



ISTANBUL MEDENİYET UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENTS OF MOLECULAR BIOLOGY AND GENETICS

Antarctic Microalga *Micractinium Simplicissimum*
ASYA46 As a Functional Ingredient for Dough
Preparation

Master's Thesis

Mihraç Görünmek

January 2024



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Supervisor

Prof. Dr. Turgay Çakmak

January 2024

THESIS JURYS APPROVAL

Mihraç Görünmek, a M.Sc. student of Istanbul Medeniyet University, successfully defended the thesis/dissertation entitled “Antarctic microalga *Micractinium simplicissimum* ASYA46 as a functional ingredient for dough preparation”, which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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Having reviewed this thesis written under my supervision, I confirm that it has been written in accordance with APA Manual of Style and used its [footnote/in text] reference format consistently throughout the entire text.

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Declaration of Originality

I hereby declare that all information in this dissertation has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conducts, I have fully cited and referenced all material and results that are not original to this work.

Signature

Mihra GÖRÜNMEK

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LIST of SYMBOLS

Na: Sodium

Mg: Magnesium

K: Potassium

Ca: Calcium

P: Phosphorus

Cu: Copper

Mn: Manganese

Fe: Iron

Zn: Zinc

Pb: Lead

As: Arsenic

Hg: Mercury

HCl: Hydrochloric acid

L: Liter

ml: Milliliter

μl: Microliter

μg: Microgram

mg: Milligram

G: Gram

u: Unit

min: Minute

nm: nanometer

M: Molarity

ppm: parts per million

ppb: parts per billion

KOH: Potassium hydroxide

HNO₃: Nitric acid

H₂O₂: Hydrogen Peroxide

%: Percentage

°C: degrees Celcius

Rpm: Revolutions per minute

w/w: Weight by weight

LIST of ABBREVIATIONS

BBM: Bold Basal Medium

HPLC: High Performance Liquid Chromatography

AAS: Atomic absorption spectroscopy

ICP-MS: Inductively coupled plasma mass spectrometry

GC-FID: Gas Chromatography flame-ionization detection

LC-MS/MS: Liquid chromatography-tandem mass spectrometry

GRAS: Generally Recognized as Safe

EFSA: European Food Safety Authority

UN: United Nations

MSG: Monosodium glutamate

MeOH: Methanol

DMSO: Dimethyl sulfoxide

AMG: Aminoglycoside

TRIS: Hidroksimetil Aminometan

MES: 2-Morpholinoethanesulphonic acid

ACN: Acetonitrile

UVR: Ultraviolet Radiation

FAME: Fatty acid methyl esters

PUFA: Polyunsaturated fatty acids

MUFA: Monounsaturated fatty acids

SFA: Saturated fatty acid

Chl: Chlorophyll

Control-GF: Microalgae-free whole wheat flour dough formulation

Control-WW: Microalgae-free oat flour dough formulation

27WW-1: 1% *C. vulgaris* ASYA27 containing, whole wheat flour dough

27WW-3: 3% *C. vulgaris* ASYA27 containing, whole wheat flour dough

27GF-1: 1% *C. vulgaris* ASYA27 containing, oat flour dough

27GF3: 3% *C. vulgaris* ASYA27 containing, oat flour dough

46WW-1: 1% *M. simplicissimum* ASYA46 containing, whole wheat flour dough

46WW-3: 3% *M. simplicissimum* ASYA46 containing, whole wheat flour dough
46GF-1: 1% *M. simplicissimum* ASYA46 containing, oat flour dough
46GF-3: 3% *M. simplicissimum* ASYA46 containing, oat flour dough
102WW-1: 1% *C. vulgaris* ARAS102 containing, whole wheat flour dough
102WW-3: 3% *C. vulgaris* ARAS102 containing, whole wheat flour dough
102GF-1: 1% *C. vulgaris* ARAS102 containing, oat flour dough
102GF3: 3% *C. vulgaris* ARAS102 containing, oat flour dough
27-MB: Lyophilized *C. vulgaris* ASYA27
46-MB: Lyophilized *M. simplicissimum* ASYA46
102-MB: Lyophilized *C. vulgaris* ARAS102

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ABSTRACT

Antarctic microalga *Micractinium simplicissimum* ASYA46 as a functional ingredient for dough preparation

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Master Thesis, Molecular Biology and Genetics Department, Molecular Biology and Genetics Program

Supervisor: Prof. Dr. Turgay ÇAKMAK

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In the current global scenario, one of the biggest challenges for the 21st century is dwindling food supplies for a growing population. The World Food Programme has reported that the number of people suffering from food insecurity will increase to 345 million in 2023, with 1 in 9 people malnourished. While meeting immediate food needs is the goal, the real need is to support long-term programs. The limited availability of arable agricultural land, the 18% share of the global agricultural system in greenhouse gas emissions, the sustainability of traditional food sources being hampered by the decline in drinking water, and the need for CO₂-neutral approaches increase the need for different sustainable and healthy food sources. At this point, microalgae are a promising candidate as an alternative food with its value-added metabolites. Microalgae stand out with their protein, lipid, carbohydrate, mineral, vitamin, fiber, and pigment content, which may vary according to environmental conditions, storage conditions, and harvesting methods. They are high-quality food candidates, especially with their carotenoid, sterol, rich amino acid structure, and polyunsaturated fatty acid content. Many microalgae species have a complete nutrient profile and outperform most plant species in terms of yield. Thousands of years of breeding and species selection work applied to traditional plants can be applied to microalgae in just a few years with advances in genetic engineering. In addition to their rich food composition, microalgae are also environmentally friendly as they grow fast, sequester carbon dioxide, do not compete with agriculture for soil, and do not need potable water as much as agriculture. Studies on the use of microalgae and their extracts in food

are increasing day by day. Many nutrients can be added to microalgae. However, the accessibility of nutrients is as important as their health effects. Unfortunately, today, foods whose formulation is enriched with any natural extract or product can appeal to a smaller audience both economically and in terms of access. At this point, it is also important that bread, which has a place in many civilizations, is accessible to all segments of society.

The main theme of the study was based on microalgae obtained from Istanbul Medeniyet University Microalgae Culture Collection (IMUASYA-IMUARAS). In this context, psychrotolerant *Chlorella vulgaris* ASYA27 and *Micractinium simplicissimum* ASYA46 were isolated from Horseshoe Island during the IV National Antarctic Science Expedition, and mesotherm *Chlorella vulgaris* ARAS102 was isolated from Nemrut Volcanic Lake within the scope of another project were used. Since *Chlorella* is recognized by both GRAS and EFSA as microalgae suitable for human consumption and the elemental biochemistry of microalgae is shaped by environmental conditions, *Chlorella vulgaris* isolated from two different environments was used for the study. *Micractinium simplicissimum* is a microalga belonging to the same class as *Chlorella* but not documented by any authorized body for human consumption. Therefore, its food potential was evaluated in comparison with two other *Chlorella*.

The thesis aimed to improve the nutritional properties of the original food matrix of whole wheat flour and gluten-free (oat) flour bread enriched with different proportions of microalgae biomass. For this purpose, the physicochemical properties of whole wheat and gluten-free flour bread fortified with *Chlorella vulgaris* ASYA27, *Micractinium simplicissimum* ASYA46, and *Chlorella vulgaris* ARAS102 at 1% and 3% were evaluated. In this context, moisture and ash content, total protein by Kjeldahl method, amino acid analysis by HPLC, chromatographic measurement of vitamins B1, B2, and B6, total diet, starch and if the content, sodium (Na), magnesium (Mg) by Atomic absorption spectroscopy (AAS), potassium (K), calcium (Ca), and phosphorus (P), copper (Cu), manganese (Mn), iron (Fe) and zinc (Zn) content, heavy metal analysis by ICP-

MS, phosphorus analysis, oil content by Soxhlet and fatty acid methyl ester analysis by GC-FID. When the nutrient profile of the bread without microalgae added and the bread with 1-3% microalgae added were compared, an increase in moisture and ash content of the bread was observed. However, protein content increased by 9-34%. Amino acid content was also enriched with the addition of microalgae. Vitamin B1, B2, and B6 also increased with the addition of microalgae. Fiber content increased by 14-73%, while starch content increased by the highest 14% compared to the control group. Total lipid content increased between 7.5 and 30%. When the fatty acid profile was analyzed, an increase in total unsaturated fatty acid content was observed.

Despite food scarcity, the search for non-traditional food continues. At this point, microalgae are a good candidate to be an ideal food source. Within the scope of the study, bread, which is accessible to everyone and has an important place in the daily diet, has increased positively in all parameters analyzed because of the additive of bread with microalgae. When the improvement results of three different microalgae in the bread profile were compared, *Micractinium simplicissimum* ASYA46, which may be a candidate for human consumption and has not been announced, was as effective as the other two microalgae. This is a good indicator of its suitability for consumption. In addition, bread formulation using both whole wheat and gluten-free oat flour was compared. The low protein content of gluten-free foods made enrichment more vital, where the addition of microalgae contributed to improving the profile.

Keywords: Antarctica, bread, dough, food, gluten-free product, microalgae

ÖZET

Hamur hazırlama için fonksiyonel bir bileşen olarak Antarktika mikroalg

***Micractinium simplicissimum* ASYA46**

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Genetik Programı

Danışman: Prof. Dr. Turgay ÇAKMAK

Ocak, 2024. 162 Sayfa.

Güncel küresel senaryoda 21. yüzyıl için en büyük sorunlardan biri giderek artan nüfusa karşın giderek azalan besin kaynaklarıdır. Dünya Gıda Programı gıda sıkıntısı çeken insanların sayısının 2023 yılında 345 milyona yükseldiğini ve her 9 kişiden 1'inin yetersiz beslendiğini rapor etmiştir. Her ne kadar acil gıda ihtiyacını karşılamak hedeflense de asıl ihtiyaç uzun vadeli programların desteklenmesidir. Ekilebilir tarım arazilerinin kısıtlı oluşu, küresel tarım sisteminin sera gazı emisyonundaki %18'lik payı, içme suyunun azalması sonucu geleneksel besin kaynaklarının sürdürülebilirliğinin sekteye uğraması ve CO₂ nötr yaklaşımlara duyulan ihtiyaç sürdürülebilir ve sağlıklı farklı besin kaynaklarına yönelik yaklaşımları artırmaktadır. Bu noktada mikroalgler sahip olduğu katma değerli metabolitler ile alternatif gıda olarak ümit verici bir adaydır. Mikroalgler çevre şartları, saklama koşulları ve hasat şekline göre değişebilen protein, lipid, karbohidrat, mineral, vitamin, lif ve pigment içeriğiyle öne çıkmaktadır. Özellikle karotenoid, sterol, zengin amino asit yapısı ve çoklu doymamış yağ asidi içeriğiyle yüksek kaliteli besin adaylarıdır. Birçok mikroalg türü besin açısından eksiksiz bir profili sahiptir ve verim açısından çoğu bitki türünden daha iyi performans sergiler. Geleneksel bitkilere uygulanan binlerce yıllık ıslah ve tür seçimi çalışmaları gelişen genetik mühendisliği ile sadece birkaç yılda mikroalglerle uygulanabilmektedir. Zengin gıda kompozisyonunun yanında hızlı büyümeleri, karbondioksit tutmaları, toprak için tarımla rekabet etmemeleri, içilebilir suya tarım kadar ihtiyaç duyulmamaları gibi çevre dostu özellikleri de mikroalglerin pozitif yönlerindendir. Gün geçtikçe de mikroalg ve ekstraktlarına yönelik gıdada

kullanımı üzerine alıřmalar artmaktadır. Mikroalg ile katkılandırılan birok besin bulunmaktadır. Ancak besinlerin saėlıėa etkileri kadar ulařılabilirliėi de nem tařımaktadır. Ne yazık ki gnmzde formlasyonu herhangi bir doėal ekstrakt ya da rn ile zenginleřtirilen besinler hem ekonomik hem de eriřim anlamında daha kk bir kitleye hitap edebilmektedir. Bu noktada birok medeniyette yeri olan ekmeklerin her kesim tarafından ulařılabilmesi de nemlidir.

alıřmanın ana teması İstanbul Medeniyet niversitesi Mikroalg Kltr Koleksiyonu'ndan (IMUASYA-IMUARAS) temin edilen mikroalgler zerine kurgulanmıřtır. Bu kapsamda IV. Ulusal Antarktik Bilim Seferi'nde Horseshoe Adası'ndan izole edilen psikrotolerant *Chlorella vulgaris* ASYA27, *Micractinium simplicissimum* ASYA46 ve bir bařka proje kapsamında Nemrut Volkanik Gl'nden izole edilen mezoterm *Chlorella vulgaris* ARAS102 mikroalgi kullanılmıřtır. *Chlorella* hem GRAS hem de EFSA tarafından insan tketimine uygun bir mikroalg olarak kabul edilmesi ve mikroalglerin element biyokimyasının evresel kořullara gre řekillenmesi nedeniyle alıřma iin iki farklı ortamdan izole edilen *Chlorella vulgaris* kullanılmıřtır. *Micractinium simplicissimum* ise *Chlorella* ile aynı sınıfa ait ancak insan tketimi asından herhangi bir yetkili organ tarafından belgelenmeyen bir mikroalgdir. Bu nedenle diėer iki *Chlorella* ile karřılařtırılarak gıda potansiyeli deėerlendirilmiřtir. Tezin amacı mikroalg biyoktlesi ile farklı oranlarda zenginleřtirilen tam buėday unlu ve glten free (yulaf) unlu ekmeėin orijinal gıda matrisinin besinsel zelliklerinin artırılmasıydı. Ama doėrultusunda *Chlorella vulgaris* ASYA27, *Micractinium simplicissimum* ASYA46 ve *Chlorella vulgaris* ARAS102 ile %1 ve %3 oranlarında katkılandırılan tam buėday ve gltensiz unlu ekmeėin fizikokimyasal zellikleri deėerlendirilmiřtir. Bu kapsamda nem ve kl ieriėi, Kjeldahl metodu ile total proteini, HPLC ile amino asit analizi, B1, B2 ve B6 vitaminlerinin kromatografik lm, total diyet, niřasta ve lif ieriėi, Atomik absorpsiyon spektroskopisi (AAS) ile sodyum (Na), magnezyum (Mg), potasyum (K), kalsiyum (Ca) ve fosfor (P), bakır (Cu), manganez (Mn), demir (Fe) ve inko (Zn) ieriėi, ICP-MS ile aėır metal analizi, fosfor analizi, Soxhlet ile yaė ieriėi ve GC-FID ile yaė aside metil ester

analizi yapılmıştır.

Mikroalg eklenmeyen ekmekler ile %1-3 oranında mikroalg eklenen ekmeklerin besin profili karşılaştırıldığında ekmeğin nem ve kül içeriğinde artış görülmüştür. Bununla beraber protein içeriği %9-34 oranında artış göstermiştir. Amino asit içeriği de mikroalg ilavesi ile zenginleşmiştir. B1, B2 ve B6 vitamini de mikroalg ilavesiyle artmıştır. Lif içeriği %14-73 oranında artış sağlarken, nişasta içeriği kontrol grubuna göre en yüksek %14 seviyelerinde artışa yol açmıştır. Total lipit içeriği %7.5-30 arasında artış sağlamıştır. Yağ aside profili incelendiğinde toplam doymamış yağ aside içeriğinde yükselme gözlemlenmiştir.

Gıda kıtlığına karşın geleneksel olmayan besin arayışı devam etmektedir. Bu noktada mikroalgler ideal besin kaynağı olmaya iyi bir adaydır. Çalışma kapsamında herkesin ulaşabileceği ve günlük beslenmede önemli bir yere sahip olan ekmeğin mikroalg ile katkılandırılması sonucu analiz edilen tüm parametrelerde olumlu anlamda artış meydana gelmiştir. Üç farklı mikroalgin ekmek profilinde meydana getirdiği iyileşme sonuçları karşılaştırıldığında özellikle insan tüketimine aday olabilecek ve anons edilmemiş olan *Micractinium simplicissimum* ASYA46'da diğer iki mikroalg kadar etkin olmuştur. Bu da tüketime uygunluğu noktasında iyi bir göstergedir. Bunun yanında hem tam buğday hem de glutensiz olan yulaf unu kullanılarak hazırlanan ekmek formülasyonu karşılaştırılmıştır. Glutensiz besinlerin düşük protein içeriği zenginleştirilmesini daha hayati hale getirmiştir, bu noktada mikroalg ilavesi profili iyileştirmeye katkı sağlamıştır.

Anahtar kelimeler: Antarktika, ekmek, gıda, glutensiz ürün, hamur, mikroalg

1. INTRODUCTION

1.1 Agriculture and Wheat: A Historical Perspective

Agriculture played a crucial role in the development and survival of early human civilizations. It enabled the transition from hunter-gatherers to settle societies, providing a continuous source of food. Archaeological excavations show that early human communities began farming in the Middle East around 10,000 years ago (Bar-Yosef, 2011). Without agriculture, people who had no choice but to hunt and gather would have had to depend on changes in seasons, migration patterns, and natural disasters for their food supply. This dependence condemned nutrition to uncertainty and variability, leading to periods of hunger (Handmer et al., 1999). However, agriculture provided a more sustainable and predictable food supply. Through the cultivation of crops and the domestication of animals, early humans were able to access food more consistently, which improved human nutritional well-being (Zeder, 2012).

As agriculture developed, so did human civilization and its dominance over the environment. With the introduction of wheat into the human diet, there have been fundamental changes in nutrition and food culture. It has contributed to the human population as a source of calories, nutrients, vitamins, and minerals. This has contributed to the establishment and progress of human civilizations (Huth et al., 2013). In addition, the processing of wheat into food has led to innovation and diversity in nutrition. For example, it led to the development of bread, which is a staple food and has a place in many cultures. Bread dates back to the ancient Egyptians and Mesopotamians. Bread consumption is embedded in many culinary cultures and is consumed all over the World (Haaland, 2007). Bread contributed to the development of ancient economies with its easy availability, storability, and portability (Pasqualone, 2018). With this, many pastries, food processing techniques, and cooking methods have emerged. In this way, the cultivation of wheat and the development of bread have been a fundamental factor

in human nutrition, food culture, and the establishment of modern civilizations (Fairweather-Tait, 2003).

In addition to food, progress has been made in agriculture, technology, and trade. It also allowed for the development of complex social structures: the specialization of labor, technology, and the arts. In short, this adventure is deeply linked to the evolution of human civilization (Klüver, 2008).

1.2 Food Crisis and Sustainable Solutions

One of the biggest challenges in nutrition is to provide sufficient/healthy food and ensure its sustainability. To address this challenge, interdisciplinary research strategies focusing on economic, environmental, and health issues are needed. Along with access to food, food security is also one of the main challenges. Poverty is a major cause of food insecurity in underdeveloped countries as it limits access to food (Mohapatra, 2020). Food insecurity can also be caused by conflict, terrorism, drought, pests, and many diseases (Alpas & Kiyamaz, 2011). In addition, this situation threatens not only individual welfare but also social welfare. The Food and Agriculture Organization of the United Nations has reported that about 820 million people suffer from chronic hunger and malnutrition (Ashagidigbi et al., 2022). The global food crisis is an urgent situation that requires comprehensive strategies. In response to this problem, it is important to address issues comprehensively to ensure food security and to support small-scale farmers by investing in sustainable agricultural practices (Himmelgreen et al., 2009).

The Relationship between Population Growth and Food Problems

As the population increases, so does the demand for food. Producing more food to meet the growing demand puts pressure on agriculture. However, dwindling water resources, changes in vegetation due to climate change, and limited arable land make food supply difficult (S. Wang et al., 2022). Population growth is not only a matter of quantity but also of resource distribution and inequality in access to food. Fresh and nutritious food suffers the most from this situation. Global warming in particular poses challenges to agriculture around the world. Rising

temperatures, changes in rainfall patterns, and extreme weather events such as droughts and floods can disrupt crop yields. The situation is worsening, especially in places such as Africa, where the food problem is most acute (Bibi & Rahman, 2023). The adoption of climate-smart agricultural systems that are integrated with climate change adaptation and mitigation strategies is recommended as a solution. In addition, the expansion of alternative food sources can also be a solution to the problem (Sniatala et al., 2023). Increasing the consumption of legume, insect, and algae-based proteins can also reduce the pressure on livestock and meet the needs of the population (Fatima et al., 2023). In short, to tackle the food crisis, a multifaceted approach should be adopted that addresses issues such as food scarcity in crowded cities, micronutrient scarcity, climate change, and population growth together (Dwivedi et al., 2023).

Exploring the Crisis: Food Scarcity in Crowded Cities

As urbanization increases, the demand for food also increases (S. Li et al., 2023). However, the scarcity of agricultural land in urban areas creates difficulties in terms of food production (X. Wang, Wang, et al., 2023). Add logistics and distribution to this situation and the situation becomes even more difficult (Kazancoglu et al., 2023). Scarcity affects not only the food security of urban populations but also social order and economic development. In response to this problem, strategies that promote sustainable urban agriculture are gaining momentum. This aims to increase local food production and reduce dependence on long-distance transportation of food (Shariatmadary et al., 2023). It is becoming attractive to turn to alternative non-agricultural foods. Algae is one of the most important food sources in this regard (Ahmad & Ashraf, 2023). For agriculture, the promotion of vertical, hydroponic, and aquaponic farming can help to maximize food production in urban areas (Oh & Lu, 2023).

