cerevisiae* is thought to be a prominent workforce for bioprocesses because of its well-characterized physiology, ease of engineering, and reputation as "generally regarded as safe." (Nielsen, 2019). However, *S. cerevisiae* biotechnological processing is becoming more difficult, in part because of its innate traits, which include low stress tolerance and limited carbon sources (Thorwall et al., 2020). Furthermore, it is challenging to satisfy the wide range of consumer demands when *S. cerevisiae* is the only strain used in controlled fermentation operations, which limits the sensory qualities of products (Steensels & Verstrepen, 2014). Because of these disadvantages, non-conventional yeasts have become popular biotechnological hosts because of their beneficial phenotypes, which include inherent stress tolerance, the capability to metabolize a variety of carbohydrates, and the release of distinct tastes. Most of the probiotic research in the literature has been conducted bacterial organisms. For example, lactic acid bacteria from the species Lactobacillus and Bifidobacterium are commonly studied and produced in probiotic dietary supplements (Saavedra, 2001). *Bifidobacterium bifidum* and *Streptococcus thermophilus* effectively reduce acute diarrheal and rotavirus transmission (Vlasova et al., 2016; Martinez. et al., 2015; Sharma et al., 2014). The positive effects seen in the intestine may be due in part to the induction of protective antimicrobials (Power et al., 2014) which could possess antagonistic properties (Cintas et al., 2001). Probiotic yeasts have gained popularity in recent years, not just for animal nutrition preparations as well as for clinical applications. Currently, the yeast *Saccharomyces boulardii* is one of the most common yeast-based probiotic dietary supplements especially prescribed to those showing diarrhea-type diarrheal intestinal symptoms. It has been reported that *S. boulardii* was discovered by experimental methods, so this yeast species offers antitoxin properties for a variety of gastrointestinal diseases (Vandenplas et al., 2009; Buts, 2009). As a result, it is regarded as a non-bacterial probiotic product. Many mechanisms have been reported to explain the vast range of health-supporting effects of food-grade yeast ingestion (Czerucka et al., 2007). Antibiotic-associated and infectious diarrhea including recurrent *Clostridium difficile*; irritable bowel syndrome; and inflammatory bowel disease are some of the diseases that probiotic yeasts are shown effective in clinical trials (Foligné et al., 2010).

This species was formerly known as *Issatchenkia orientalis* and was later renamed *Pichia kudriavzevii* by Kurtzman et al. (2008). This species is distinguished by the production of spherical ascospores.

Initially, it was suggested that *Candida krusei*, which is frequently used to refer to clinical isolates, was *P. kudriavzevii*'s asexual form (anamorph), as they shared the same sequences in the D1/D2 sections of the 26S ribosomal DNA (Kurtzman et al., 2008). The non-conventional yeast *Pichia kudriavzevii* is found in traditionally fermented foods in the world and is widely distributed in natural habitats. Chelliah (2016) reported that several experimental evaluations on *Pichia kudriavzevii* resulted in technological advantages for sustainable bioenergy production of this yeast species. It has also been isolated as an indigenous microbiota in olive fermentation and pickled wax gourd (Golomb et al., 2013, Wu et al., 2016). When nutritional constraints are induced, *P. kudriavzevii* exhibits dimorphic transitions marked by the establishment of pseudohyphae and the creation of biofilms (Van Rijswijck et al., 2014), (Gómez-Gaviria & Mora-Montes, 2020). According to Chu et al. (2023), extremely low pH stress also causes pseudohyphae to grow and multicellular clusters to gradually evolve; these processes are incompatible with mass transfer bioprocess development. This phenotype differs from *C. albicans* (Villa et al., 2020). Because the creation of biofilm is thought to be linked to invasive growth and food spoiling, it has gained interest in both medical studies and the industry of food. *P. kudriavzevii* is found in many naturally fermented foods across the world, which may be partially attributed to its strong resilience to environmental challenges. This makes the species a promising strong framework for chemical biosynthesis. According to Chelliah (2016), no reports has been found on probiotic evaluation of *P. kudriavzevii.* Moreover, apart from Gumustop and Ortakci (2022) no reports have been found on evaluating the bioreactor processability and biomass optimization of probiotic candidate *P. kudriavzevii* strains. We isolated a novel *P. kudrivzevii* FOL-27 strain from fermented plant material called “Shalgam” and performed in vitro gastrointestinal survival experiments to determine fundamental probiotic characteristics along with bioreactor trials to optimize biomass development of this novel strain. To our knowledge, no reports have been published on this novel isolate with regards to its simulated gastric survival and bioprocess development capacity.