Micronutrient Scarcity: A Hidden Aspect of Food Insecurity

Micronutrient scarcity is a hidden aspect of food insecurity that often goes

unnoticed but has significant impacts on the health and well-being of populations (Saquee et al., 2023). Micronutrients are essential vitamins and minerals required in small amounts for proper body function, growth, and development (Khader, 2021). However, many people worldwide suffer from micronutrient deficiencies, which can lead to serious health problems such as stunted growth, weakened immune systems, and impaired cognitive development (X. Wang, Dou, et al., 2023). To address micronutrient shortages, it is crucial to promote diverse and nutrient-rich diets, fortify staple foods with essential micronutrients, improve access to health care and nutrition education, and implement sustainable agricultural practices that prioritize nutrient-rich crops (Malézieux et al., 2023). In recent years, there has been increasing interest and innovation in alternative foods as a potential solution to problems such as hunger, population growth, food shortages in crowded cities, and the negative impact of climate change on agriculture (Barrett, 2021). One of these alternatives is non-agricultural foods produced using methods that do not require traditional agricultural practices (Nyyssölä et al., 2022). These foods can be derived from a variety of non-conventional sources such as insects, algae, and plant-based proteins (X. Zhang et al., 2024). These alternative food sources have the potential to provide nutritious and sustainable options to address food scarcity and reduce the environmental impact of conventional agriculture (Tal, 2018). Furthermore, non-farm foods can be grown in smaller areas and are less dependent on specific climatic conditions, making them accessible to communities in urban areas or regions with limited agricultural land (Rathi, 2022).

1.3 Whole Wheat Bread and Gluten Intolerance

Food accessibility refers to the availability and affordability of safe and nutritious food for every individual in each community (Murrell & Jones, 2020). It is also the physical and economic availability of recommended and needed food items by consumption standards (Chen & Antonelli, 2020). This can be hindered by many factors such as income level, transportation, and geographical location (Negi &

Trivedi, 2021). Individuals who cannot access recommended and essential healthy foods turn to different alternatives. These individuals often seek foods that are high in calories, high in saturated fat, sodium and added sugar (Popkin & Ng, 2022). Areas with this convenience can be called food swamps and include fast-food restaurants, grocery stores, and establishments that serve unhealthy/processed foods (Smets et al., 2022). Unfortunately, the ease of access to unhealthy foods is also leading to an increase in diet-related diseases (Verde et al., 2023). Obesity, diabetes, and cardiovascular disease are the most prominent problems in the relationship between diet and disease (La Sala & Pontiroli, 2020). One proposed solution to the problem is to increase the availability of healthy alternative foods. One of the current approaches is to enrich bread, which is one of the basic foods accessible to everyone, and to consume whole wheat bread (Tebben et al., 2018). In recent years, demand for high-fiber whole wheat bread has increased as consumers have become more health-conscious (Collar, 2008). Whole wheat bread is healthier than white bread because it contains whole wheat grain with nutrient-rich bran and germ, and provides benefits with its high fiber content, soluble vitamin and mineral content, phytochemicals, and essential amino acid profile (Slavin, 2000). In addition, whole wheat bread helps with weight management by providing a longer-lasting feeling of satiety compared to refined bread (Bonnema & Slavin, 2014). Also, whole wheat bread has a lower glycemic index than refined bread, meaning it causes a slower and more gradual rise in blood sugar levels (Koksel et al., 2023). In general, wheat varieties differ in terms of gluten level, taste, texture, and protein content (Mastromatteo et al., 2014). Gluten has attracted attention in recent years with the increase in intolerant individuals (Roszkowska et al., 2019). Individuals with gluten intolerance need to consume gluten-free bread. These individuals cannot digest and process gluten, a protein found in wheat, barley, and rye (Demirkesen & Ozkaya, 2022). Three different conditions can occur as a result: Celiac disease, non-celiac gluten sensitivity, and wheat allergy (Elli et al., 2015). Gluten intolerance causes bloating, diarrhea, indigestion, skin flaking, itching, and many other digestive problems in people who

consume gluten. The increase and seriousness of the condition have led to the development of gluten-free bread and increased demand for the product (Bozoglu, 2012).

1.4 Antarctic Microalgae

Microalgae are photosynthetic, filamentous, and colony-forming, with cell sizes ranging from 1-100 microns, and exhibit remarkable physiological adaptations that enable them to survive and thrive in various ecological niches including freshwater, marine environments, and even extreme conditions such as hypersaline lakes and Antarctica (Queiroz et al., 2020). Microalgae have developed adaptive strategies to cope with changes in their chemical and physical environment (Kholssi et al., 2023). These strategies include the synthesis of a wide range of compounds through different metabolic pathways, such as pigments, lipids, proteins, and polysaccharides (Dolganyuk et al., 2020). In addition, microalgae have developed morphological adaptations such as cell wall structures and pigment compositions that contribute to their classification within different taxonomic groups (González-Hourcade et al., 2023).

Microalgae are a vital part of the food chain in aquatic ecosystems, serving as primary producers and providing food for a variety of organisms. Microalgae are known as versatile cell factories. They are also benthic or planktonic, floating in water (W. Zhang et al., 2020). They contribute to the production of oxygen, and the removal of carbon dioxide from the atmosphere and play an important role in nutrient cycling (Cheng et al., 2006). Microalgae are also known for their ability to produce a wide range of bioactive compounds, including chlorophyll and pigments such as carotenoids, lipids, proteins, and polysaccharides (de Vera et al., 2018). Besides their ecological importance, microalgae are of great interest due to their potential biotechnological applications (Odjadjare et al., 2017). Some examples of these applications include their use as biofuels, food supplements, pharmaceuticals, and aquaculture (Ansari et al., 2021). Microalgae also can

remove pollutants and nutrients from water, making them useful in wastewater treatment and bioremediation (S. F. Ahmed et al., 2022). Overall, microalgae are a fascinating group of organisms with the ability to thrive in a variety of habitats and have enormous potential for ecological and economic purposes (Renuka et al., 2018).

With their high adaptation capabilities, microalgae serve as a fundamental food source in most ecosystems in different geographies including Antarctica (Lyon & Mock, 2014). Microalgae in Antarctica have adapted to survive and thrive in extremely cold temperatures, high salinity, low carbon-nitrogen availability, and low light conditions found in the region (Teoh et al., 2013a). These adaptations include the production of antifreeze proteins to prevent ice formation, the ability to regulate their metabolic processes in response to changing environmental conditions, and the production of UV-absorbing compounds to protect against high levels of ultraviolet radiation (Montuori et al., 2023). Antarctic microalgae have also been found to produce unique bioactive compounds with potential industrial and pharmaceutical applications. This allows Antarctic microalgae to overcome their harsh environment and continue to photosynthesize, grow, and reproduce (Lauritano & Coppola, 2023). In addition to their adaptations for survival, Antarctic microalgae play a crucial role in the Antarctic ecosystem. They form the base of the food chain, providing food and energy for higher trophic levels such as krill, fish, birds, and marine mammals (Hay & Kubanek, 2002).

1.5 Microalgae as Food

Microalgae have been part of the human diet for thousands of years. The earliest use as food dates back 14,000 years ago in Chile (Villarruel-López et al., 2017). In the last few centuries, many written documents have been found proving its use as a food source. Documents have also been found showing that the Aztecs and Mesoamericans used *Spirulina* collected from Lake Texcoco (De Obeso Fernandez Del Valle & Scheckhuber, 2021). *Nostoc* and *Chlorella* were used to survive during the famine in China 2000 years ago (Han et al., 2013). Although the use of

microalgae as food goes back a long way, biotechnological developments began in the 1940s. In 1952, the Algae Culture Symposium organized at Stanford University was the first of its kind and provided momentum to the field. In 1974, *Spirulina* was introduced by the UN as a good food for the future (Khatoon & Pal, 2015). Much work was then done on the use of microalgae as food. Dihe, a cake containing algae, was widely used in Africa (Gohara-Beirigo et al., 2022a). Several microalgae are commonly consumed as tablets, powders, capsules, and additives to foods (Torky et al., 2023). In the USA, the concept of Generally Recognized as Safe (GRAS) comes to the fore. GRAS is the status given to any chemical or substance, including all organisms, that is considered safe for human consumption. There are two main criteria for GRAS status: documented consumption by humans over many years and scientific evidence that the proposed substance is safe (Burdock & Carabin, 2004). Some of the microalgae designated GRAS by the FDA are *Arthrospira platensis*, *Chlamydomonas reinhardtii*, *Auxenochlorella protothecoides*, *Chlorella vulgaris*, *Dunaliella bardawil* and *Euglena gracilis* (Torres-Tiji et al., 2020a). The GRAS concept applies to the USA and other countries have their regulations. Microalgae considered safe according to the European Food Safety Authority (EFSA) of the European Union are: *Dunaliella salina*; *Auxenochlorella protothecoides*, *Auxenochlorella pyrenoidosa*, *Chlorella luteoviridis*, *Chlorella pyrenoidosa*, *Chlorella sorokiniana*, *Chlorella vulgaris*, *Graesiella emersonii*, *Haematococcus pluvialis*, *Heterochlorella luteoviridis*, *Nannochloropsis oculata*, *Odontella aurita*, *Parachlorella kessleri*, *Scenedesmus vacuolatus*, and *Odontella aurita* (Cruz & Vasconcelos, 2023). Several factors contribute to the increasing popularity of microalgae as a food source (Matos, 2017). Interest in microalgae has increased in recent years because of the need for additional food sources, among others, and has contributed to rebalancing the interaction between environmental, human food, and animal feed sustainability (Viegas et al., 2021). Moreover, microalgae offer a valuable source of various nutritional compounds with high added value, such as lipids, proteins, pigments, antioxidants, vitamins, and minerals. These

compounds have been found to have many benefits for human and animal health, including antioxidant, antibacterial, antiviral, and anti-inflammatory activities (Nasser et al., 2018). Microalgae food products have been predominantly marketed as dietary supplements, but there is now a growing trend towards the inclusion of microalgae in a variety of food products such as pasta, snack foods, candy bars or chewing gum and beverages (Laamanen et al., 2021).

1.6 Value-Added Nutrients of Microalgae

Essential amino acids, essential fatty acids, phytosterols, phytosterols, prebiotics, polyphenols, carotenoids, and vitamins cannot be synthesized by the human body, so they must be obtained from both traditional (fruits and vegetables) and non-traditional foods such as microalgae. Microalgae are characterized by both high productivity and rich nutrient content compared to traditional terrestrial food sources (Di Lena et al., 2020).

The protein content of microalgae is of high potential for humans when compared in terms of protein digestibility and biological value (de Carvalho Carneiro et al., 2019). In particular, the diversity of amino acid content, which has almost all amino acids, and the ratio of amino acids gives a favorable result compared to reference foods (León-Vaz et al., 2023). This makes microalgae a high-quality source of protein. Microalgae are also an important source of carbohydrates in terms of sugar, cellulose, starch, and other polysaccharides (de Carvalho Silvello et al., 2022). The availability of digestible carbohydrates also ensures low limitations in use and applications (Olguín et al., 2022). The lipid content of microalgae can range from 25% to 40%, depending on the growth condition, and can be up to 85% of the dry biomass (Olguín et al., 2022). The lipid profile is characterized by essential unsaturated acids such as linoleic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (Boelen et al., 2013). It has a higher fat concentration than fish oil, less contamination, and higher purity after extraction (Santigosa et al., 2021). Microalgae are also a source of almost all vitamins (Tandon et al., 2017). This increases the nutritional value of their biomass.

Exposure to high oxygen stress has enabled microalgae to develop more effective protection systems against radical oxidizers (Ugya et al., 2020). These include pigments such as carotene, phycobiliprotein, and chlorophyll. This makes microalgae stand out with their antioxidant properties (Mulders et al., 2014).

Microalgae as a Protein Source

Protein is very important for human nutrition. People meet their protein needs with the help of many foods. The intake of essential amino acids that the human body cannot synthesize is also important (G. Wu, 2009a). However, the scarcity in protein supply because of population growth has triggered the search for new sources. Protein-energy malnutrition, one of the most important drivers of global hunger, is on the rise and the need for single-cell proteins (SCP) to solve this problem is increasing (X. Zhang et al., 2022). Microalgae, macroalgae, fungi, bacteria, and yeast are important for SCP. Microalgae are particularly preferred with their high and rich amino acid profile and low nucleic acid content. Microalgal protein has attracted attention as a sustainable source with potential health benefits (Bratosin et al., 2021).

Some species can contain up to 50-60% protein on a dry basis (Qazi, Ballance, Kousoulaki, et al., 2021). Microalgae protein is rich in essential amino acids (Andreeva et al., 2021). Research shows that microalgae proteins have favorable amino acid profiles that are like or even exceed those of other high-quality plant proteins. For example, it has a composition comparable to other traditional protein sources such as soybeans and eggs (Acquah et al., 2020). On the other hand, ribulose biphosphate carboxylase-oxygen (Rubisco), which is vital for photosynthetic organisms, can also be a source of dietary protein (Grácio et al., 2023).

Microalgal proteins exhibit many bioactivities besides their nutritional properties. Antioxidant, anti-inflammatory, anticarcinogenic, antitumor, and antimicrobial are some of them (Sheih et al., 2009). This group, called bioactive peptides, has many properties. Their amino acid sequence and chain lengths are the determining factors here. More research is needed to fully characterize them to determine their

nutritional value and amino acid availability (R. Kumar et al., 2022). This characterization is necessary to understand their potential as a new protein source and to ensure their suitability for human consumption. The use of microalgae as an alternative protein source is also of interest due to the current global scenario where sustainable and environmentally friendly protein sources are needed. Furthermore, the use of microalgae as a protein source is valuable as it has the potential for many by-products (Kusmayadi et al., 2021).

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1.6.2 Microalgae Proteins: Challenges and Handicaps

Although microalgae protein has great potential for human consumption, it has several challenges and handicaps. Chief among these is the high cost of large-scale production of microalgae protein. The production process requires significant infrastructure and resources to grow and extract large quantities of microalgae (Soto-Sierra et al., 2018). Another challenge is the variability in protein content among different microalgae species and strains (Bleakley & Hayes, 2017). This variability makes it difficult to standardize and optimize the production process. Furthermore, extraction of microalgae protein can be difficult due to the presence of rigid cell walls and complex cellular structures (Machado et al., 2022). These factors can hinder efficient protein extraction and limit the overall yield. Furthermore, the scalability of microalgae protein production is another barrier. While small-scale production of microalgae protein has been successful, scaling up the process to meet the demands of a growing population poses significant challenges (Villaró et al., 2023). Researchers are constantly exploring ways to address these challenges and overcome the handicaps associated with microalgae protein development. One potential solution to the high cost of large-scale production is the application of improved cultivation and harvesting

methods. By optimizing the cultivation process and developing efficient harvesting techniques, researchers aim to reduce microalgae protein production (Khan et al., 2018). Another approach to combat variability in protein content among different microalgae species and strains is genetic manipulation and strain selection (Sproles et al., 2021). By identifying and altering specific genetic traits responsible for protein content, researchers can work to standardize and optimize the production of microalgae proteins across different species. Furthermore, the scalability of microalgae protein production is also being addressed through the development of integrated biorefinery systems. These systems aim to maximize the utilization of microalgae biomass by co-producing value-added products alongside protein extraction. Researchers are working towards a more holistic and sustainable approach to microalgae biorefinery, integrating multiple processes such as lipid extraction and biofuel production (Slegers et al., 2020). Overall, while challenges exist, ongoing research and technological advances offer promising solutions to advance microalgae protein production toward commercial viability. As the field continues to evolve, the future of microalgae protein as a sustainable and high-quality dietary protein looks bright.

1.6.3 Prospects of Microalgae Protein

The prospects of microalgae protein are promising, as ongoing research and advances in technology continue to address the challenges and handicaps associated with its production (Bhattacharya & Goswami, 2020). By optimizing cultivation and harvesting methods, improving genetic manipulation techniques, and developing innovative extraction technologies, researchers are working to reduce production costs, increase protein yields, and improve the overall efficiency of microalgae protein production (Amorim et al., 2021). These developments will not only make microalgae protein a more economically viable and sustainable alternative to traditional protein sources such as fishmeal and soybeans but will also help meet the growing demand for high-quality and environmentally friendly protein sources (Bhatnagar et al., 2024). Furthermore,

the unique properties of microalgae protein make it an attractive option for a variety of applications.

For example, microalgae protein could be incorporated into functional food products due to its high nutritional value and potential health benefits (Caporgno & Mathys, 2018). Furthermore, microalgae protein has the potential to be used in the development of animal feed formulations, reducing dependence on traditional feed sources, and contributing to a more sustainable livestock industry (Madeira et al., 2017). The future of microalgae protein has great potential in addressing food and protein scarcity issues by providing a sustainable and environmentally friendly solution to meet the growing global demand for protein (Kim et al., 2019). In conclusion, microalgae protein is an emerging and promising source of sustainable dietary protein. Given the ongoing research and advances in technology, microalgae protein holds great promise as a future protein source. Thereby, microalgae protein has the potential to be an important and sustainable alternative to traditional protein sources and has the potential to revolutionize the protein industry by providing a sustainable and environmentally friendly solution to meet the growing global protein demand (Ahmad & Ashraf, 2023).

Antarctic microalgae play a crucial role in the polar ecosystem and studying their protein profile is essential to understanding their unique adaptations and potential applications in various fields, including biotechnology, food production, and pharmaceuticals. By analyzing the protein profile of Antarctic microalgae, scientists can gain insight into the molecular mechanisms that allow these organisms to survive extreme environments such as low temperature, high salinity, and limited sunlight. This information could also help identify specific proteins with valuable properties, such as antioxidant or anti-inflammatory effects, and could be used in the development of new therapies and products that benefit human health and well-being (Pihlanto et al., 2017). In conclusion, studying the protein profile of Antarctic microalgae is important for understanding their adaptations, discovering valuable properties that can benefit human health and well-being, and exploring their potential as sustainable sources of nutrition and

biofuel (Biomolecular et al., 2022).

1.7 Microalgal Carbohydrates

Microalgae are organisms that can produce carbohydrates as a major component. Carbohydrates in microalgae cells can be both structural and storage compounds. The high carbohydrate profile of microalgae is one of the aspects of interest. For example, beta-glucan is in demand in the market with the potential for many biotechnological uses. *Chlorella vulgaris* and *Desmodesmus* sp. are particularly prominent in terms of carbohydrates (Ferreira et al., 2021). The adaptability of microalgae carbohydrates, whose profile can change according to different growth conditions, is also an important parameter in terms of biotechnological application potential. In summary, the rich carbohydrate profile of microalgae offers rich opportunities for various industrial and biotechnological applications, and further research on the cultivation and utilization of microalgal carbohydrates is warranted.

1.7.1 Microalgae Carbohydrates for Human Consumption

Microalgal carbohydrates are used to produce several compounds such as hydrocolloid, agar, alginate, carrageenan, and fructan (Mišurcová et al., 2012). These carbohydrates offer functional properties such as thickening, gelling, and stabilizing, making them suitable for use in a wide range of food applications (Ravindran & Rajauria, 2021). In addition, microalgal carbohydrates offer potential health benefits as they are low in calories, high in dietary fiber, and contain bioactive compounds with antioxidant and immune-boosting properties (Barros de Medeiros et al., 2022). Microalgal carbohydrates have also been found to have a low glycemic index, making them suitable for individuals with diabetes or those following a low-glycemic diet (Khemiri et al., 2022). The incorporation of microalgal carbohydrates into functional beverages, food products, and animal feeds has shown promising results (Camacho et al., 2019). Therefore, the use of microalgae carbohydrates in food products and animal feeds has the potential to

improve nutritional value, enhance functional properties, and promote overall health and well-being (Nagarajan et al., 2021).

Consequently, the use of microalgal carbohydrates in human consumption has the potential to provide nutritional value, and functional benefits, and enhance health and well-being. Microalgal carbohydrates offer a sustainable and innovative solution to meet the growing demand for food products with enhanced nutritional profiles and functional properties. Overall, microalgal carbohydrates have the potential to revolutionize the food industry by providing a sustainable and nutritious alternative to traditional carbohydrate sources. The use of microalgae carbohydrates in human consumption has the potential to revolutionize the food industry by providing a sustainable and nutritious alternative to traditional carbohydrate sources, while also having the potential to address the limitations and challenges faced by agriculture, pharmaceuticals, nutrition, and other industries in developing solutions that meet the needs of the human body. It can contribute to the development of new functional foods and nutraceuticals that promote health and well-being (Shahid et al., 2020).

The potential applications of microalgal carbohydrates are diverse and promising. They can be used in functional foods and beverages, animal feed, and even in the production of renewable biochemicals and biofuels (Markou et al., 2012). As a result, the development of microalgae carbohydrates presents both opportunities and challenges. Researchers are actively working to optimize cultivation conditions, employ genetic and metabolic engineering strategies, and take advantage of advanced extraction techniques to increase the carbohydrate content of microalgae and overcome the challenges associated with their development.