2. Materials and Methods

2.1. DNA isolation and PCR Fingerprinting

P. kudriavzevii FOL-27 culture was medium in YPS or yeast extract peptone sucrose, is a complete medium for yeast growth and incubated aerobically at 30 ℃ for 24h with 225 rpm. DNA extraction was performed according to DNA extraction kit manufacturer’s protocol. The DNA of *P. kudriavzevii* FOL-27 was kept at 4°C upon isolation. The conserved region of 5.8S ITS rRNA was amplified using ITS1 and ITS4 primers. D1/D2 domains of the 26S rRNA region was amplified using NL1 and

NL4 primers. The nucleotide sequence of primers used in the PCR runs were shown in Table 1. The individual components of PCR mix were purchased from Transgen Biotech. PCR reagents and final concentrations are shown in Table 2. PCR cycling started with thermal cycling and the first denaturation of samples at 95°C for 5 min. The denaturation phase started at 94°C for 30 seconds followed by annealing which occurred at following temperatures of 55°C (ITS1/4), 52.5°C (NL1/4). For extension phase, specimens were extended at 78°C for 2 minutes. All three phases were repeated 36 times. Samples were incubated at 72°C for 10 min for the last extension step. Then, samples were stored at 4°C. After PCR amplification, specimens were run on 1% agarose gel with 140 voltage. NL primer amplified DNA product was further processed for sanger sequencing application. Sanger sequencing reads were evaluated by using the NCBI's BLAST tool.

Table 1. Primer names and their sequences.
Tablo 1. Primer adları ve bunların sekansları.

Primer Name	Sequence
ITS1	TCCGTAGGTGAACCTGCGG
ITS4	TCCTCCGCTTATTGATATGC
NL1	CGCATATCAATAAGCGGAGGAAAAG
NL4	GGTCCGTGTTTCAAGACGG

Table 2. Components of PCR reactions.
Tablo 2. PCR reaksiyonlarının bileşenleri.

Component	Volume (µL)	Final Concentration
Template DNA	2	-
Forward primer (10 µM)	1	0.2 µM
Reverse primer (10 µM)	1	0.2 µM
10X EasyTaq® buffer	5	1X
2.5 mM dNTPs	4	0.2 µM
EasyTaq® DNA polymerase	1	2.5-5 units
Nuclease-free water	36	-
Total volume	50	-

2.2. Inoculum preparation

YPS was prepared with the following w/w inclusions of 1% yeast extract, 2% peptone, and 2% sucrose in a shake flask. First, a cryovial of *P. kudriavzevii* FOL-27 stored at -80°C was subculture in a 10 mL YPS broth at 30°C for 24h. After pre-inoculation, a 1% v/v of sub-cultured FOL-27 was inoculated into a 50 mL of YPS media placed in Erlenmeyer flask followed by incubation at 30°C for 24-h with 225 rpm shaking. At the end of incubation optical density of samples was measured at 600 nm by using Shimadzu UVmini-1240 spectrophotometer.

2.3. Cultivation in batch bioreactor

For batch fermentation, bioreactor was filled with 650 mL of YPS media which was inoculated with a 50 mL of pre-cultivated *P. kudriavzevii* FOL-27. Since batch fermentation doesn't require any fresh media to feed in and cultures to harvest while running the process neither fresh media was added nor grown cells were removed from the bioreactor. The temperature, pH and dissolved oxygen (DO) levels were controlled at 30°C, 5.5, and 25% during the entire batch fermentation process, respectively. The samples were taken every 2h until the first 6-h of the fermentation, and the fifth sampling was carried out at 22h for OD_{600} measurements to determine biomass development of FOL-27. Upon cease of fermentation, another 50 mL of fermentative were taken, and the samples were centrifuged to discard the supernatant after which they were put into the incubator until they dry out to determine dry weight of *P. kudriavzevii* FOL-27.

2.4. Cultivation in fed-batch bioreactors

Fed-batch bioreactor trials were carried out to evaluate the performance boost in terms of biomass yield and to test the effect of two different DO levels on growth and performance of *P. kudriavzevii* FOL-27 strain. Fed-Batch bioreactor fermentations started off with 700 mL of YPS media. A 50 mL of pre-cultivated *P. kudriavzevii* FOL-27 strain was inoculated into bioreactors. The temperature and pH of the bioreactors were maintained at 30°C, and 5.5 respectively, while dissolved oxygen levels were adjusted to 25% (control) or 12.5% (treatment) through the entire fermentation process. A sterile 33.3% sucrose solution was prepared for the fed-batch phase and feed pumps were kicked in from 6h onset of fermentation. Fed-batch fermentations were allowed to continue for 20h in which samples were taken every hour until 12h after which samples were taken every two hours for OD measurements at 600 nm. Fed-Batch bioreactor trials were performed in duplicates.

2.5. Simulated gastric and bile juice

To evaluate the survival of FOL-27 under simulated gastric juice and simulated bile juice, modified methods from Sun & Griffiths (2000), Yetiman et al. (2022), Klaenhammer & Kleeman (1981), Song et. al. (2003) were applied. To prepare SGJ, YPS media was adjusted to pH conditions of control (no acid supplementation), pH = 1.5, pH = 2, and pH = 3 by adding HCl solution. To prepare SBJ, YPS broth was supplemented with control (no bile), 0.2%, 1%, and 2% (w/v) ox bile extract (Sigma, Germany). Later, each treatment condition was inoculated with *P. kudriavzevii* FOL-27 fresh culture followed by incubation at 30°C x 200 rpm shaking conditions for 48 h. The final optical cell densities were achieved by using Shimadzu UVmini-1240 spectrophotometer at a wavelength of 600 nm. The trials were conducted in 3 reps and results were shown as relative cell density ratio (OD_{600} t_{final}/OD_{600} t_0). Statistical analysis was conducted using the analysis of variance (ANOVA) and Tukey tests in excel.

3. Results and Discussion

3.1. PCR and sanger sequencing

The DNA samples amplified with NL primers are shown in Figure 1. However, there was nothing observed on the sample that was amplified with ITS primers. For this reason, the PCR product that was amplified with NL primer was sent for sequencing. The BLAST tool (Altschul et al., 1990) was used to examine the Sanger sequencing results. Examining genetic links among *Pichia* species has been made possible by phylogenetic analysis of gene sequences. The PCR fragment of the NL primer was found on the CK5 large subunit ribosomal RNA gene of *Pichia kudriavzevii* strain with 99.47% similarity and a 0.0 E-value score, according to BLAST data. Furthermore, when we checked the BLAST result, the close species was found *Pichia occidentalis* have 99.27% homology.

Nuclear DNA reassociation was used to identify *Issatchenkia occidentalis* as a unique species among strains of *P. kudriavzevii* and the taxonomy database presently lists *Issatchenkia occidentalis* as a synonym for *P. occidentalis* (Kurtzman et al., 2008).