The future of microalgae carbohydrates holds great promise and potential. The growing demand for sustainable and renewable sources of food, feed, biofuels, pharmaceuticals, bioplastics, and food additives presents a significant opportunity for the development of microalgae carbohydrates (Debnath et al., 2021). Moreover, advances in genetic and metabolic engineering techniques will enable

the manipulation of microalgae to produce high amounts of carbohydrates with desired properties (Ran et al., 2019). In addition, the integration of process intensification strategies and downstream processing technologies will improve the efficiency and cost-effectiveness of microalgae carbohydrate production. Furthermore, the search for new microalgae species with higher carbohydrate content and improved cultivation strategies will contribute to the expansion of the microalgae carbohydrate industry (Cea-Barcia et al., 2014). Finally, the use of microalgae in wastewater treatment and nutrient recovery processes contributes to the sustainability of microalgae carbohydrate production. Overall, the prospects of microalgae carbohydrates are promising, with opportunities for innovation, collaboration, and sustainable development in various industries.

1.7.2 Carbohydrate Profile of Antarctic Microalgae

Antarctic microalgae exhibit a diverse and intriguing carbohydrate profile. These microalgae species have adapted to extreme cold and harsh environmental conditions, leading to unique carbohydrate compositions (Morales-Sánchez et al., 2020). Their carbohydrate profiles are mainly composed of polysaccharides such as cellulose, hemicellulose, and various types of algal polysaccharides. These carbohydrates contribute to the structural integrity and energy storage of Antarctic microalgae. In addition, Antarctic microalgae have been found to have high levels of storage carbohydrates, particularly in the form of starch and glycogen, which serve as energy reserves for survival during prolonged periods of low light and nutrient availability. The carbohydrate profile of Antarctic microalgae also includes unique sugars and sugar derivatives such as xylenes, mannans, fucans, and sulfated polysaccharides (İnan et al., 2023). These carbohydrates have been found to possess various bioactive properties, including antioxidant, anti-inflammatory and anticoagulant activities. They have potential for applications in the pharmaceutical, nutraceutical, and cosmetic industries (Mehariya et al., 2021).

1.8 Microalgal Lipids

Microalgal lipids are of great interest due to their potential use as a sustainable and environmentally friendly source of renewable energy and valuable compounds. The lipid content of microalgae can vary depending on the species and growing conditions (Bellou et al., 2014). Several factors affect the lipid content of microalgae, including nutrient availability, light intensity, temperature, and stress conditions. One of the most attractive components of microalgae is the ability to produce biofuels from their lipids (Rehman et al., 2022). Lipid production by microalgae is influenced by several factors such as the cost of reactor design, nutrient substrates, scalability, and energy demands. In addition, the cultivation of microalgae has attracted interest due to the ability to synthesize biologically active substances, rapid biomass growth, and the capacity to adjust their biochemical composition according to cultivation conditions (Moreno-Garcia et al., 2019). Compared to heterotrophic microorganisms, unicellular photosynthetic microalgae have the unique ability to produce biomass from inorganic substances and mineral elements by utilizing light energy during photosynthesis (K. Zhang et al., 2014).

Fatty acids are the building blocks of lipids and play a vital role in determining the nutritional and functional properties of extracted lipids. Different microalgae species exhibit diverse fatty acid compositions and specific fatty acids are of particular interest due to their health benefits and industrial applications (Maltsev et al., 2021). Common fatty acids found in microalgal lipids include palmitic acid, oleic acid, linoleic acid, and α -linolenic acid (Arutselvan et al., 2021). These fatty acids contribute to the sensory properties, oxidative stability, and nutritional value of the extracted lipids (Demets et al., 2021). Furthermore, the fatty acid profile of microalgae can influence the quality of biodiesel derived from their lipids (Deshmukh et al., 2019). While high concentrations of saturated or monounsaturated fatty acids are desirable for biodiesel production due to their favorable fuel properties, the presence of polyunsaturated fatty acids can negatively affect the oxidative stability and cold flow properties of biodiesel

(Kateryna Maltseva et al., 2021). Therefore, understanding the fatty acid profile of microalgae is essential to optimize lipid extraction and lipid utilization processes for various industrial applications, including biodiesel production.

Considered an environmentally friendly alternative, microalgae biomass production technologies play a vital role in reducing environmental pollution, utilizing carbon dioxide, releasing oxygen, and conserving water resources. Microalgae, which can be prokaryotic (e.g. cyanobacteria) or eukaryotic (e.g. green algae), act as efficient converters of solar energy into biomass (Ghayal & Pandya, 2013). As fossil fuel depletion continues to impact the environment, there is a growing emphasis on biofuels as a renewable, non-toxic, and environmentally friendly alternative. This approach aims to reduce dependence on fossil fuels and mitigate the environmental impacts resulting from their use. Potential products derived from microalgae, including biodiesel, hydrogen, biogas, synthesis gas, pigments, and fatty acids, contribute to the diverse applications of microalgae cultivation.

1.8.1 Microalgal Lipids for Human Consumption

Microalgae have shown great potential in lipid production as they can produce lipids much faster compared to traditional oil crops such as soybeans. In addition, microalgae have a short growth and cultivation cycle of only 15 days, which is much faster than the annual cycle of soybeans (Taelman et al., 2015). Microalgae are not only valuable for biofuel production but also have potential applications in other industries such as agriculture (Ganesan et al., 2020). For example, polyunsaturated fatty acids found in microalgae lipids are essential nutrients for aquatic animals and humans (Remize et al., 2021). Furthermore, recent advances in the cultivation of microalgae using heterotrophic methods show promise for large-scale oil production (Morales-Sánchez et al., 2017). These advances in microalgae lipid production open new possibilities for the use of microalgae lipids as dietary supplements for human consumption. With their high lipid content and potential to produce polyunsaturated fatty acids, microalgae lipids have the

potential to be a valuable source of health benefits and nutrients for human consumption (Bélignon et al., 2016). As a result, microalgae lipids hold great promise for various applications, including biofuel production and nutritional supplementation for human consumption. Their ability to produce high-value compounds such as polyunsaturated fatty acids, antioxidants, and natural lipids makes them a valuable resource for various industries, including nutraceuticals, pharmaceuticals, and cosmetics (Banskota et al., 2019). Furthermore, the sustainable and environmentally friendly nature of microalgae cultivation makes them an attractive alternative to traditional lipid sources (Sati et al., 2019). Research and development efforts are focused on overcoming challenges related to microalgae lipid production, such as optimizing cultivation strategies, improving lipid extraction techniques, and scaling up production processes (Singh et al., 2016). Once these challenges are overcome, microalgae lipids have the potential to become a major player in the global lipid market, offering a sustainable and renewable source of high-quality lipids with diverse applications in various industries (Carvalho et al., 2022). As a result, the use of microalgae for lipid production offers numerous advantages, including fast growth rates, high oil yields, and the ability to thrive in a variety of environments.

1.8.2 Lipid Profile of Antarctic Microalgae

Antarctic microalgae have unique adaptations to survive in extreme cold environments (Margesin et al., 2008). One of these adaptations is the accumulation of polyunsaturated fatty acids, which help increase membrane fluidity and protect against ice crystal growth. These polyunsaturated fatty acids, such as omega-3 and omega-6, have important health benefits and are essential for human nutrition (Ramesh Kumar et al., 2019). It has been found to have a high content of eicosapentaenoic acid and docosahexaenoic acid, which are highly valued for their anti-inflammatory and cardiovascular health benefits. In addition, Antarctic microalgae were found to accumulate significant amounts of phytol, a compound with potential applications in biopharmaceutical production due to its

antioxidant properties (X. Li et al., 2019). These unique adaptations and lipid profiles make Antarctic microalgae a valuable resource for producing high-quality lipids with diverse applications in the nutraceutical industry (Udayan et al., 2022). Although microalgae lipid production has enormous potential, there are still many challenges that need to be addressed. These include developing efficient and cost-effective cultivation systems for Antarctic microalgae, optimizing lipid extraction methods to ensure maximum lipid recovery, and increasing lipid productivity (Park et al., 2019).

1.9 Microalgae Mineral Content

Microalgae also contain a wide variety of minerals (Dolganyuk et al., 2020). These minerals play important roles in various biological processes and are essential for the growth and development of microalgae (Fox & Zimba, 2018). Microalgae can be a valuable source of these essential minerals due to their ability to bioaccumulate and bind heavy metals (Arunakumara & Zhang, 2007). Through their natural growth processes, microalgae can absorb and accumulate minerals from their surrounding environment. These minerals are then stored in the cells of microalgae, making them easily accessible for human consumption (Barbera et al., 2017). Consuming microalgae rich in essential minerals can increase the nutritional value of different foods. In addition, studies have shown that microalgae can be used to replace common ingredients such as sodium chloride (salt) in food products (Bernaerts et al., 2019). This can help reduce sodium intake while still providing essential minerals. This high mineral content makes them a valuable source of essential minerals for human consumption (Rzymiski et al., 2019). However, it is important to note that the bioavailability of these minerals in microalgae may vary depending on the food matrix and their interactions with other compounds. Therefore, further research is needed to determine the bioaccessibility and bioavailability of microalgae minerals for optimal physiological functions in humans (Uribe-Wandurraga, Igual, et al., 2020).

The mineral content of microalgae can vary depending on the species and

growing conditions (Santhakumaran et al., 2020). Some minerals in microalgae include iron, magnesium, calcium, potassium, and phosphorus (Fithriani & Melanie, 2022). These minerals are important for microalgae and have potential health benefits for humans (Gharibzahedi & Jafari, 2017a). Microalgae can also accumulate some trace minerals such as selenium and zinc, which have been shown to have antioxidant and immune-boosting properties. (Mazo et al., 2007) Several factors, including the nutrient composition of the growth environment, light intensity, and pH levels influence the mineral content of microalgae. Therefore, understanding the mineral content of microalgae is crucial for their utilization in various industries such as nutrition, pharmaceuticals, and cosmetics (Mobin et al., 2019).

1.10 Microalgae Vitamin Content

Microalgae are an excellent source of natural vitamins, offering a wide range of essential nutrients to support human health (Sathasivam et al., 2019). Microalgal vitamins have been shown to have important roles in human metabolism and health maintenance, including regulation of cell growth and differentiation, immune function, and protection against oxidative stress (Del Mondo et al., 2020). They contain a wide range of water-soluble and fat-soluble vitamins, making them a valuable source of essential nutrients (Hagan & Anyangwe, 2023). The high vitamin content of microalgae, including vitamins A, B-complex, C, and E, makes them a valuable resource for enhancing their nutritional value and potential health benefits in various industries such as nutraceutical, food, and pharmaceutical industries (De Roeck-Holtzhauer et al., 1991). They contain important vitamins such as vitamin A, which plays a critical role in vision and immune function, as well as various forms of the vitamin B complex, which is essential for energy production, brain function, and metabolism. Microalgae are also rich in vitamin C, a powerful antioxidant that helps protect against oxidative stress and boost immune function. Microalgae also contain vitamin E, a powerful antioxidant that plays a role in protecting cells from damage and supporting cardiovascular health. The high vitamin content of microalgae makes them a valuable resource for

enhancing their nutritional value and potential health benefits in various applications (Loy & Chu, n.d.). The small size of microalgae, their high growth rate, and their ability to produce bioactive compounds make them a valuable resource for enhancing their nutritional value and potential health benefits in various industries such as food, nutrition, pharmaceuticals, and cosmetics by exploiting their biotechnological advantages through methods for cultivation and isolation of microalgae vitamins. Furthermore, advances in technology, such as stable isotope dilution analysis using LC-MS/MS, have enabled accurate quantification of vitamin content in microalgae (Pantami et al., 2020). To fully exploit the potential of microalgae as a source of microalgal vitamins for human consumption, it is crucial to continue research and development in the areas of cultivation techniques, extraction methods, and processing technologies to guarantee high quality and bioavailability. Microalgae have emerged as a promising and sustainable source of microalgal vitamins for human consumption (Chacón-Lee & González-Mariño, 2010). In conclusion, microalgae are a promising and sustainable source of microalgal vitamins for human consumption.

1.11 Secondary Metabolites of Microalgae

Microalgae are single-celled photosynthetic organisms that play an important role in various biotechnological applications. These applications include biofuel production, food and feed supplements, pharmaceutical products, cosmetics, and environmental remediation (Orejuela-Escobar et al., 2021). Metabolites produced by microalgae are of particular interest in these applications. These metabolites include bioactive compounds with potential therapeutic properties such as anti-inflammatory, antimicrobial, and antioxidant activities (Pagels et al., 2022). Moreover, metabolites derived from microalgae show promising results in improving human health and reducing the risk of degenerative diseases (De Morais et al., 2015). Secondary metabolites from microalgae have great potential for the development of new pharmaceutical and nutraceutical products (Hassan et al., 2022). Research and exploration of secondary metabolites from microalgae

open new opportunities for the development of innovative pharmaceutical and nutraceutical products due to their unique properties and potential therapeutic applications.

1.11.1 Microalgal Chlorophylls and Carotenoids

Microalgae are microscopic organisms that play an important role in various ecosystems. They are primary producers, converting sunlight, water, and carbon dioxide into organic matter through the process of photosynthesis. One of the key components involved in photosynthesis is chlorophyll, a pigment that gives microalgae their green color. Chlorophyll is responsible for capturing light energy and converting it into chemical energy, which allows microalgae to grow and develop (J. C. da Silva & Lombardi, 2020). Microalgal chlorophyll is a good food known as a detoxifying agent (Piotrowska-Niczyporuk et al., 2017). Research shows that the consumption of microalgal chlorophyll can positively affect human health. These potential health effects include antioxidant properties, detoxification support, anti-inflammatory effects, antigenotoxic, antimutagenic, and potential cancer-fighting abilities (da Silva Ferreira & Sant'Anna, 2017a). Its consumption has been reported to improve the liver by increasing bile secretion (Bedirli et al., 2009). It has been found to regulate carbohydrate, protein, and lipid metabolism in humans (Ramos-Romero et al., 2021). It has been stated that the addition of easily absorbable and water-soluble chlorophyll (CHL) to the diet has anticarcinogenic benefits (Hamouda et al., 2022). Another study with CHL determined that it had a suppressive effect on the progression of lung cancer in mice (D. Zhong et al., 2020).

Microalgae are a rich source of carotenoids, and pigments with many health benefits and commercial applications. Carotenoids play a vital role in microalgae, contributing to their coloration and protecting them against harmful environmental conditions such as excessive light and oxidative stress (X. M. Sun et al., 2018). Carotenoids also play an important role in the coloration of microalgae and their protection against environmental stress (Henríquez et al., 2016). By determining

the carotenoid content in microalgae, researchers can better understand the nutritional value and potential applications of these organisms. Furthermore, this knowledge can contribute to the development of innovative processes for the large-scale extraction and purification of carotenoids from microalgae, enabling their use in various industries such as food, cosmetics, and pharmaceuticals. The commercial use of microalgae for carotenoid production holds great promise due to their high carotenoid content and various health benefits (Novoveská et al., 2019).

1.11.2 Microalgal Phenolic Compounds

In recent years, there has been increasing interest in the potential health benefits of microalgae due to their high phenolic content. Microalgae play an important role in the phenolic content of various biotechnological, commercial, and industrial sectors (Hajimahmoodi et al., 2010). These phenolic compounds, also known as polyphenols, have been shown to have antioxidant, anti-inflammatory, and anticancer properties (Vieira et al., 2021). Furthermore, phenolic compounds found in microalgae have been linked to cardiovascular and cancer prevention and have shown potential in the prevention of neurodegenerative diseases such as Alzheimer's and Parkinson's (Parameswari. & Lakshmi, 2022). Their diverse bioactivities and potential health benefits make microalgae phenolic compounds an attractive source for the development of nutraceuticals, functional foods, and pharmaceutical products (M. Silva et al., 2022). The abundance and diversity of phenolic compounds in microalgae make them a valuable resource for the development of novel functional food products with enhanced health benefits. Furthermore, studies have shown that different microalgae species exhibit differences in their phenolic content, with some species containing higher concentrations of specific phenolic compounds than others. Overall, the study and utilization of microalgae phenolic content have the potential to revolutionize industries such as pharmaceuticals, nutraceuticals, and functional foods by providing a sustainable and bioactive source of compounds with diverse health

benefits.

1.11.3 Fiber Content of Microalgae

Decoding microalgae fiber content has the potential to unlock new possibilities for developing functional foods with improved digestive health and increased satiety. Incorporating microalgae fiber into food products can contribute to increased dietary fiber intake, which is beneficial in supporting healthy digestion and preventing chronic diseases such as obesity, diabetes, and cardiovascular conditions (De Jesus Raposo et al., 2016). Furthermore, microalgae fiber has unique properties such as high water retention capacity and gel-forming ability, which can improve the texture, mouthfeel, and stability of food products (Uribe-Wandurraga, Zhang, et al., 2020). Furthermore, microalgae fiber contains bioactive compounds such as antioxidants and polysaccharides, which show positive effects on gut microbiota and immune function (Y. Sun et al., 2014). These properties make microalgae fiber a promising ingredient for the development of functional foods aimed at improving digestive health and overall well-being (Zhou et al., 2023). The phycobiliprotein content of microalgae also contributes to their potential health benefits and applications in various industries (Manirafasha et al., 2016). These pigmented proteins have been found to possess antioxidant, anti-inflammatory, and anticancer properties, making them valuable in the development of dietary supplements, pharmaceutical products, and cosmetic formulations (Hlima et al., 2023).

1.11.4 Microalgal Mycosporine-like Amino Acid

Microalgal mycosporine-like amino acid content is an important parameter to study as it can provide insight into the ability of algae to withstand stressful environmental conditions (Raj et al., 2021). These mycosporine-like amino acids act as natural sunscreen compounds, protecting algae from harmful UV radiation (Sen & Mallick, 2021). This content has been observed in several microalgae species and plays an important role in maintaining the osmotic balance of

organisms, especially those involved in photosynthesis. In addition, the mycosporine-like amino acid content from microalgae has also been found to have antioxidant and anti-inflammatory properties, making them potential candidates for the development of natural and sustainable skincare products (Shick & Dunlap, 2003). Analyzing microalgal mycosporine-like amino acid content is essential to understanding the mechanisms of algal adaptation to stressful environmental conditions and their potential applications in industries such as cosmetics, pharmaceuticals, and biotechnology (Pliego-Cortés et al., 2019). The isolation and characterization of mycosporine-like amino acids from marine macroalgae have been investigated, but further research is needed to fully understand the distribution, content, and species of these compounds in different microalgae species (Xiong et al., 1999). By creating a comprehensive database on microalgae mycosporine-like amino acid content, researchers can improve their understanding of the distribution, contents, and types of these compounds in different microalgae species. The database will facilitate further research into the potential applications of microalgal mycosporine-like amino acids in various industries, including cosmetics, pharmaceuticals, and biotechnology (Y. Sun et al., 2020). Furthermore, the presence of mycosporine-like amino acids in microalgae makes it a promising ingredient for food use as these compounds have been linked to UV protection, antioxidant properties, immune-enhancing effects, and potential anti-inflammatory activities.

1.12 Bioactivity Properties of Microalgae

Microalgae are a valuable source of biologically active molecules in cosmetics, pharmaceuticals, energy, agriculture, nutraceuticals, and functional foods (Mimouni et al., 2012). Among these activities, antioxidant, anti-inflammatory, anticancer, antidiabetic, antimicrobial, antibacterial, antiviral, antihypertensive, and antifungal properties are the main ones (Tabarzad et al., 2020a). Bioinformatics has emerged as a vital tool to better understand the structure and activity of bioactive compounds derived from microalgae. By utilizing

bioinformatics, researchers can potentially develop bioactive compounds with enhanced efficacy and reduced negative effects. In addition to bioinformatics, advances in genetically modified techniques for cultivating microalgae have proven to be instrumental in harnessing their potential, especially in utilizing them for bio-based processes in various applications. Continued progress in bioengineering-related research plays an important role in the realization of green technology to produce microalgae-derived bioactive compounds, in line with the growing demand for sustainable and environmentally friendly solutions (Tejano et al., 2019).

The multifaceted potential of microalgae, including their anti-diabetic, antibacterial, antiviral, and antifungal properties, underscores the need for continued research and investigation into their therapeutic applications (Ibrahim et al., 2023). Moreover, the different bioactive compounds found in microalgae position them as a promising source for nutraceutical and pharmaceutical development (Chu & Phang, 2019). Consequently, the evolving landscape of microalgae research provides a compelling opportunity to further investigate their therapeutic potential and develop innovative approaches that can exploit the diverse pharmacological properties of microalgae-based bioactive compounds.