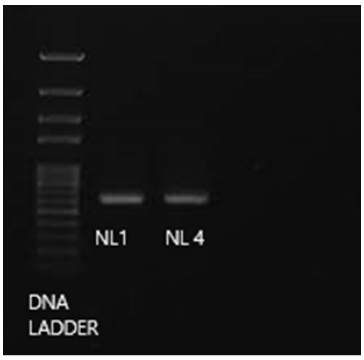

Figure 1. Gel picture of NL primers amplified DNA fragments.
Şekil 1. NL primerleri ile amplifiye edilmiş DNA parçalarının jel resmi.

3.2. Biomass in batch cultivation

The biomass evolution of *P. kudriavzevii* FOL-27 over time is shown in Fig 2. The samples were taken for the first 6 hours, after last sample was taken at around 22h after which stationary phase has been approached. Results indicated the lag and log phases of *P. kudriavzevii* FOL-27 was achieved at around 3 and 7 h, respectively (Figure 2).

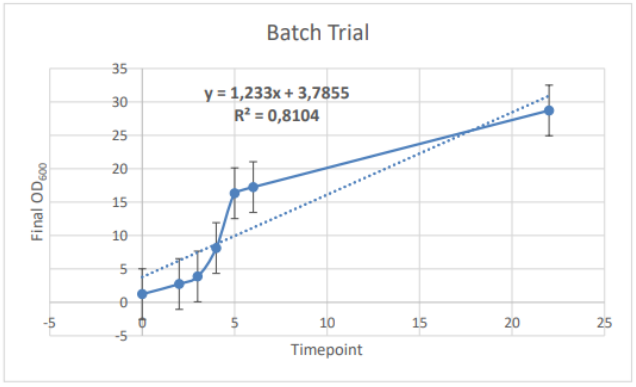

Figure 2. Evolution of biomass over time for batch fermentations.
Şekil 2. Batch fermantasyonlar için biyokütlenin zaman içindeki gelişimi.

Throughout the batch trial pO_2 and pH were controlled. The pH decline started at 8th hour perhaps due to acid production in the environment and base pump dosed NaOH until pH level reaches pH=5.5. The stirrer maintained a constant mixing to avoid sedimentation and uniformity across the medium (Figure 4). During the batch trials, the doubling-time, maximum specific growth rate, and final cell densities achieved were 101.8 min, 8.202 h^{-1} and 28.7 respectively.

Figure 3. The evolution of pO_2, pH, agitation, temperature, base pump duration over time. The graph shows the pH level (blue), stirrer (pink), base pump duration (dark blue), total flow(grey).
Şekil 3. pO_2, pH, çalkalama, sıcaklık, temel pompa süresinin zaman içindeki gelişimi. Grafik pH seviyesini (mavi), karıştırıcıyı (pembe), temel pompa süresini (koyu mavi), toplam akışı (gri) gösterir.

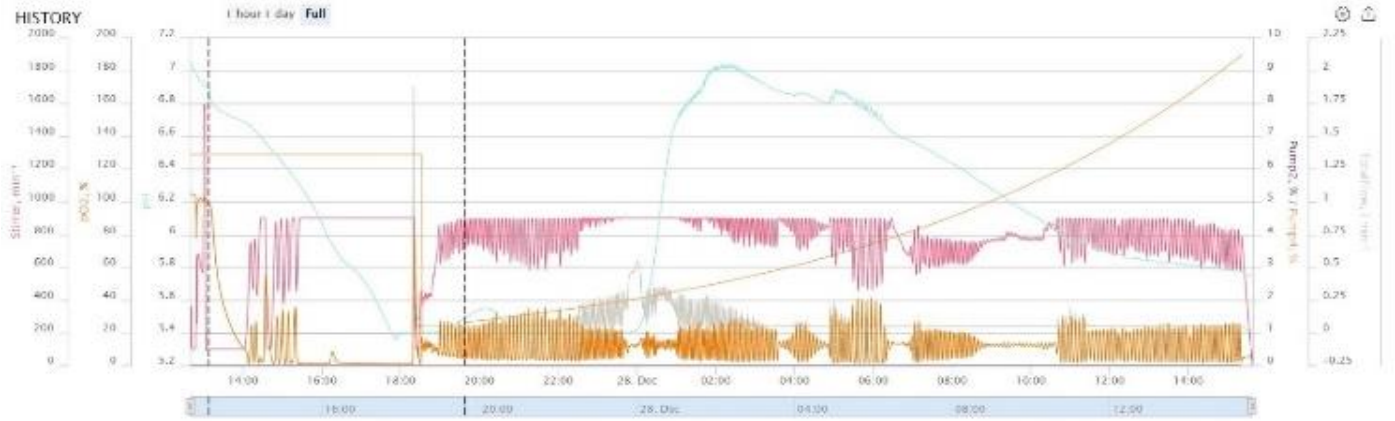

Figure 4. The evolution of pH (light blue) and base pump duration (orange line), dissolved oxygen (pO_2) (orange), airflow rate (grey), and stirrer rate (pink) against time during fed-batch cultivation. pO_2 set to 12.5%.
Şekil 4. Beslemeli kesikli ekim sırasında pH'ın (açık mavi) ve baz pompa süresinin (turuncu çizgi), çözünmüş oksijenin (pO_2) (turuncu), hava akış hızının (gri) ve karıştırıcı hızının (pembe) zamana karşı gelişimi. pO_2 %12.5'e ayarlanmıştır.

3.3. Biomass in fed-batch cultivation

Fed-batch trials (FBT) at 25% pO_2 or 12.5% pO_2 yielded doubling-time, maximum specific growth rate, and final cell densities of 90.18 min, 3.95 h^{-1}, 22.51 and 88.8 min, 2.83 h^{-1}, 26.6, respectively (Figure 5 and 6). Similar biomass yields were achieved in the first 12 hours of bioreactor cultivation. Later, biomass yield was increased perhaps due to feeding with 33.3% sucrose. The stirrer and airflow intake through the cascade system were used to modify pre-set levels of dissolved oxygen throughout the fed-batch operation.

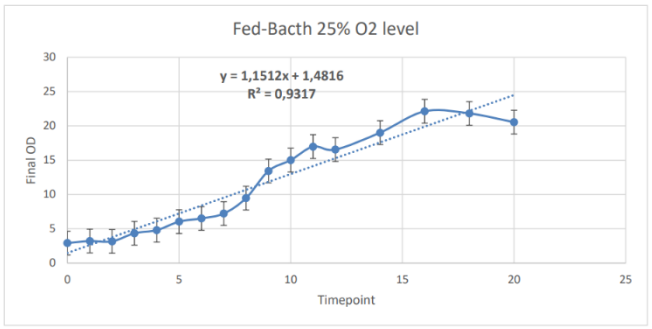

Figure 5. Evolution of biomass over time in fed-batch 25% pO_2 level bioreactor.
Şekil 5. Beslemeli kesikli %25 pO_2 seviyeli biyoreaktörde biyokütlenin zaman içindeki gelişimi.

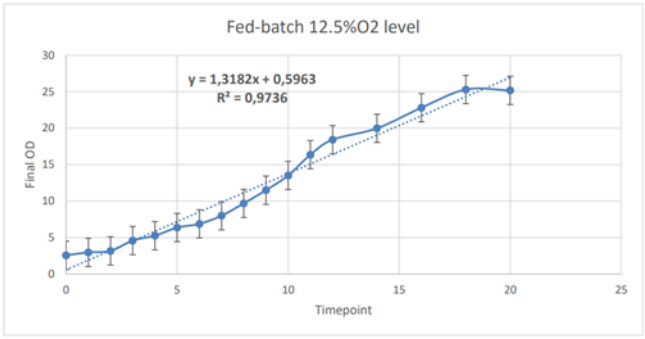

Figure 6. Evolution of biomass over time in fed-batch at 12.5% pO_2 level bioreactor.
Şekil 6. Beslemeli kesikli %12.5 pO_2 seviyeli biyoreaktörde biyokütlenin zaman içindeki gelişimi.