1.12.1 Antioxidant Profile of Microalgae

Microalgae have attracted attention as a potential source of nutraceuticals due to their numerous health benefits (Galasso et al., 2019). This is because microalgae can produce a variety of biomolecules with bioactive properties such as beta carotene, lutein, astaxanthin, chlorophyll, phycobiliprotein, sulfated polysaccharides, phenolics, and polyunsaturated fatty acids (Gouveia et al., 2010). These bioactive compounds found in microalgae have antioxidant properties that play an important role in protecting the body against oxidative stress and damage caused by free radicals (Rezayian et al., 2019). This antioxidant activity helps to neutralize harmful molecules in the body, reducing the risk of chronic diseases such as cardiovascular diseases, cancer, and

neurodegenerative disorders (Khavari et al., 2021). In addition, microalgae contain other antioxidant compounds such as phycobiliproteins and polyunsaturated fatty acids that contribute to their overall antioxidant capacity. Microalgae are also a potential source of bioactive compounds with direct anti-tumor effects (Abd El-Hack et al., 2019). The antioxidant activities of microalgae products, their potential to ameliorate disease progression, and the underlying molecular mechanisms are of interest to the scientific community (Yu et al., 2022). However, more extensive research is needed to better understand the antioxidant effects of microalgae and their applications in disease prevention and treatment (Dixon & Wilken, 2018). Furthermore, microalgae production and the extraction of bioactive compounds from them are ongoing research areas. This research aims to identify and validate the beneficial effects of these bioactive compounds, examine their potential in disease prevention and treatment, and assess their impact on the environment.

1.12.2 Anti-inflammatory Profile of Microalgae

The anti-inflammatory properties of microalgae have been established by many studies, making them a potential source of natural anti-inflammatory agents (Montero-Lobato et al., 2018). These anti-inflammatory properties are attributed to the presence of bioactive compounds such as carotenoids, phycobilins, and sulfated polysaccharides in microalgae. These compounds have shown inhibitory effects on inflammatory mediators and pathways, including the production of pro-inflammatory cytokines and activation of inflammatory signaling pathways (Sibi & Rabina, 2016). Microalgae have also shown the ability to reduce the expression of pro-inflammatory genes and enzymes involved in inflammation. These findings suggest that microalgae have the potential to be used in the development of anti-inflammatory therapeutics and may contribute to the management and prevention of inflammatory diseases.

1.12.3 Anticancer Profile of Microalgae

Microalgae show promising anticancer properties, making them a potential tool for cancer prevention (Martínez Andrade et al., 2018). Studies have demonstrated the ability of microalgae-derived compounds to inhibit tumor growth, induce apoptosis (programmed cell death) in cancer cells, and suppress cancer cell proliferation and metastasis (Alateyah et al., 2022). These anticancer properties are attributed to the rich bioactive compounds found in microalgae, such as phycocyanin, polysaccharides and bioactive peptides. Compounds derived from microalgae have also been shown to have the potential to improve the efficacy of conventional cancer treatments such as chemotherapy and radiation therapy by increasing their sensitivity to treatment and reducing their side effects (Jabeen et al., 2017).

1.12.4 Antimicrobial Profile of Microalgae

Microalgae have been found to possess significant antimicrobial capabilities, making them a potential natural alternative to synthetic antibiotics (Falaise et al., 2016). Bioactive compounds such as peptides and polysaccharides have been shown to show activity against a variety of bacteria and fungi, including antibiotic-resistant strains (Ayswaria et al., 2023). These compounds have the potential to address the growing problem of antibiotic resistance and contribute to the development of new treatments for microbial infections.

1.12.5 Antibacterial Profile of Microalgae

potential candidates for development. Research has shown that some microalgae species produce bioactive compounds with antibacterial activity against a variety of pathogens, including both gram-positive and gram-negative bacteria (Pradhan et al., 2014). These antibacterial compounds have the potential to combat antibiotic-resistant bacteria and provide alternative options for treating bacterial infections (Cepas et al., 2021). Moreover, the antibacterial properties of microalgae can be attributed to the presence of bioactive compounds such as

antimicrobial peptides, fatty acids, pigments, and polysaccharides in their cellular composition.

1.12.6 Antiviral Profile of Microalgae

Microalgae have also been studied for their antiviral properties. Research has shown that some species of microalgae have antiviral activity against a variety of viruses, including human pathogenic viruses (Carbone et al., 2021). These antiviral properties can be attributed to the presence of bioactive compounds such as polysaccharides, proteins, and lipids, which have been found to inhibit viral replication in microalgae and prevent viral attachment to host cells (Chaisuwan et al., 2021).

1.12.7 Antifungal Profile of Microalgae

Microalgae show promise as a natural alternative to conventional antifungal drugs due to their ability to inhibit the growth of various fungi (Schmid et al., 2022). Research has shown that some bioactive compounds found in microalgae disrupt fungal cell membranes and inhibit key enzymes involved in fungal metabolism (Scaglioni et al., 2019). Furthermore, microalgae have shown potential antifungal properties against common fungal pathogens such as *Candida albicans* and *Aspergillus* species (Battah et al., 2014). As the potential of microalgae in various therapeutic areas continues to be uncovered, it is imperative to further investigate their pharmacological properties and applications in clinical research studies.

1.12.8 Antidiabetic Profile of Microalgae

Microalgae have been found to play a role in the treatment of diabetes. Studies have shown that microalgae can regulate blood glucose levels, improve insulin sensitivity, and reduce oxidative stress in individuals with diabetes (Songserm et al., 2022). These effects are attributed to bioactive compounds found in microalgae, such as peptides and polysaccharides, which have been found to have potential antidiabetic properties. These compounds may help control blood

sugar levels and offer a natural alternative to conventional antidiabetic drugs (Siahbalaei et al., 2021). The potential of microalgae as antidiabetic therapy is particularly important given the global increase in the prevalence of diabetes and the need for effective, natural-based therapies to combat the disease and its associated complications (Hussein et al., 2023). Furthermore, the use of microalgae in the production of nutraceuticals and functional foods provides an opportunity to incorporate these beneficial compounds into daily diets, supporting better glycemic control and overall diabetes management. Studies have shown that microalgae, especially brown and red seaweeds, have anti-diabetic properties (Agarwal et al., 2023). These properties include the ability to regulate blood glucose levels, improve insulin sensitivity, and reduce oxidative stress. These beneficial effects can be attributed to bioactive compounds found in microalgae, such as fluorotannins, fucosterol, and polysaccharides (Kargin & Bilgüven, 2022). These compounds have been found to inhibit key enzymes involved in carbohydrate and lipid metabolism, potentiate insulin signaling pathways, and modulate glucose uptake in cells. In addition, bioactive compounds derived from microalgae have been shown to have the potential to reduce postprandial blood glucose concentrations (Kawee-Ai et al., 2019). The use of microalgae in the treatment of diabetes is promising due to its potential to regulate blood glucose levels, improve insulin sensitivity, and reduce oxidative stress.

1.12.9 Antihypertensive Profile of Microalgae

The antihypertensive properties of microalgae are attributed to their bioactive compounds such as peptides and pigments, which have been shown to inhibit angiotensin-converting enzyme (ACE) and lower blood pressure levels by promoting vasodilation (Jiang et al., 2021). Peptides such as valine, proline, tyrosine, aspartic acid are the best known. Microalgae may play a role as a reservoir of antihypertensive peptides, although this is relatively uncommon. This property of many microalgae species has also been reported in studies (Pekkoh et al., 2022).

2. MATERIAL & METHODS

2.1 Microalgal strains and culture conditions

In this study, *Chlorella vulgaris* ASYA27 and *Micractinium simplicissimum* ASYA46 isolated from Horseshoe Island in Antarctica in 2020, and *Chlorella vulgaris* ARAS102 isolated from Nemrut volcanic lake in Türkiye in 2016 were used from the microalgae culture collection of Istanbul Medeniyet University (<https://www.imuasya.org/>). Laboratory liquid stock cultures of ASYA27 and ASYA46 were grown in a climate cabinet (Fitotron SGC120) set at 15 °C, at a light intensity of 50 $\mu\text{mol photons/s/m}^2$ (Philips TL-D 36W/840 fluorescent, ~3700 lumens shelf top illumination; 16 h day/8 h night), in a 250 ml flask, on a shaker (VWR, M5000) at 120 rpm, 100 ml 8.82 mM NaNO₃; 0.17 mM CaCl₂·2H₂O; 0.3 mM MgSO₄·7H₂O; 0.43 mM K₂HPO₄; 1.29 mM KH₂PO₄; 0.43 mM NaCl and P-IV metal solution, soil water and vitamin supplemented Bold-3N medium. ARAS102 stock culture was maintained in 100 ml 3N-BBM medium in a 250 ml erlen mayer on a shaker (VWR, M5000) at room temperature (23-25 °C) with a light intensity of 50 $\mu\text{mol photons/s/m}^2$ (Philips TL-D 36W/840 fluorescent, ~3700 lumens shelf top illumination; 16 h day/8 h night) and a speed of 120 rpm.

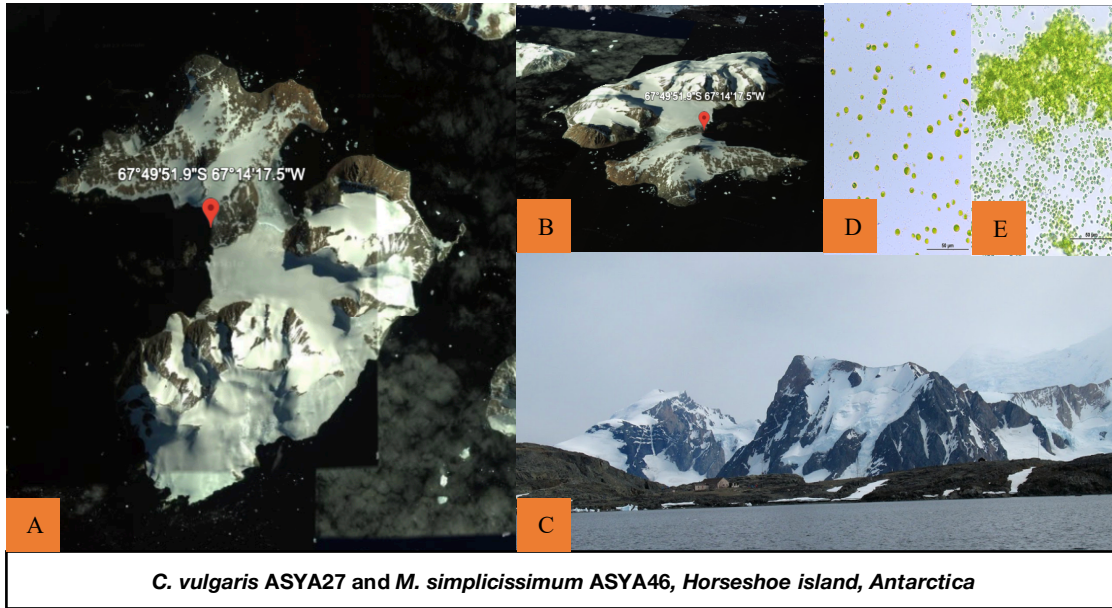


Figure 1. Location map of the sampling area and microscopic image of the isolates. Satellite

images (A, B) and real view (C) of the Horseshoe island are given along with microscopic images of *C.vulgaris* ASYA27 (D) and *M.simplicissimum* (E).

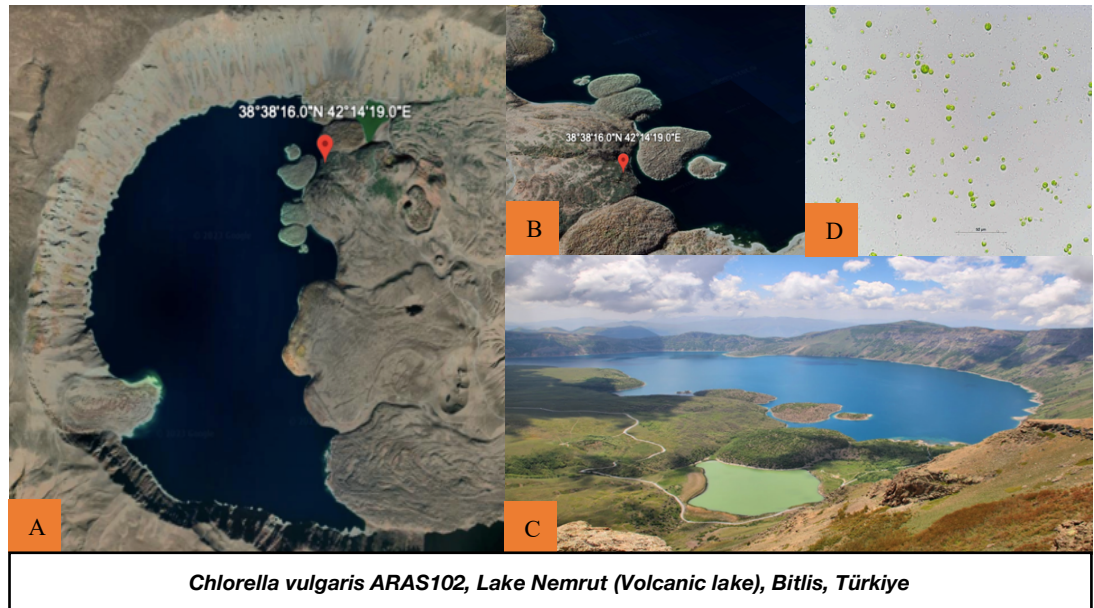


Figure 2. Location map and image of the sampling area (A, B, C) and microscopic image of *Chlorella vulgaris* ARAS102 (D).

2.2 Biomass production

Although the isolation locations of all species were known, growth was monitored at 4, 15, and 25 °C to find the optimal growth temperature. The samples were cultured according to the results obtained. ASYA27 and ASYA46 were grown in a 30L photobioreactor containing 3N-BBM with pocket light system, ventilation, and cryostat connection, 15 °C/100 µmol light setting (Fig. 1). ARAS102 was grown in a 19L glass carboy with 25 °C/100 µmol light in a 3N-BBM medium. Microalgae were harvested in the late exponential phase after 28 days of growth. The harvested samples were dried in a lyophilizer and prepared for analysis.

2.3 Dough Preparation

While oat flour was used for gluten-free bread, whole wheat flour was used for gluten bread. In both dough formulations, 27% skim milk, 60% flour, 1% yeast,

3% salt, 2% sugar, and 7% olive oil were used. The breads were prepared 30 g each and 1% (0.3 g) and 3% (0.9 g) microalgae were used. A control group was formed by omitting microalgae in gluten-free and whole wheat flour bread. The doughs were baked in a 180 °C oven for 25 min, then lyophilized and made suitable for biochemical analysis. The experimental groups are presented in the table below.

Table 1. Dough formulations and naming of experimental groups.

Sample Name	Description
Dough (WW)	27% skim milk, 60% whole wheat flour, 1% yeast, 3% salt, 2% sugar and 7% olive oil
Dough (GF)	27% skim milk, 60% oat flour, 1% yeast, 3% salt, 2% sugar and 7% olive oil
Control-WW	Microalgae-free whole wheat flour dough formulation
Control-GF	Microalgae-free oat flour dough formulation
27- WW-1	1% <i>C. vulgaris</i> ASYA27 containing, whole wheat flour dough formulation
27- WW-3	3% <i>C. vulgaris</i> ASYA27 containing, whole wheat flour dough formulation
27-GF-1	1% <i>C. vulgaris</i> ASYA27 containing, oat flour dough formulation
27-GF-3	3% <i>C. vulgaris</i> ASYA27 containing, oat flour dough formulation
46- WW-1	1% <i>M. simplicissimum</i> ASYA46 containing, whole wheat flour dough formulation
46- WW-3	3% <i>M. simplicissimum</i> ASYA46 containing, whole wheat flour dough formulation
46-GF-1	1% <i>M. simplicissimum</i> ASYA46 containing, oat flour dough formulation
46-GF-3	3% <i>M. simplicissimum</i> ASYA46 containing, oat flour dough formulation
102- WW-1	1% <i>C. vulgaris</i> ARAS102 containing, whole wheat flour dough formulation
102- WW-3	3% <i>C. vulgaris</i> ARAS102 containing, whole wheat flour dough formulation
102-GF-1	1% <i>C. vulgaris</i> ARAS102 containing, oat flour dough formulation
102-GF-3	3% <i>C. vulgaris</i> ARAS102 containing, oat flour dough formulation
27-MB	Lyophilized <i>C. vulgaris</i> ASYA27
46-MB	Lyophilized <i>M. simplicissimum</i> ASYA46
102-MB	Lyophilized <i>C. vulgaris</i> ARAS102

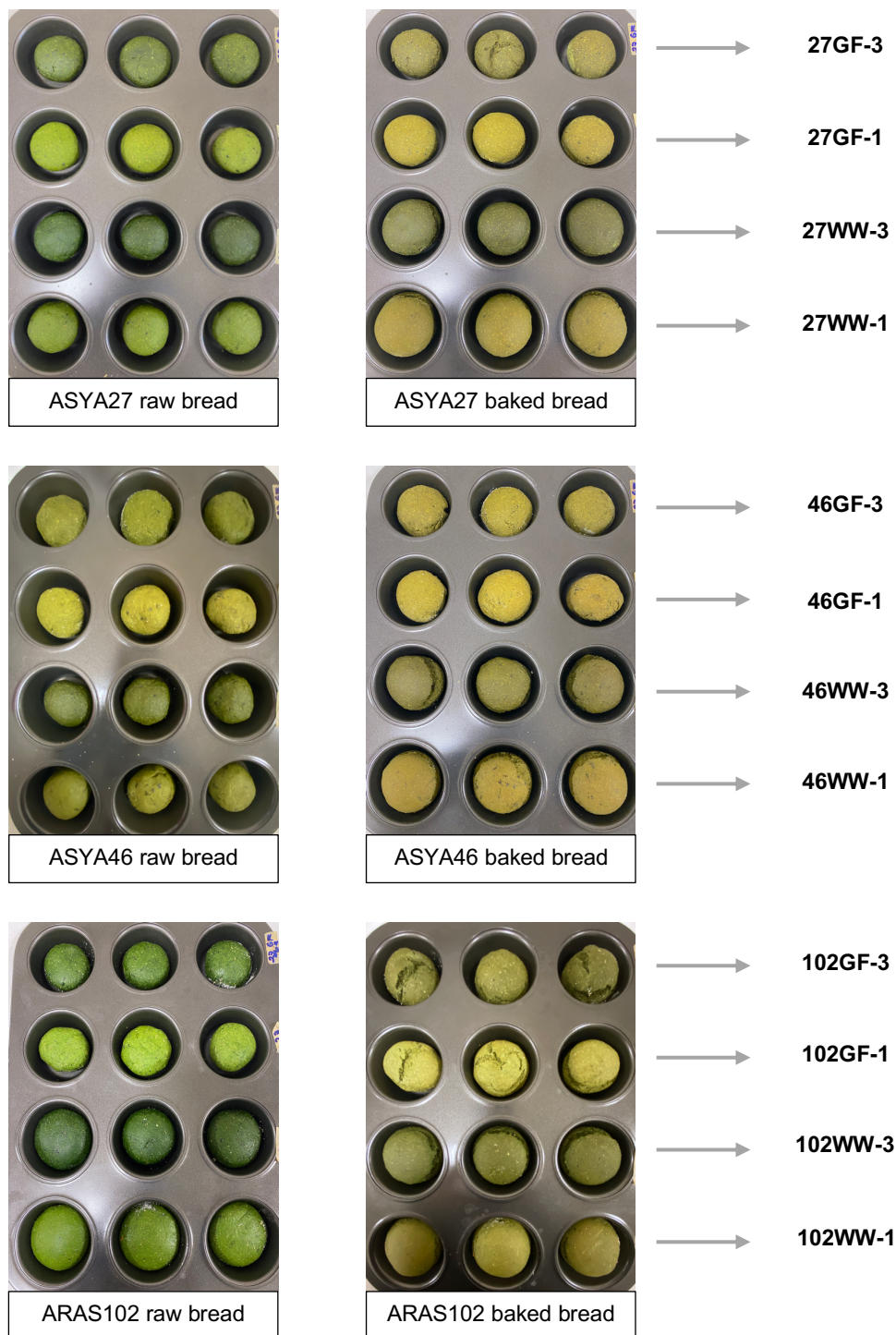


Figure 3. Appearance of bread enriched with microalgae before and after baking

2.4 Moisture

Moisture was determined by drying the sample in an oven at 105 °C for 2 hours (to constant weight). The average moisture values were used to calculate the chemical composition as a percentage of total dry matter.

1. 4 g of sample was weighed into tared petri dishes.
2. They were then placed in an oven at 105 °C for 2 hours.
3. The petri dishes were cooled in a desiccator for 2 hours and then weighed.