The pH was oscillating at 5.4 at the onset of the batch operation, as shown in Figures 4 and 7. At that point, the base pump starts in and raises the pH to 5.5. When the pH rises to above 5.5, the base pump shuts down. At around 10h, the base pump resumes to adjust the pH of the fermentate in the vessel.

At around 6h after the inoculation, a pH bump was seen (Figure 7) perhaps because of *P. kudriavzevii* FOL-27 producing more alkaline characteristic metabolite than acidic. According to ANOVA results, no significant difference seen between means of biomass in FBT at 25% pO_2 or 12.5% pO_2 level (p=0.83).

Figure 7. The evolution of pH (light blue) and base pump duration (orange line), dissolved oxygen (pO_2) (orange), airflow rate (grey), and stirrer rate (pink) against time during fed-batch cultivation. pO_2 set to 25%.
Şekil 7. Beslemeli kesikli ekim sırasında pH'ın (açık mavi) ve baz pompa süresinin (turuncu çizgi), çözünmüş oksijenin (pO_2) (turuncu), hava akış hızının (gri) ve karıştırıcı hızının (pembe) zamana karşı gelişimi. pO_2 %25'e ayarlanmıştır.

3.4. Survival against SGJ and SBJ

The SGJ survivability trials indicated that *P. kudriavzevii* FOL-27 can proliferate in pH=3 at a similar rate to control condition of pH=6.5 ($p > 0.05$). When the pH was below 2; however, the viability of *P. kudriavzevii* FOL-27 significantly declined ($p<0.05$). According to ANOVA, viability of *P. kudriavzevii* FOL-27 between pH=2 vs pH=1.5 was significantly different ($p< 0.05$). (Table 3).

Table 3. Survival of *P. kudriavzevii* FOL-27 against SGJ.
Tablo 3. P. kudriavzevii FOL-27'nin yapay mide ortamında hayatta kalması.

pH	Relative Cell Density Ratio (OD_{600} t_{final}/OD_{600} t_0)	Standard Deviation
1.5	2.03^c	±0.5
2	13.8^b	±2.0
3	25.2^a	±4.2
6.5 (control)	24.9^a	±5.7

P. kudriavzevii FOL-27 can grow in YPS media with a bile salt concentration of 0.2 percent. Furthermore, when the bile concentration is greater than 1%, *P. kudriavzevii* FOL-27 is still alive. Moreover, when the bile salt concentration is between 1% and 2%, no significant difference in survival of *P. kudriavzevii* FOL-27 was achieved (p=0.86) (Table 4).

Table 4. Survival of *P. kudriavzevii* FOL-27 under SBJ.
Tablo 4. P. kudriavzevii FOL-27'nin yapay safra ortamında hayatta kalması.

Bile Salt Concentration (%)	Relative Cell Density Ratio (OD_{600} t_{final}/ OD_{600} t_0)	Standard Deviation
0 (control)	19.2^a	±0.77
0.2	18.1^a	±0.61
1	18.3^a	±1.26
2	19.0^a	±2.63

P. kudriavzevii has been identified as a potential candidate for ethanol production (Díaz-Nava et al., 2017). *P. kudriavzevii* is an unusual yeast that can withstand a variety of stresses, including low pH, elevated temperature, and high salt concentrations. In the manufacture of xylonic acid, lactic acid, and succinic acid, the potential for genetic engineering of *P. kudriavzevii* for organic acid synthesis has been established (Ndubuisi et al., 2020). *P. kudriavzevii* can use glucose, fructose, and glycerol as carbon sources. On the other hand, *P. kudriavzevii* ITV-S42, does not use sucrose or xylose sugars and ferments ethanol when sugar concentrations are high (Díaz-Nava et al., 2017). Ndubuisi et al. (2020) demonstrated that the first 7.5 hours of *P. kudriavzevii* LC375240 growth weas practically identical across 30°C and 37°C. In the present study, the biomass development of *P. kudriavzevii* FOL-27 was evaluated at 30°C and a stationary phase was reached at 10 h. The results of SGJ and SBJ tests suggested that *P. kudriavzevii* FOL-27 could be a probiotic yeast strain owing to the resilience and survivability seen in acid and bile conditions mimicking human gastric digestion system. A previous study on probiotic characterization of isolated yeasts from Iranian traditional dairies showed that *Pichia fermentans* and *Pichia kudriavzevii* yeast strains showed probiotic potentials because of exhibiting antibacterial and antifungal properties (Saber et al., 2019). *P. kudriavzevii* FOL-27 is resistant to bile salts at concentrations comparable to other *P. kudriavzevii* strains examined, for example M31, M30, M29, M28, M26, O6, G6, G5 (Greppi et al., 2017). Similar to FOL-27, those strains survived satisfactorily at pH=2. Previous research has shown that the carbon source has a significant impact on *P. kudriavzevii* kinetic characteristics (Díaz Nava et al., 2017). Conditions of oxygen limitation encourage ethanol production but not biomass development in some yeast species. As a result, two-step fermentation methods have been designed: an aerobic stage to produce vast amounts of biomass, followed by a low-oxygen stage to increase ethanol production and the kinetic study was carried out on *P. kudriavzevii* 4A in an oxygen-limited environment. (Galafassi et al., 2010). *P. kudriavzevii* FOL-27 exhibited tolerance to 12.5% oxygen level. Chen et al. (2010) reported that raw milk isolates of *P. kudriavzevii* strains of BY15 and BY10 demonstrated probiotic capacity with regards to their survivability in SGJ and SBJ. The probiotic properties of the *P. kudriavzevii* FOL-27 strain used have not been tested before. Therefore, acid-bile tolerance tests were carried out on the strains.

4. Conclusion

Probiotics research has been rapidly evolving in recent years, such as the use of probiotic yeast strains, which has been underutilized so far but is becoming increasingly attractive. The most recent research demonstrates the immense potential of probiotic yeast in the food market, as well as the application of their special characteristics that are not present in probiotic bacteria. Many of the properties of the most well-known probiotic yeast, *S. cerevisiae* var. *boulardii,* have been studied, including positive effects on human health and negative or positive impacts on food products. In this study, the potential probiotic activity of a newly isolated *P. kudriavzevii* FOL-27 strain was investigated under simulated gastric juice and simulated bile juice conditions. We found that FOL-27 is can tolerate high ox-bile salt and low pH conditions mimicking the human gastrointestinal conditions. *P. kudriavzevii* FOL-27 was also subjected to batch reactor and fed-batch reactor conditions to characterize its growth kinetics. Fed-batch fermentation trials using an exponential feeding regimen with 33.3% sucrose supplementation yielded significantly more final biomass than batch trial, as determined by optical density at 600 nm wavelength. Our study demonstrates the preliminary data for probiotic potential of *P. kudriavzevii* FOL-27 and provides tools to improve the biomass development and fermentation growth kinetics of this newly isolated yeast strain. *P. kudriavzevii* FOL-27 carries potential durableness in harsh conditions of SGJ and SBJ, and possesses bioprocess compatibility, leading further probiotic characterizations via in vitro and in vivo experiments. *P. kudriavzevii* is a food fermentation starter culture that can be used to increase fermentation efficiency and achieve controlled food processing. It is commonly found in and dominates spontaneously fermented foods, which lead to distinctive flavors and demonstrate its probiotic potential.

5. Acknowledgment

This work was a capstone project for bachelor of degree.

6. Conflict of Interest

Authors declare no conflict of interest.

7. References

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