The calculation was made with the formula below:

$$\text{Moisture \%} = \frac{\text{Final weight} - \text{Petri tare}}{\text{Sample}} \times 100$$

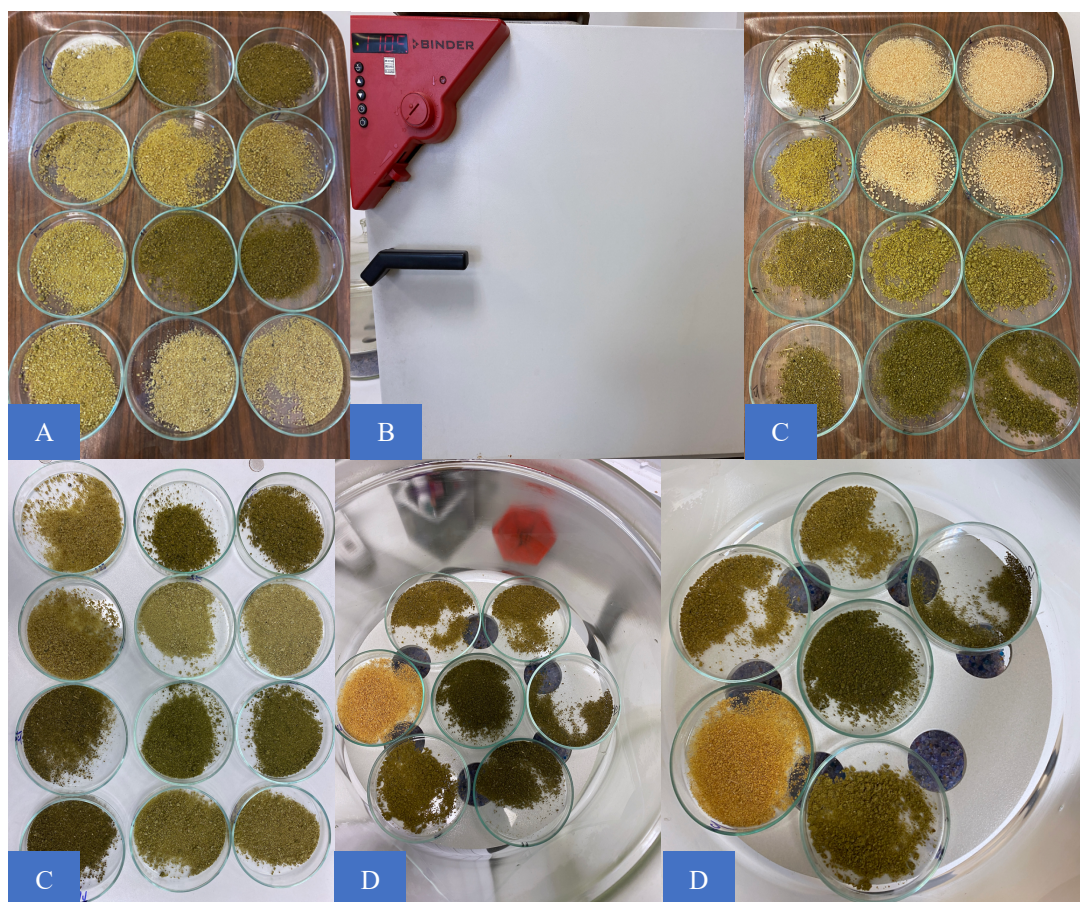


Figure 4. Stages of moisture analysis

2.5 Ash

For ash analysis, 2-3 g samples were weighed into tared crucibles and recorded. The crucibles were then subjected to incineration for 6 hours in a muffle furnace set at 550 °C. At the end of six hours, the crucibles were removed from the muffle furnace, cooled in a desiccator for about 2 hours, and then weighed. The following formula was used to find the amount of ash:

$$\text{Ash \%} = \frac{W2 - W0}{W1} \times 100$$

W0: Crucible weight

W1: Sample weight

W2: Last weighing



Figure 5. Stages of ash analysis.

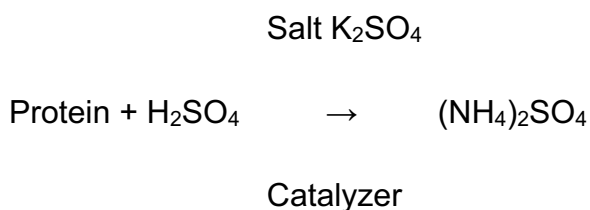
A: Samples weighed into cruses; **B:** Incinerated samples; **C-D:** Disposal to muffle furnace; **a:** 27-WW/1 ash; **b:** ASIA27-WW/3 ash; **c:** ASYA27-GF/1 ash; **d:** ASYA27-GF/3 ash; **e:** ASYA46-WW/1 ash; **f:** ASYA46-WW/3 ash; **g:** ASYA46-WW/1 ash ASYA46-GF/1 ash; **h:** ASYA46-GF/3 ash; **j:** ARAS102-WW/1 ash; **k:** ARAS102-WW/3 ash; **l:** ARAS102-GF/1 ash and **m:** ARAS102-GF/3 ash.

2.6 Protein

Total nitrogen was determined by the Kjeldahl method after acid digestion (AOAC 991.20), ammonium addition, steam distillation, and titration with 0.1 N HCl. The protein content was calculated using a nitrogen-to-protein conversion factor of N × 6.25.

Sample preparation / Incineration

During the combustion process, the nitrogen or protein present in the sample is converted to ammonium sulfate according to the formula below.



Salt

Since the decomposition temperature of most of the samples is incinerated and the boiling point of the concentrated sulfuric acid is high, the boiling point of the acid needs to be raised. Potassium sulfate was used for this purpose. This salt is contained in Kjeltabs together with the catalyst.

Distillation

After incineration all samples were converted to ammonium sulfate. This reagent was therefore used as a distillation control. The principle of the distillation process is to convert ammonium to ammonia in the presence of alkali, i.e. NaOH, and then titrate it with a standard acid solution by steam distillation into a flask containing boric acid.

Calculation

The results obtained were expressed in g/100g nitrogen. Factors were used to convert nitrogen to protein according to the matrix.

$$\text{Nitrogen \%} = \frac{1.4007 \times M \times F \times F \times A - B}{W} \times 100$$

Protein (%) = NxF

1.4007: Atomic weight of nitrogen corresponding to 0.1 ml of 0.1 N HCl

M: Acidity molarity (mol/L)

F: Standard Kjeldahl factor

A: Titrant expenditure (ml)

B: Control expenditure (ml)

W: Sample weight (g)

The % nitrogen value is multiplied by factors to obtain the protein value.

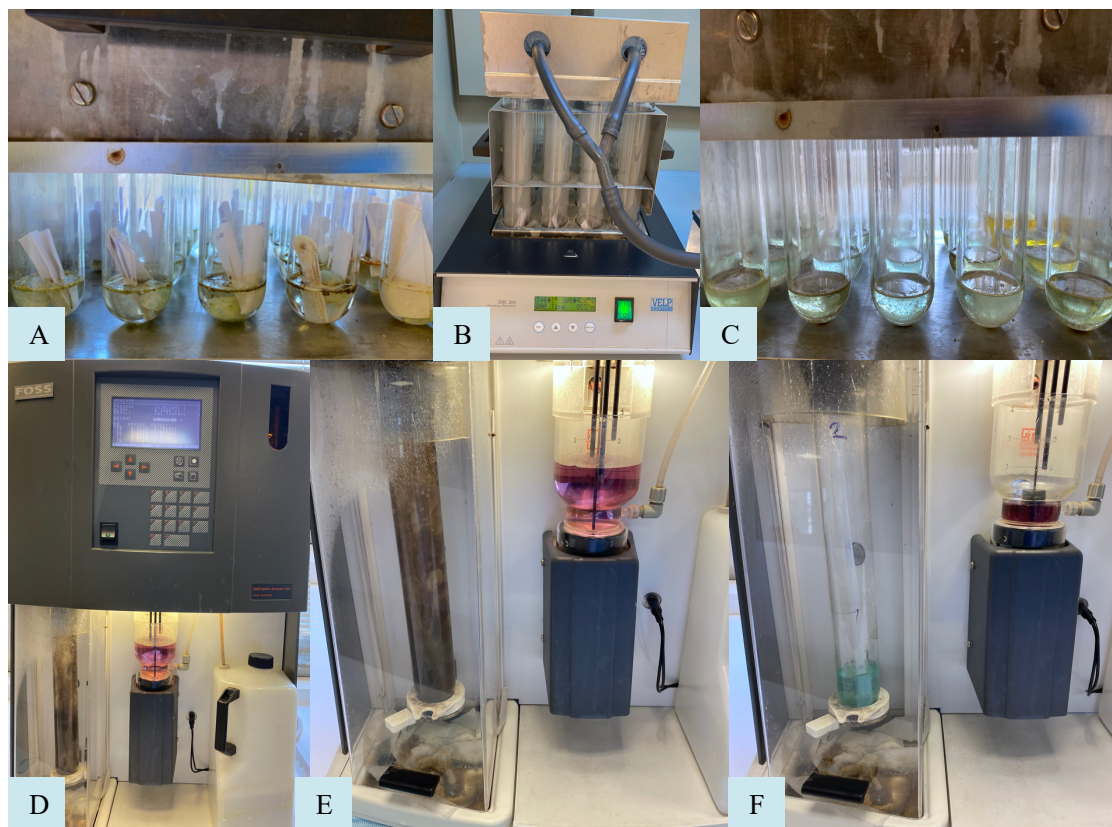


Figure 6. Stages of protein analysis.

2.7 Amino Acid Analysis

The method covers 16 amino acids (aspartic acid, glutamic acid, serine, glycine, arginine, arginine, histidine, threonine, lysine, alanine, proline, hydroxyproline, leucine, isoleucine, cysteine, tyrosine, phenylalanine, valine, methionine).

Environmental conditions that may cause oxidation (high temperature, UV light, etc.) were avoided during sample preparation.

Chemicals, Solutions, and Preparation

- Preparation of 0.1 N HCl solution: 8.28 ml of HCl was added to 1 liter of water.
- Preparation of 6 N HCl solution: 496.8 ml HCl was added to 1 liter of water.
- Preparation of derivatization solution (Edman reagent): 100 ml ACN was added to 1.2 ml phenylisothiocyanate (PITC) and stirred.
- Preparation of 0.02 M ammonium acetate solution: 0.31 g of ammonium acetate was weighed and dissolved with 200 ml of water.
- Preparation of Acetonitrile-Methanol-Triethylamine (ACN:MeOH: TEA) mixture: 100 ml acetonitrile, 50 ml methanol, and 20 ml triethylamine were mixed.

Analysis

Preparation of the Standard

10 mg of each amino acid standard was weighed into a 100 ml balloon jug. 100 ppm stock standard solution was prepared by dissolving with 0.1 N HCl and adding to the volume.

Working standards were prepared by making necessary dilutions from the stock standard solution.

Sample Preparation

1. 0.1-1 g of homogenized sample was taken into a 50 ml sealed analysis bottle and hydrolyzed in an oven at 1100C for 24 hours by adding 20 ml of 6 N HCl

solution. After reaching room temperature, it was filtered through ordinary filter paper.

2. 0.2 ml of the filtrate was taken and evaporated at 500C under nitrogen gas.
3. 0.5 ml of ACN:MeOH:TEA mixture and 0.1 ml of derivatization solution (PITC) were added and derivatized in a 400C oven for 30 minutes.
4. After evaporation at 400C under nitrogen gas, 5 ml of 0.02 M ammonium acetate was added.
5. Filtered through a 0.45 μ filter and placed in vials

Device Operating Conditions

Mobile Phase

1. Mobile Phase A: 0.78 g sodium dihydrogen phosphate dihydrate and 0.88 g disodium hydrogen phosphate dihydrate were dissolved with water and adjusted to pH:6.9. The volume was made up to 1 liter with distilled water.
2. Mobile Phase B: Acetonitrile (HPLC pure)
3. UV detector, 254 nm
4. Flow Rate 1 ml/m

Gradient Program (Injection Time: 40 min.

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0.01	96	4
15.00	84	16
22.00	65	35
26.00	60	40
28.00	40	60
30.00	40	60
33.00	40	60
33.01	96	4
40.00	96	4

Calculation

The difference between parallel results should not be more than 15%.

When calculating the linear range, R²=0.99 between concentrations.

The calculation was performed by comparing the sample to the standard by peak area or concentration. The concentration of the sample was calculated from the linear calibration line.

Since the standard concentration was expressed in $\mu\text{g/ml}$, it was calculated with the following formula:

$$C = a \times V/m \times k$$

a: sample concentration

k: slope for standard curve

V: dilution factor (ml)

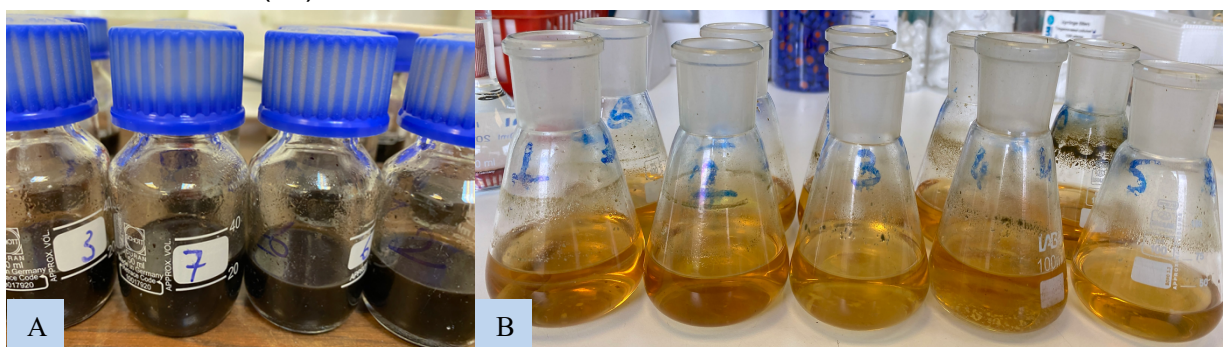


Figure 7. Stages of amino acid analysis.

2.8 Analysis of vitamin B1

Thiamine, a water-soluble vitamin group, is found in different forms (Thiamine, TNP, TPP, TTP) in foods. The pH range in which its highest stability is 2- 4. Therefore, it is the type of vitamin with the highest loss when examined or analyzed in foods with low acidic properties. Although the UV range for thiamine is between 245-254 nm, it is used to analyze foods with high amounts of thiamine. The UV detector is insufficient to detect Thiamine because it is found in low amounts in natural foods. Therefore, the more sensitive Fluorescence detector is used. However, since Thiamine alone does not show fluorescence, it is converted to Thiochrome by adding Potassium ferricyanide.

For maximum fluorescence, the pH of the mobile phase and the prepared sample should be between 7-7.5. If the sample pH is higher than the mobile phase pH, it causes the retention time to shift. Thiamine is not present in foods in free form or the form of TNP and TPP. Therefore, alpha-amylase, clara-diastrase, papain, acid

phosphatase, and beta-glucosidase enzymes are used to free and analyze these bonds.

Water of appropriate purity should be used in experiments and in the preparation of solutions, and the chemicals used should be of analytical purity. Ambient conditions that may cause oxidation (high temperature, UV light, etc.) should be avoided in sample preparation.

15% (w/w) NaOH solution: Weigh 15 g NaOH into a beaker and add 100 ml distilled water. Care was taken not to overheat when adding water.

Preparation of potassium ferricyanide solution: 0.25 g of potassium ferricyanide was added to 25 ml of distilled water.

Preparation of 0.1 N HCl solution: 8.28 ml HCl was added to 1 liter.

Enzyme Preparation:

- Acid phosphatase: For 1 mg, the potency is between 4-5 U.
- 125 mg was taken for 50 samples and made up to 50 ml with distilled water.
- Beta-Glucosidase: For 1 mg, the detection value is between 2-3 U
- 250 mg was taken for 50 samples and made up to 50 ml with distilled water.

Standard Thiamine stock solution:

- 10 mg of thiamine vitamin standard was weighed into a 100 ml flask.
- It was dissolved with some 0.1 N HCl.
- Necessary dilutions were made from the stock standard and 0.05- 0.1- 0.2 ppm standards were prepared.
- While preparing the standards, 25 ml was taken and 1.5 ml of Potassium ferricyanide 1% solution (in 15% NaOH) was added. The pH was then adjusted to 7-7.1 with 15% ortho-phosphoric acid.

Preparation of the Test Sample:

- 10 g of homogenized sample was taken into a 100 ml balloon jug and 60 ml of 0.1 N HCl solution was added.
- Autoclaved at 121 C for 30 minutes.
- It was then cooled to 37 degrees C and the pH of the sample was adjusted to 4.5 with sodium acetate solution.

- Then 250 mg clara-diestase, 50 mg papain, 20 mg alpha-amylase, 0.5 ml acid phosphatase, and 1 ml beta-glucosidase enzyme were added.
- It was then kept in a shaking water bath at 45 degrees for 3-18 hours.
- After the sample volume had cooled, the volume was made up to 100 ml with deionized water and filtered through ordinary filter paper. From the filtrate, 25 ml was taken into polyethylene tubes.
- 1.5 ml of Potassium ferricyanide 1% solution (in 15% NaOH) was added.
- The pH of the sample was then adjusted to 7.0-7.1 with 15% ortho-phosphoric acid. The sample was filtered through a 0.45-micron filter.
- The dilution factor for thiamine was calculated as $100 + \text{final volume}/25$.
- HPLC

Calculation and Display of Results

The calculation was done by comparing the standard with the sample based on peak area or concentration. The concentration of the sample was calculated from the linear calibration line generated. Since the standard concentration was expressed in $\mu\text{g/ml}$, it was calculated with the following formula.

$$C = a \cdot V / m \cdot k$$

a: sample concentration

k: slope for standard curve

V: Dilution factor (ml)

m: sample weight (g)

2.9 Analysis of vitamin B2

Riboflavin, a water-soluble vitamin, is present in foods in different forms (Riboflavin, FMN, and FAD). Although Riboflavin is stable against heat and oxidation, the pH range in which its stability is the highest is 2-5. It starts to degrade above pH 7. Under pH 5, FMN and FAD are converted into Riboflavin. Riboflavin is present in foods in free form or the form of FAD and FMN, so it is analyzed by freeing these bonds using alpha-amylase, clara-diestase, papain, acid phosphatase, and beta-glucosidase enzymes. In sample preparation, it is necessary to avoid ambient conditions (high temperature, UV light, etc.) that may

cause oxidation.

Preparation of 0.1 N HCl solution:

- 8.28 ml HCl is added to 1 liter.

Enzyme Preparation:

- ⇒ Acid phosphatase: For 1 mg, the potency is between 4-5 U.
- ⇒ 125 mg was taken for 50 samples and made up to 50 ml with distilled water.
- ⇒ Beta-Glucosidase: For 1 mg, the potency is between 2-3 U
- ⇒ 250 mg was taken for 50 samples and made up to 10 ml with distilled water.
- ⇒ Standard Riboflavin stock solution:
- ⇒ 10 mg of Riboflavin vitamin standard was weighed into a 100 ml flask.
- ⇒ It was dissolved with some 0.1 N HCl.
- ⇒ Necessary dilutions were made from the stock standard and 0.05- 0.1- 0.2 ppm standards were prepared.

Test Sample Preparation:

- ⇒ 10 g of the homogenized sample was taken into a 100 ml flask and 60 ml of 0.1 N HCl solution was added.
- ⇒ It was placed in an autoclave at 121 degrees for 30 minutes.
- ⇒ It was then cooled to 37 degrees and the pH of the sample was adjusted to 4.5 with sodium acetate solution.
- ⇒ 250 mg clara-diestase, 50 mg papain, 20 mg alpha-amylase, 0.5 ml acid phosphatase, and 1 ml beta-glucosidase enzyme were added.
- ⇒ It was then kept in a 45-degree shaking water bath for 3 hours.
- ⇒ After cooling, the sample volume was made up to 100 ml with deionized water filtered through a 0.45-micron filter and placed in vials.
- ⇒ HPLC.

HPLC Conditions

- ⇒ Mobile phase: Prepared by mixing 750 ml deionized water and 250 ml methanol.
- ⇒ Detector: Fluorescence Detector

- ⇒ Excitation: 445 nm
- ⇒ Emission: 545 nm
- ⇒ Column Analytical reversed-phase column
- ⇒ Injection Volume: 20 µl
- ⇒ Flow Rate: 1 ml/min

Calculation of Results

The calculation was done by comparing the standard to the sample based on peak area or concentration. The concentration of the sample was calculated from the linear calibration line. Since the standard concentration is expressed in µg/ml, it was calculated with the following formula:

$$C = a \cdot V / m \cdot k$$

a: sample concentration

k: slope for standard curve

V: Dilution factor (ml)

m: sample weight (g)

2.10 Analysis of vitamin B6

Vitamin B6, which belongs to the water-soluble vitamin group, is found in various forms in foods in the form of free pyridoxine, pyridoxal, and pyridoxamine.

However, it is mostly found in natural foods such as phosphate, glycoside, and oligo-glucoside PL-phosphate, PN-phosphate, PM-phosphate, PN-glucoside, and PN-oligoside.

Therefore, alpha-amylase, alpha-diastase, papain, acid phosphatase, and beta-glucosidase enzymes are used to free these bonds and analyze them.

Since the PL form overlaps with other matrices when analyzing vitamin B6, the PL form is converted to the PN form.

Preparation of 0.1 N HCl solution:

- 8.28 ml HCl was added to 1 liter.

Enzyme Preparation:

- Acid phosphatase: For 1 mg, the potency is between 4-5 U.
- Weighed 125 mg for 50 samples and added to 50 ml with distilled water.

- Beta-Glucosidase: The potency for 1 mg is between 2-3 U
- For 50 samples, 250 mg was weighed and made up to 10 ml with distilled water.

Preparation of standards

- 10 mg Riboflavin vitamin standard was weighed into a 100 ml flask.
- It was dissolved with some 0.1 N HCl.
- Necessary dilutions were made from the stock standard and 0.05- 0.1- 0.2 ppm standards were prepared.

Preparation of the Test Sample:

- 5-10 g of well homogenized sample was taken into a 100 ml flask and 60 ml of 0.1 N HCl solution was added.
- Autoclaved at 121 °C for 30 minutes.
- The pH of the sample was then adjusted to 4.5 with sodium acetate solution and 250 mg clara-diestase, 50 mg papain, 20 mg alpha-amylase, 0.5 ml acid phosphatase, and 1 ml beta-glucosidase enzyme were added.
- It was then kept in a 45-degree shaking water bath for 18 hours.
- The sample volume was made up to 100 ml with deionized water and filtered through 0.45-micron filter paper.
- The PL form was converted to PN form according to the following method.
- HPLC.

HPLC Conditions:

Mobile phase:

- 11 g potassium dihydrogen phosphate and 0.5 g octanesulfonic acid sodium salt monohydrate was weighed and added to 940 ml distilled water.
- Then 60 ml of Acetonitrile (ACN) was added.
- The pH was adjusted to 2.85 with 85% orthophosphoric acid.
- Filtered on 0.45% filter paper.

Post-column derivatization

- Produced with 1% sodium bisulfite solution.
- Flow rate 0.1 ml/min

- Detector: Fluorescence Detector
- Excitation 290 nm
- Emission:395 nm
- Column Analytical reversed-phase column
- Injection Volume: 20µl
- Flow Rate: 1 ml/min

Calculation and Display of Results

The calculation was done by comparing the standard to the sample based on peak area or concentration. The concentration of the sample was calculated from the linear calibration line. Since the standard concentration is expressed in µg/ml, it was calculated with the following formula:

$$C=a \times V/m \times k$$

a: sample concentration

k: slope for standard curve

V: Dilution factor (ml)

m: sample weight (g)

2.11 Total dietary fiber determination

When the principle of total dietary fiber analysis is examined: gelatinization, hydrolysis, and depolymerization of starch in samples incubated at 100 °C with α-amylase. It is then incubated at 60 °C with protease to dissolve proteins. Then treated with aminoglucosidase to hydrolyze the starch fragments to glucose and ethanol to separate the depolymerized protein and glucose from the starch. The residues are filtered, washed with ethanol and acetone, dried, and weighed. One of the sample replicates is then used to determine protein and the other to determine ash. The results obtained are subtracted from the total amount of residue to arrive at the total dietary fiber value.

MES/TRIS Buffer Preparation

19.52 g 2(N-morpholino) ethane sulfonic acid (MES) (Megazyme cat. no. B-MES250) and 14 g tris (hydroxymethyl)aminomethane (TRIS) (Megazyme cat. no. B-TRIS500) were dissolved in 1.7 L deionized water and pH adjusted to 8.2

with 6.0 N NaOH. It was then diluted to 2 L with water and kept at room temperature for use.

Procedure

- Weighed 1 g of sample in tall-form beakers.
- 40 mL of MES-TRIS buffer solution was added to each beaker and mixed with a magnetic stirrer.
- To degrade starches, 50 μ l of α -amylase solution was added to each sample, the beakers were covered with aluminum foil and incubated in a water bath at 100 °C/35 min.
- After the samples were removed from incubation, the water bath was set to 60 °C.
- Then 100 μ l of protease was added to each sample covered with aluminum foil and incubated at 60 °C/30 min.
- Add 5 ml of 0.561 N HCl solution to the samples removed from the water bath (pH: 4.1-4.8 via 5% NaOH solution or 5% HCl solution). Then 200 μ l of aminoglucosidase enzyme was added and incubated in a water bath at 60 °C/30 min.
- The volume was then made up to 200 ml with technical ethanol (96%).
- Incubated again at 60 °C/1 hour in a water bath.
- For the filtration step, 0.1 mg aliquots of crucibles containing celite were taken. (Celite was used because it prevents clogging of the pores and provides more comfortable filtration).
- The celite bed in the crucible placed in the Nuche erlen was dispersed with distilled water (~3 ml), the samples taken from the water bath were filtered and the beakers were washed with ethanol and the remaining samples were filtered.
- The residue in the crucible was washed with ethanol and acetone. The crucibles containing the residue were then dried overnight in an oven set at 103 °C.
- The dried crucibles were cooled in a desiccator for about 1 hour and then weighed.

- After weighing the samples, they were treated in a muffle furnace at 550 °C/4 hours for ash analysis.
- The total diet was calculated after subtracting the ash result and the protein content previously determined by the Kjeldahl method from the residue.

Calculation:

$$\text{Dietary Fiber (\%)} = (R_1 + R_2/2) - (p - A) / m_1 + m_2/2 \times 100$$

R_1 = residue weight 1 from m_1 ; R_2 = residue weight 2 from m_2 m_1 = sample weight 1; m_2 = sample weight 2; A = ash weight from R_1 ; p = protein weight

2.12 Starch quantitative analysis

The principle of the analysis is to enzymatically break down the starch in the sample to glucose, convert the glucose to NADPH, and calculate the amount of starch by the amount of NADPH produced.

- 0.1 g of the ground sample was weighed into a 100 ml volumetric flask.
- After adding 20 ml DMSO and 5 ml HCl (8 M), the samples were covered with parafilm and incubated in a water bath at 60 °C/30 min.
- After cooling the samples to 20-25 degrees Celsius, the volume was made up to 100 ml with distilled water. It was then adjusted to pH:4-5 with 5 M NaOH.
- 0.1 ml of the samples filtered through ordinary filter paper were transferred to spectrophotometer cuvettes.
- 0.1 ml distilled water and 0.2 ml Enzytech AGS buffer were added to the samples. 0.1 ml distilled water for the blank and 0.1 ml Starch (HK) Assay Kit for the starch sample were added to the cuvettes.
- The samples were covered with parafilm and incubated in a water bath at 60 °C/30 min.
- To the incubated samples 1 ml of Enzytec NADH/ATP enzyme and 1 ml of distilled water were added. Since no enzyme was added to the blank, 1.2

ml of distilled water was added to equalize the volume. After waiting for 5 min, absorbance was measured at 340 nm.

- Finally, 0.020 ml of Glucose-6-phosphate enzyme was added to the cuvettes and absorbance was measured at 340 nm after one hour at room temperature.

Calculation:

$$\text{Starch amount (g/l)} = (V \times \text{MW}/e \times d \times v \times 1000) \times \Delta A$$

V= final volume (ml)

v= sample volume (ml)

MW= molecular weight (Starch=162.1 g/mol)

e= Absorption coefficient of NADPH ($6.3 \text{ l} \times \text{mmol}^{-1} \times \text{cm}^{-1}$ for 340 nm)

2.13 Mineral analysis

Atomic absorption spectroscopy (AAS) is based on the absorption of UV radiation by free atoms in the gaseous state. The sample to be analyzed is reduced to ash and then dissolved in an aqueous solution. After aspiration of the resulting solution, it is aerated and mixed with combustible gases (approx. 2100-2800 °C) and released to form a cloud.

Radiation is passed through the atomized sample and the absorption of the radiation is measured at specific wavelengths corresponding to the mineral under study.

The concentration of the mineral under study is determined by the intensity of the peaks in the spectrum. This highly sensitive method allows the detection of an element down to 1 ppm. Elements such as Zn, Mn, Mg, Ca, Cr, Fe, Li, K, and Na can be determined directly by this method.

- Approximately 0.3-1.0 g of the homogenized samples were weighed into the sample chambers of the microwave unit. Then 6.5 ml of 65% nitric acid (HNO_3) and 1.5 ml of 30% hydrogen peroxide (H_2O_2) were added. The lids of the sample containers were tightened tightly placed inside the device and turned on. In the microwave digestion device, digestion was performed with the program suitable for the matrix as shown in the table below.

Step	Ramp time (min)	Temperature (°C)	Waiting time (min)
1	5	90	5
2	5	90	5
3	5	120	5
4	5	120	5
5	5	150	5
6	5	150	5
7	5	175	5
8	5	175	5

- *Standard Preparation*

Mineral stock standard solution (1000 mg/L)

Intermediate standard (10 mg/L): 0.5 ml of the stock solution was taken in 50 ml balloon jugs and the volume was made up to 50 ml with 1% nitric acid.

Working standard: 1.0, 2.0, 4.0 ml of the intermediate standard was added to three separate 50 ml flasks. The volume was then made up to 50 ml with distilled water.

Working standard 1: (0.2 mg/L)

Working standard 2: (0.4 mg/L)

Working standard 3: (0.8 mg/L)

Blank solution was subjected to the same treatments as the working standards.

- *Reading Conditions in AAS*

The desired mineral lamp was installed in the AAS instrument. After the lamp was lit, it was waited for about 1/2 hour to reach the appropriate energy level. According to the conditions suitable for the mineral (For example for zinc; Wavelength: 219 nm, Slit: 0.7 nm).

Flame conditions: Acetylene-air mixture

Blank, standard and samples were injected into the instrument (dilution of samples was performed as needed), followed by three injections for each sample.

- *Calculations*

Mineral (mg/kg) = Sample absorbance * sample volume * dilution ratio * standard concentration / sample amount (g)* standard absorbance

Inductively coupled plasma mass spectrometry (ICP-MS) instrument was used for heavy metal analysis. Among many techniques, ICP-MS is the ideal technique for toxic/heavy metals due to its high throughput and low amounts of detection lipid.

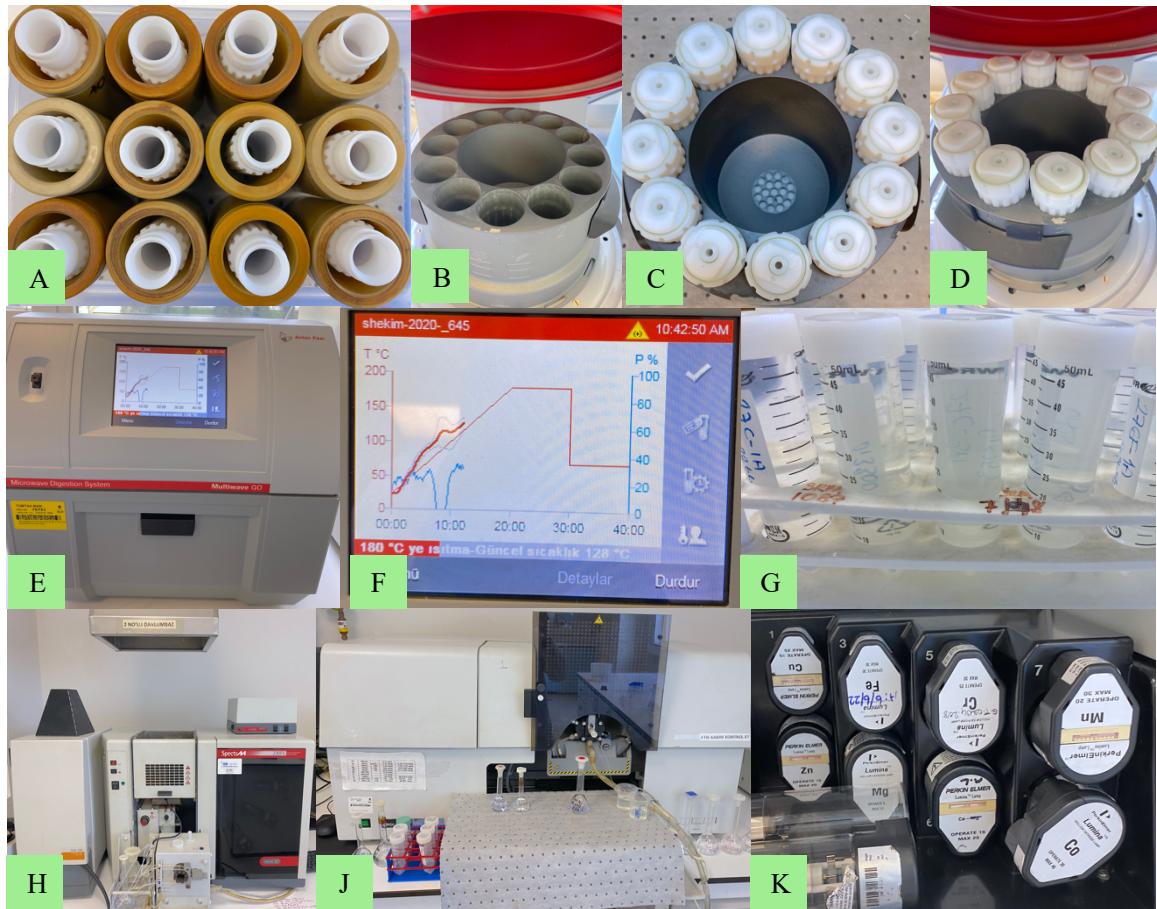


Figure 8. Stages of mineral analysis.

2.14 Heavy Metal Analysis

The end products obtained in the mineral analysis described above were also used in the ICP-MS device. In particular, the heavy metals lead (Pb), arsenic (As) and mercury (Hg), which are likely to be present in algae, were analyzed.

Standard preparation

Stock standard solutions (1000 mg/L) were purchased commercially.

⇒ Intermediate standard A (10 mg/L): For lead, mercury and arsenic, 0.5 ml of the 1000 mg/L stock standard solution was taken into 50 ml balloonjugs and the volume was made up to 50 ml with 1% nitric acid.

⇒ Intermediate standard B (100 µg/L): For lead, mercury and arsenic, 0.5 ml of the 10 mg/L "Intermediate standard A" solution was taken in a 50 ml flask and the volume was made up to 50 ml with 1% nitric acid.

After the elements to be analyzed were prepared as above, the working standards were prepared as follows.

Table 2. The working standards of heavy metal analysis

Amount of intermediate standards (ml)	Element concentration (ppm)	Element concentration (ppb)
0,5	0,1	1
1,0	0,2	2
2,0	0,4	4
4,0	0,8	8
8,0	1,6	16
16	3,2	32

Reading Conditions in ICP-MS

After the device was properly prepared for use, the elements and isotopes to be analyzed were selected in the relevant method. The isotopes of the elements are given in the table below. After the weighing and preparation volume of the samples were entered into the sample section on the software, the device automatically performed the calculation.

Table 3. Recommended isotopes for element selection

Element	Mass
Lead	204, 206, 207, 208
Mercury	199, 200, 201, 202
Arsenic	75

2.15 Phosphorus Analysis

The final product obtained in the mineral analysis was also used for this analysis.

Sample and Standard Preparation

Preparing molybdovanadate solution

20 g ammonium molybdate was dissolved in 200 ml hot water and cooled. Then 1 g of ammonium metavanadate was dissolved in 125 ml of hot water, cooled and 140 ml of concentrated nitric acid was added. After mixing both solutions, the volume was made up to 1 liter with water.

Phosphorus standard preparation

Stock standard: KH_2PO_4 was weighed 0.2196 g after drying at 105 °C for 1 h and the volume was made up to 1 l with water.

Working standard:

Standard1: $50 * 1/100 = 0.5 \text{ mg/ml}$

Standard2: $50 * \text{KH}_2\text{PO}_4 \text{ 2/100} = 1 \text{ mg/ml}$

Standard3: $50 * 4/100 = 2 \text{ mg/ml}$

Standard4: $50 * 8/100 = 4 \text{ mg/ml}$

Standard5: $50 * 16/100 = 8 \text{ mg/ml}$

Standard6: $50 * 20/100 = 10 \text{ mg/ml}$

Preparation of the test solution

5 ml of molybdovanadate solution was added to 5 ml of sample solution and made up to 25 ml with distilled water. A blank was then prepared using only the chemicals. Samples and standards were then read at 400 nm wavelength.

The phosphorus content of the samples was determined from the standard curve.

The result gave the % phosphorus.

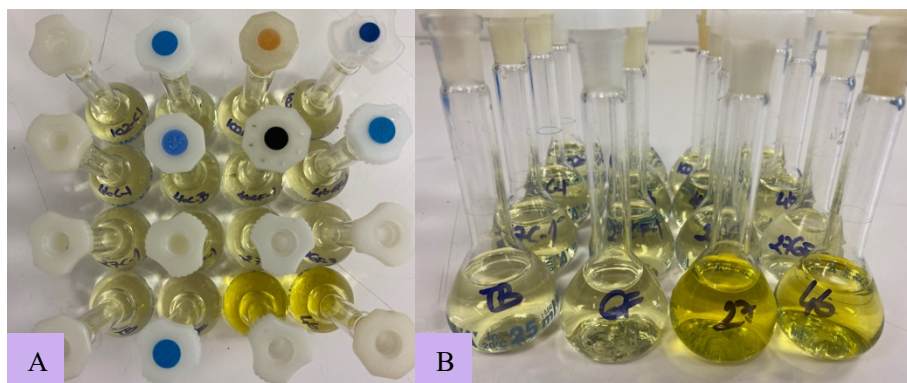


Figure 9. Stages of phosphorus analysis.

2.16 Fat Determination by Acid Hydrolysis

- Weighed 0.5 g of the homogenized sample into a glass beaker. Then 100 -120 mL of acid solution (4.0 N HCl) was added to the sample.
- Then it was hydrolyzed for 4 hours with the help of a 200 °C heater and stirrer.
- The mixture was then brought to room temperature and 50 mL of distilled water was added.
- The sample was filtered through filter paper and washed with distilled water (50 x 2) and the filter paper was dried in an oven at 105 °C for 1 hour.

Soxhlet Extraction

- It was put into the extractor cartridge together with the filter paper obtained because of acid hydrolysis.
- Cartridges were placed in the device. 6 buttons were placed in the "rinsing" position.
- Each button was set to a "boiling" position. The magnetic part was connected to the cartridge adapter.
- The buttons were moved to the "rinsing" position again.
- The extraction vessels were brought to constant weight and weighing values were taken.

After extraction, the extraction vessels were dried in an oven at approximately

105 °C for 30 minutes. Then they were taken to the desiccator, cooled to room temperature, and weighed and the percentage of oil extracted was calculated.

Calculation

W3: Weight of the oil collection container at the end of extraction and after cooling in the desiccator

W2: Initial weight of the oil collection container (Clean and pre-dried)

W1: Sample weight

$$\% \text{Oil} = \frac{W3 - W2}{W1} \times 100$$

After the determination of oil by acid hydrolysis, esterification was performed.

2.17 Fatty Acid Methyl Ester (FAME) Analysis

Preparation of 2.0 N Potassium Hydroxide (KOH) Solution

- Dissolve 11.22 g KOH with methyl alcohol in a 100 mL balloon flask.

Sample Preparation

- The oils obtained by the above-mentioned Acid Hydrolysis Fat Determination Method were used for this step. The sample was homogenized by thorough mixing.
- Then 0.1 g sample was weighed into a centrifuge tube, 0.5 mL 2.0 N KOH and 5 mL heptane were added and vortexed for 1 min. Then dry sodium sulfate was added with a spatula tip and vortexed again.

Gas Chromatography Working Conditions

- Detector temperature 260 °C
- Time constant 200
- Range: 1
- Injector temperature: 280 °C
- Oven temperature program held at 120 °C for 2 minutes, increased to 220 °C at 5 °C per minute and held for 10 minutes.
- Carrier gas: Helium
- Carrier gas flow rate: 0.5 mL/min
- Air flow rate: 450 mL/min
- Hydrogen flow rate: 45 mL/min

- Attenuation -4
- Injection: automatic
- Injection volume 1µL
- Analysis time: 32min
- Split mode: flow
- Ratio: 50mL/min

Analysis With GC-FID

Mix fatty acid standard solution was injected into the GC-FID and fatty acid methyl esters were checked for their retention time. Sample injection was then performed (three times).

Calculation

The fatty acid composition in the sample was found as a relative percentage. Trans fat, Omega 3, Omega 6, saturated fat, monounsaturated fat, polyunsaturated fat was calculated according to the following formulas and the results were reported in g/100g.

$$\text{Trans fatty acids} = \frac{\text{Total trans fatty acids (\%)}}{\text{Total fat in the sample}} \times 100$$

$$\text{Trans fatty acids} = \frac{\text{Omega3 fatty acids total}}{\text{Total fat in the sample}} \times 100$$

$$\text{Trans fatty acids} = \frac{\text{Omega6 fatty acids total}}{\text{Total fat in the sample}} \times 100$$

$$\text{Saturated fat} = \frac{\text{Total saturated fatty acids (\%)}}{\text{Total fat in the sample}} \times 100$$

$$\text{Monounsaturated fat} = \frac{\text{Total monounsaturated fatty acids (\%)}}{\text{Total fat in the sample}} \times 100$$

$$\text{Polyunsaturated fat} = \frac{\text{Total polyunsaturated fatty acids (\%)}}{\text{Total fat in the sample}} \times 100$$

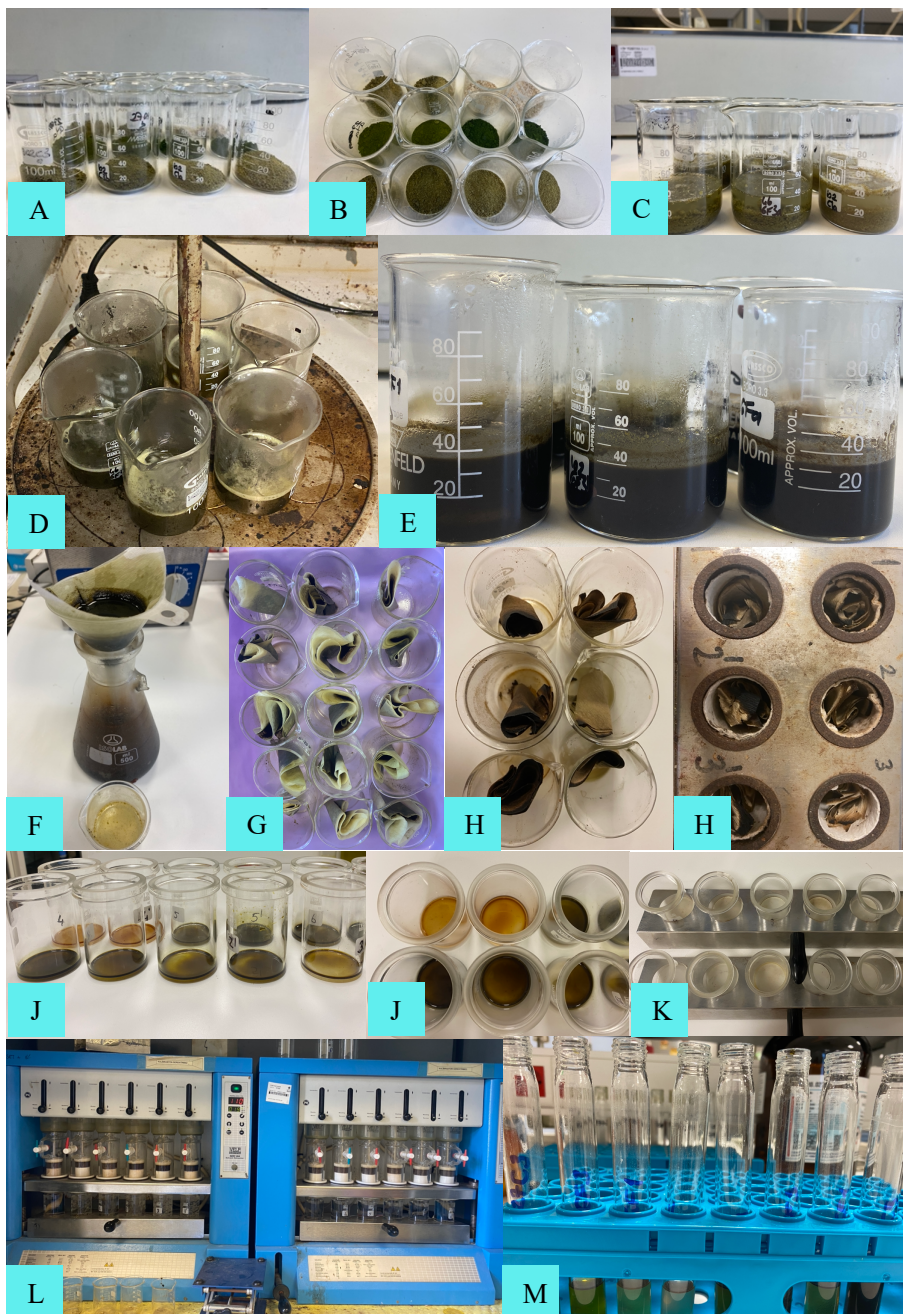


Figure 10. Stages of FAME analysis.

3. RESULTS

3.1 Growth monitoring at different temperatures

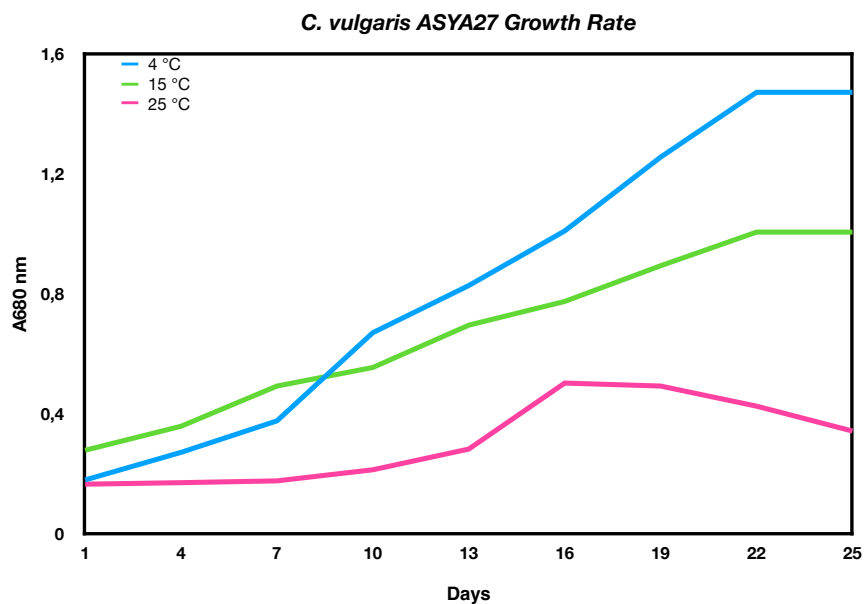


Figure 11. Growth rate of *C. vulgaris* ASYA27

To determine the optimum temperature level for the growth, all microalgae were incubated at 4, 15, and 25 °C. When it was grown at 4 °C, the microalga *C. vulgaris* ASYA27 reached its maximum concentration in 22 days with approximately 1.5- and 2.5 times higher biomass than those grown under 15 and 25 °C.

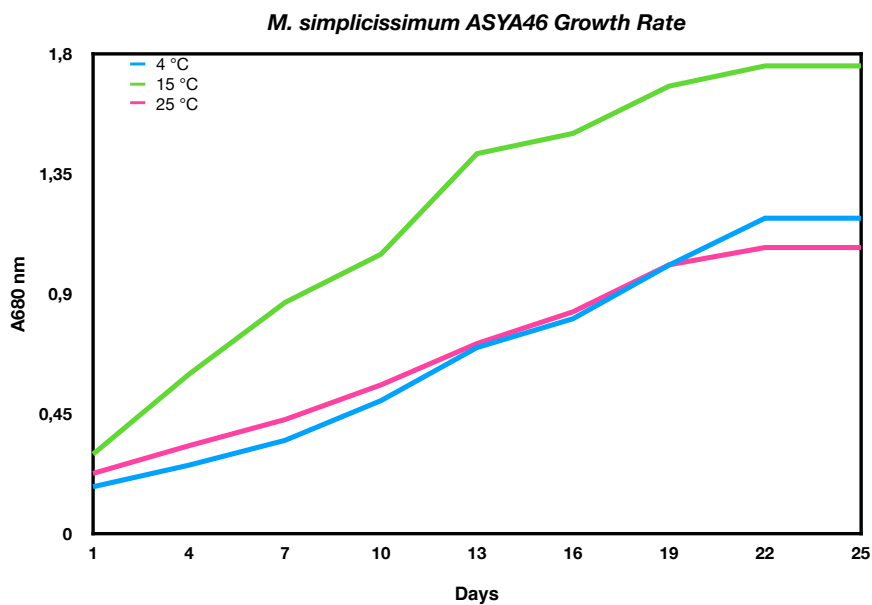


Figure 12. Growth rate and Fatty acids profile of ASYA46

M. simplicissimum ASYA 46 showed the best growth when it was incubated at 15 °C. The microalga biomass reached its maximum level on day 22 with approximately 1.7-fold higher cell density when compared to those incubated at 4 °C and 25 °C.

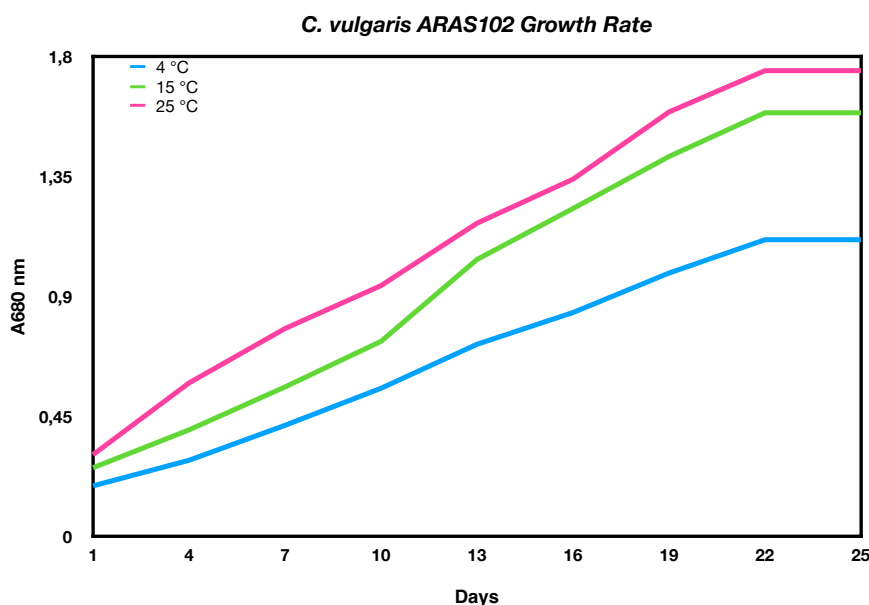


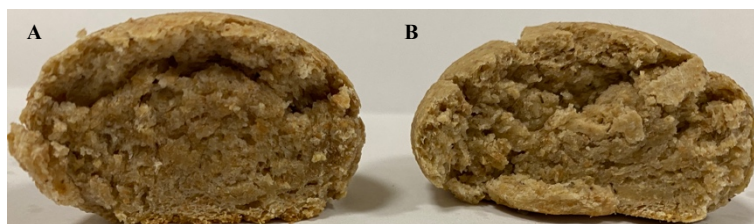
Figure 13. Growth rate and Fatty acids profile of ASYA46

When the growth graph for *C. vulgaris* ARAS102 at different temperatures was examined, the highest absorbance values were reached at 25 °C growth condition. The stationary stage was reached on the 22nd day for all three temperature conditions.

Considering the optimal growth efficiency of the strains, the psychrotolerant microalgae *C. vulgaris* ASYA27 and *M. simplicissimum* ASYA46 were grown under 15 °C and the mesophilic microalga *C. vulgaris* ARAS102 was grown under 25 °C for the following experiments.

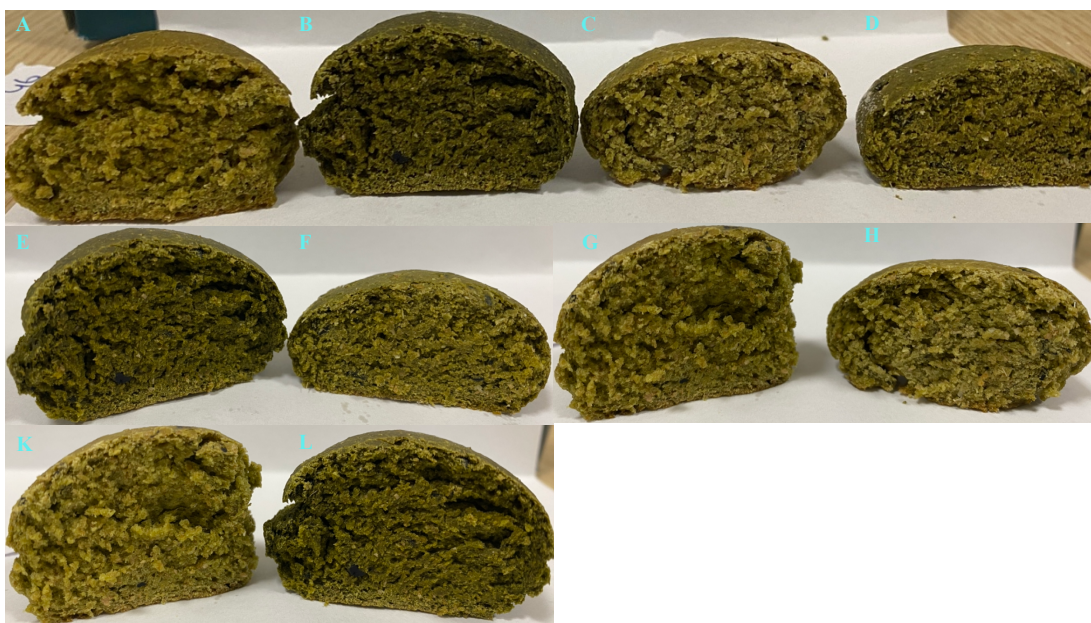
3.2 Dough

Control Group



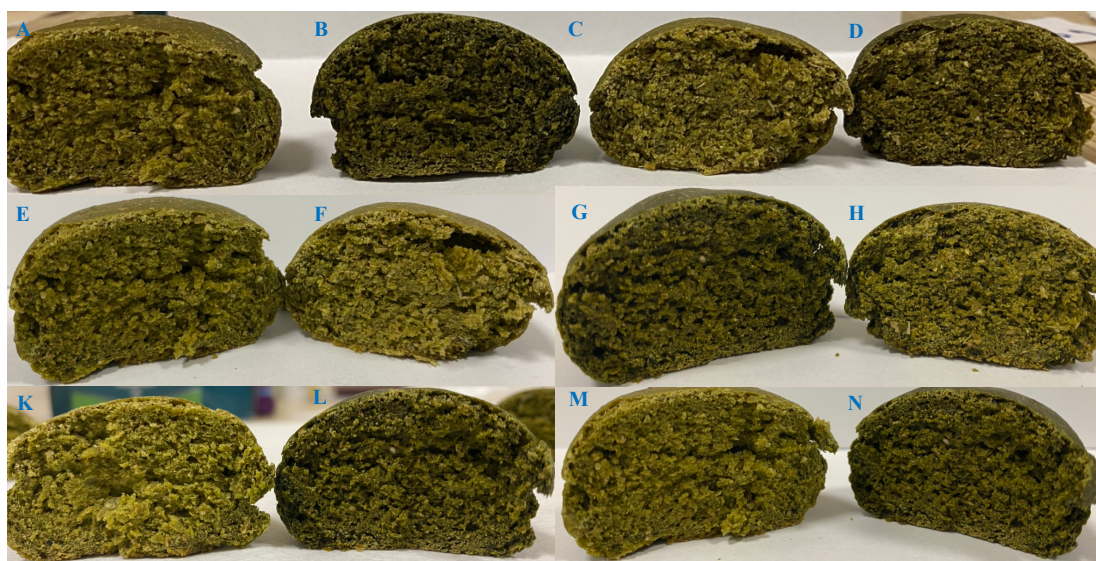
When the control group of whole wheat (a) and gluten-free (b) breads were examined; it was observed that the bread prepared with whole wheat was puffier.

Microalgae-containing dough formulation

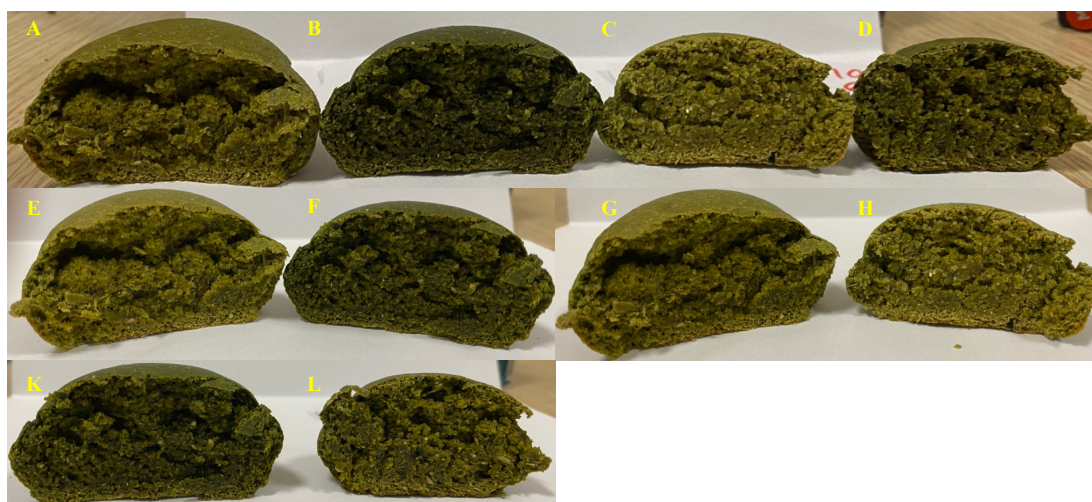


M. simplicissimum ASYA46 containing dough formulation **A:** 46-C/1; **B:** 46-C/3; **C:** 46-GF/1; **D:** 46-GF/3; **E:** 46-C/1; **F:** 46-C/3; **G:** 46-C/1; **H:** 46-GF/1; **K:** 46-C/3; **L:** 46-GF/3

When the bread enriched with *C. vulgaris* ASYA27 was examined, darkening in color was observed as expected as the microalgae percentage increased. However, it was observed that bread formulations prepared with whole wheat flour were fluffier. In addition, the color intensity of whole wheat flour bread was more vivid compared to bread prepared with gluten-free flour, regardless of the percentage.



C. vulgaris ASYA27 containing dough formulation **A:** 27-C/1; **B:** 27-C/3; **C:** 27-GF/1; **D:** 27-GF/3; **E:** 27-C/1; **F:** 27-GF/1; **G:** 27-C/3; **H:** 27-GF/3; **K:** 27-C/1; **L:** 27-C/3; **M:** 27-GF/1; **N:** 27-GF/3



C. vulgaris ARAS102 containing dough formulation **A:** 102-C/1; **B:** 102-C/3; **C:** 102-GF/1; **D:** 102-GF/3; **E:** 102-C/1; **F:** 102-C/3; **G:** 102-C/1; **H:** 102-GF/1; **K:** 102-C/3; **L:** 102-GF/3

Figure 14. ASYA27, ASYA46 and ARAS102 containing dough formulation.

The above statements also apply to bread formulations enriched with *M. simplicissimum* ASYA46, and *C. vulgaris* ARAS102. In general, the most intense color was observed in breads doped with *C. vulgaris* ARAS102. Puffiness was higher in breads prepared with whole wheat flour.

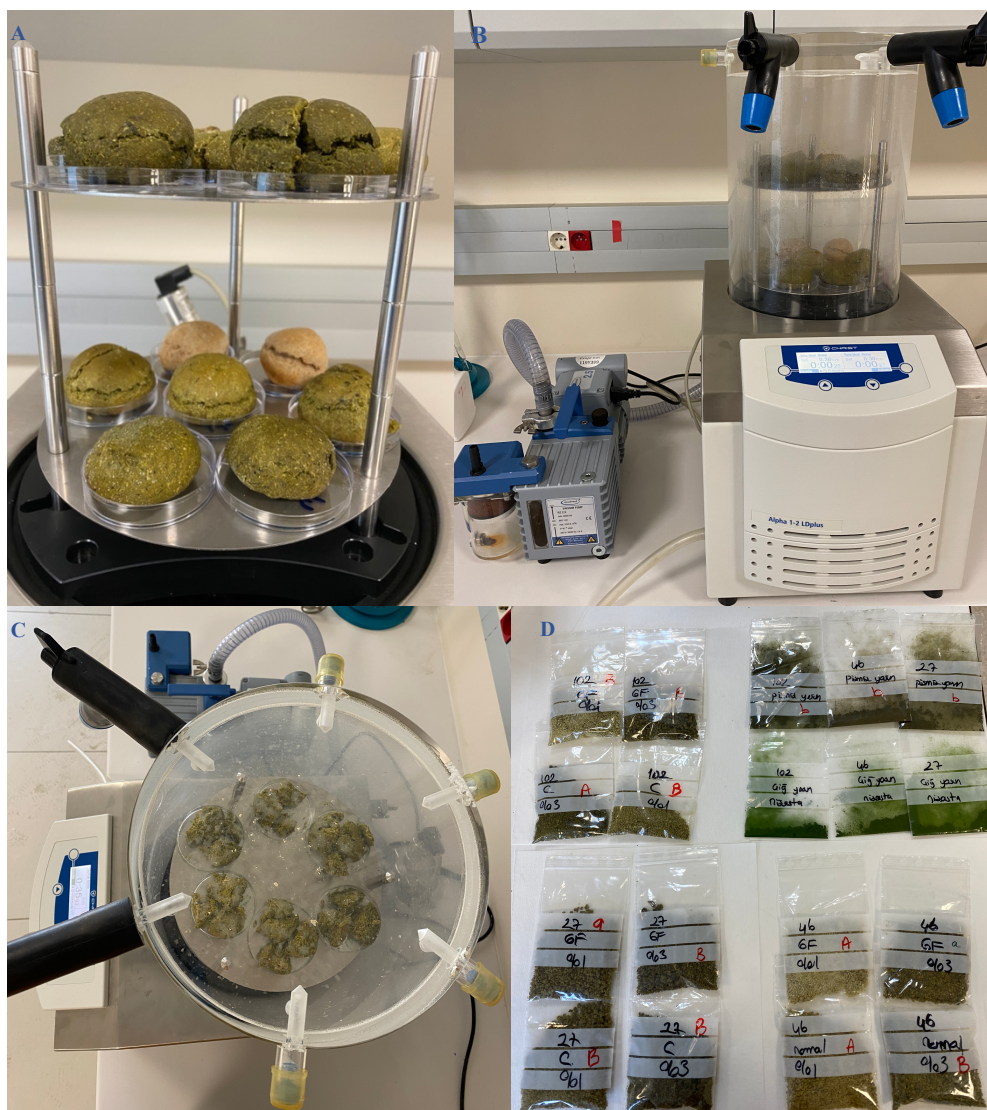


Figure 15. The lyophilization stage of bread

The lyophilized breads (A-B-C) were powdered with the help of a blender and packaged according to their groups (D). Although the samples were lyophilized, they were stored in a 4 °C refrigerator for the duration of the analysis just in case.

3.3 Minerals

Table 4. Mineral content of bread enriched with microalgae and control group.

Samples	Na (mg/1000 g)	K (mg/1000 g)	Mg (mg/1000 g)	Zn (mg/1000 g)	Ca (mg/1000 g)	Mn (mg/1000 g)	Fe (mg/1000 g)	Cu (mg/1000 g)	P (mg/1000 g)
Control-WW	3755 ± 0,33	1505 ± 0,07	846,5 ± 0,32	14,4 ± 0,33	599,3 ± 0,52	16,4 ± 0,24	28,3 ± 0,36	2,4 ± 0,33	1144,6 ± 0,21
Control-GF	3924,7 ± 0,38	1491,1 ± 0,23	826,2 ± 0,09	16,7 ± 0,22	648,3 ± 0,23	13,3 ± 0,07	46,1 ± 0,42	1 ± 0,09	1114,6 ± 0,21
27- WW-1	4136,2 ± 0,39	1647,1 ± 0,14	976,1 ± 0,12	16,3 ± 0,24	769,4 ± 0,37	18,5 ± 0,22	41,4 ± 0,27	11 ± 0,18	1361,4 ± 0,45
27- WW-3	4312,8 ± 0,77	1682,2 ± 0,15	1004,1 ± 0,09	18,1 ± 0,29	861 ± 0,39	20,5 ± 0,25	46,5 ± 0,42	14,3 ± 0,16	1423,5 ± 0,16
27-GF-1	4444,1 ± 0,28	1712,9 ± 0,18	910,1 ± 0,17	18,4 ± 0,36	781,4 ± 0,28	15,8 ± 0,11	72,2 ± 0,26	15,3 ± 0,16	1377,4 ± 0,15
27-GF-3	4562,7 ± 0,52	1790,3 ± 0,04	1054,1 ± 0,09	18,7 ± 0,47	867,6 ± 0,74	16,5 ± 0,35	74,9 ± 0,48	17 ± 0,42	1441,4 ± 0,20
46- WW-1	4542,1 ± 0,21	1670,2 ± 0,05	976,4 ± 0,39	16,1 ± 0,20	844,6 ± 0,40	24,4 ± 0,26	31,2 ± 0,16	4,5 ± 0,28	1289,7 ± 0,25
46- WW-3	4577,8 ± 0,40	1906,1 ± 0,03	1066 ± 0,06	18,4 ± 0,24	877,3 ± 0,61	27,5 ± 0,29	50,5 ± 0,38	8,9 ± 0,45	1448,4 ± 0,38
46-GF-1	4442 ± 0,62	1788,2 ± 0,04	927,1 ± 0,16	20,4 ± 0,28	820,4 ± 0,38	16,9 ± 0,39	88,2 ± 0,59	6,6 ± 0,42	1210,6 ± 0,32
46-GF-3	4501,6 ± 0,39	1828,3 ± 0,12	1095,1 ± 0,09	21,2 ± 0,20	865,4 ± 0,24	18,3 ± 0,30	107,5 ± 0,27	9,7 ± 0,23	1423,2 ± 0,06
102- WW-1	4121,5 ± 0,63	1721,2 ± 0,16	1017,1 ± 0,09	16,8 ± 0,23	881,3 ± 0,29	24,5 ± 0,14	32,1 ± 0,40	4,2 ± 0,19	1448,4 ± 0,26
102- WW-3	4212,6 ± 0,47	1832 ± 0,22	1186 ± 0,05	17,6 ± 0,29	987,9 ± 0,69	25,2 ± 0,13	57,5 ± 0,29	5 ± 0,32	1536,5 ± 0,35
102-GF-1	4612,2 ± 0,32	1621,5 ± 0,18	1046,1 ± 0,09	19,6 ± 0,30	899,7 ± 0,60	13,8 ± 0,27	71,7 ± 0,29	5,5 ± 0,40	1411,6 ± 0,26
102-GF-3	4651,4 ± 0,31	1854,4 ± 0,04	1096,1 ± 0,08	21,4 ± 0,38	956,3 ± 0,22	15,3 ± 0,21	100,3 ± 0,20	6,5 ± 0,24	1528,2 ± 0,12
27-RM/MB	1778,6 ± 0,69	3739,2 ± 0,29	3576,1 ± 0,08	73,5 ± 0,17	7833,1 ± 0,50	117,4 ± 0,31	656,3 ± 0,58	106,1 ± 0,14	8504,4 ± 0,45
46-RM/MB	1737,9 ± 0,47	4118,2 ± 0,05	3429 ± 0,05	52,2 ± 0,19	7080,7 ± 0,56	114,2 ± 0,27	506 ± 0,46	115,4 ± 0,35	6672,5 ± 0,35
102-RM/MB	1815,1 ± 0,20	4257,4 ± 0,46	4385,1 ± 0,16	38,5 ± 0,21	10255 ± 0,16	111,4 ± 0,29	634,3 ± 0,33	31,3 ± 0,32	9123,5 ± 0,15

Breads and seaweeds were evaluated for nine different minerals and 3 different heavy metals. Mercury, lead, and arsenic content were found to be below the detection limit of 0.013 ppm. This shows that the products and seaweeds do not pose a risk in terms of heavy metals. When the minerals were analyzed, an increase was observed in every mineral without exception compared to the control group. Detailed evaluation is presented in the graphs below.

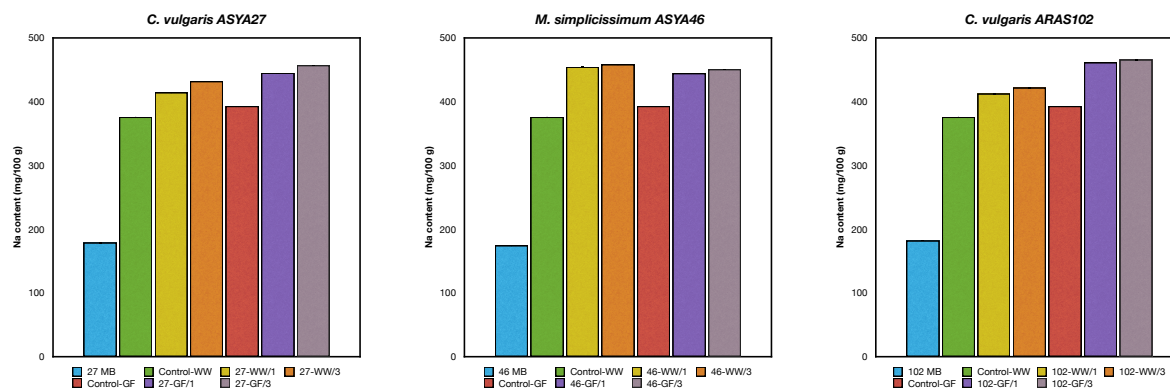


Figure 16. Na content

The Na content of all three microalgae strains was somewhat similar. All three microalgae caused significant increases in the Na content of dough preparations. The increase in sodium content with microalgae percentage was seen in all three microalgae. The highest increase of 21.9% compared to the control group occurred in whole wheat bread enriched with 3% *M. simplicissimum* ASYA46. The largest change was observed in gluten-free breads with 16.3% and 18.5% in breads supplemented with *C. vulgaris* ASYA27 and *C. vulgaris* ARAS102, respectively.

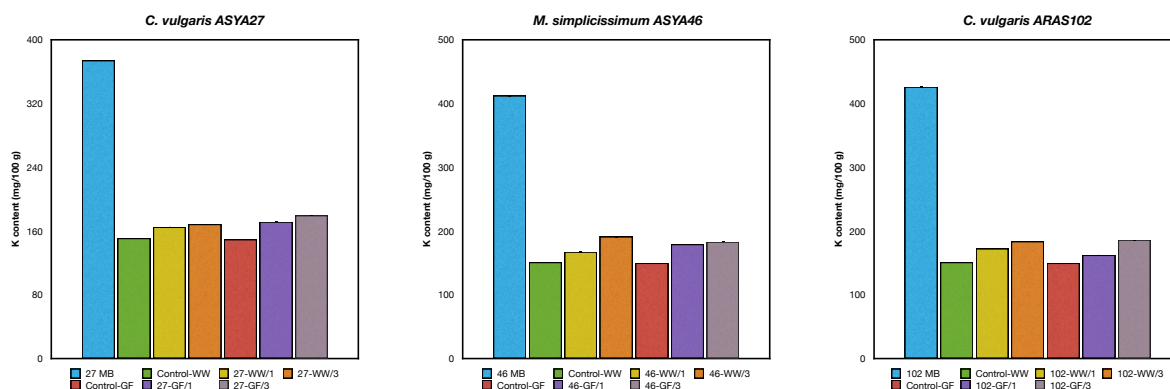


Figure 17. K content

The potassium content of bread enriched with *C. vulgaris* ASYA27 increased in parallel with the microalgae percentage. The greatest change compared to the control group was observed in the 3% microalgae supplementation of the gluten-free group with 20.1%. Whole wheat bread stood out with 26.6% in bread supplemented with *M. simplicissimum* ASYA46 and gluten-free group with 24.4% in bread supplemented with *C. vulgaris* ARAS102. As expected, the highest potassium increases were observed in breads supplemented with 3% microalgae.

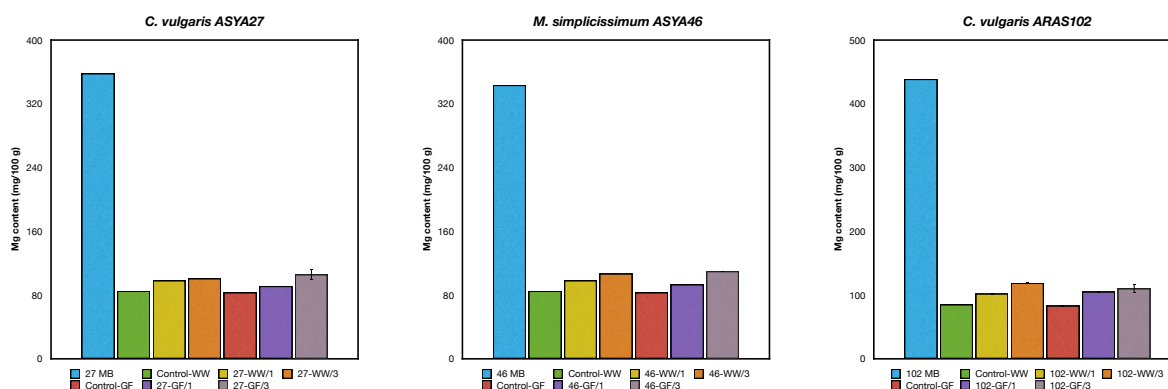


Figure 18. Mg content

The magnesium content of the microalga *C. vulgaris* ASYA27 was recorded as 3.57 mg/g. When this microalga was added to whole wheat at 1% or 3%, the total Mg concentration increased by 15.3% and 18.6%. When gluten-free bread was made with *C. vulgaris* ASYA27, an increase of 10.2% and 27.6% in Mg concentration was recorded with the addition of 1% and 3% microalga to the dough preparation. For *M. simplicissimum* ASYA46, the increase in the gluten-free group stood out, while for *C. vulgaris* ARAS102, the whole wheat bread group stood out.

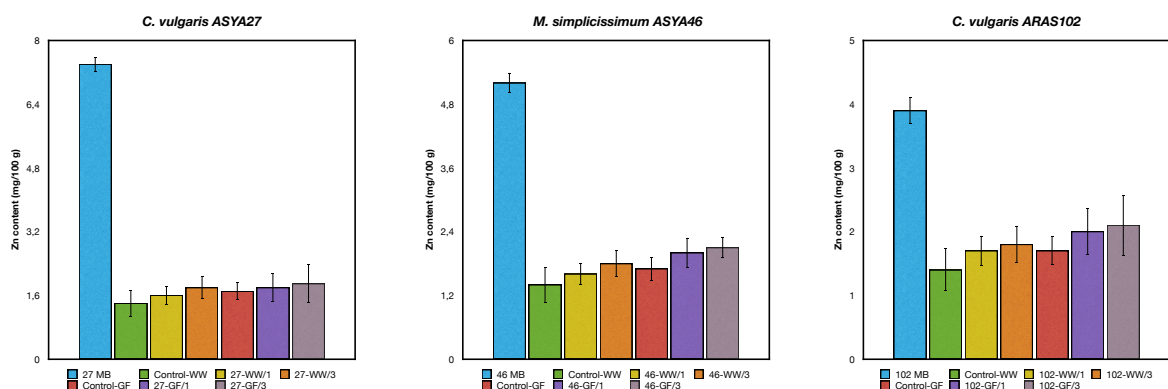


Figure 19. Zn content

The zinc content of bread enriched with *C. vulgaris* ASYA27 increased with the percentage of microalgae. The most striking increase compared to the control group was seen in the group with 3% microalgae addition to whole wheat bread with 25.3%. In general, a change between 10.5% and 25.3% was observed. The most remarkable increase occurred in gluten-free bread to which *C. vulgaris* ARAS102 was added with 28.7%. *M. simplicissimum* followed with a 27.7% zinc increase in whole wheat bread. Overall, an increase of 11.4-28.7% was calculated compared to the control.

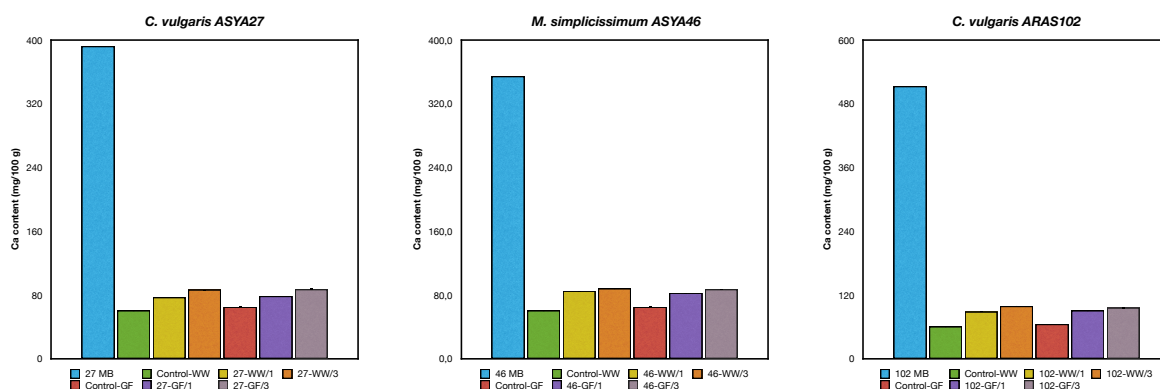


Figure 20. Ca content

Among the microalgae, the prominent species in terms of calcium was *C. vulgaris* ARAS102 with 512.7 mg/100. In parallel, the most remarkable increase occurred in whole wheat bread supplemented with *C. vulgaris* ARAS102 at 3%. Among the other species, *C. vulgaris* ASYA27 showed the highest change in whole wheat bread with 43.7%, while *M. simplicissimum* ASYA46 increased in whole wheat bread with 46.4%. The most striking change for all three microalgae occurred in whole wheat bread. The highest increase in gluten-free bread occurred in the group to which *C. vulgaris* ARAS102 was added at 47.5%.

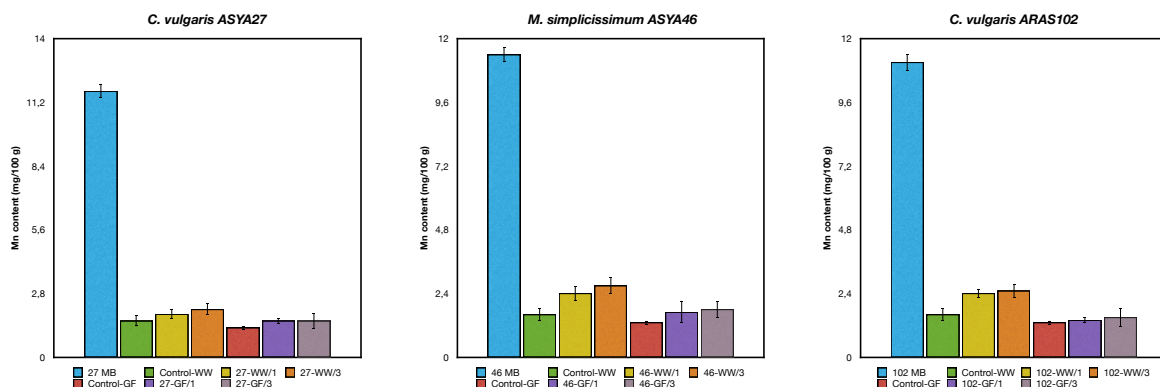


Figure 21. Mn content

The manganese increases by 67.8% in whole wheat bread to which *M. simplicissimum* ASYA46 was added was the most remarkable change. Increases ranged from 27.0% to 67.8%. The manganese content of all three microalgae was measured at almost similar levels. Supplementation of whole wheat bread with 3% microalgae gave the highest results for all three microalgae. The prominent group for gluten-free was the group with 3% *M. simplicissimum*. In addition, the manganese content in microalgae was found to be 10 times higher than the manganese content of the control group bread.

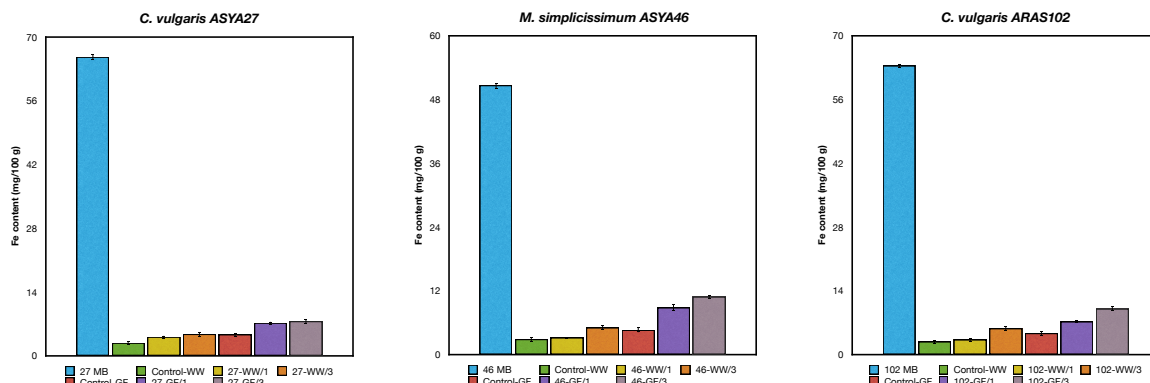


Figure 22. Fe content

The iron content of bread enriched with *C. vulgaris* ARAS102 increased in parallel with the percentage of microalgae. The most remarkable change of 117.7% compared to the control group was observed in the 3% microalgae fortification of the gluten-free flour group. In general, a change between 13.2% and 117.7% was observed compared to the control group. The iron content of *C. vulgaris* ASYA27 and ARAS102 was higher than that of *M. simplicissimum* ASYA46. The greatest change provided by the three microalgae occurred in the gluten-free bread group.

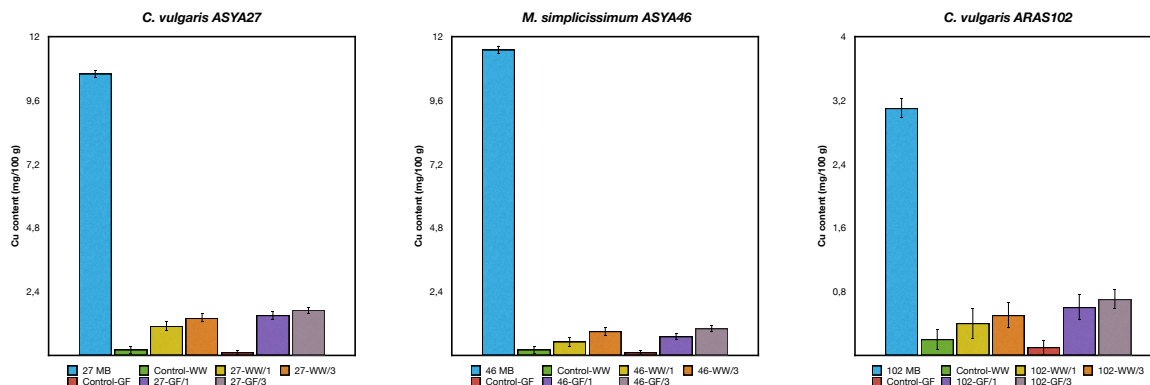


Figure 23. Cu content

Copper was the element that led to the most remarkable increase among minerals. While the copper content of Antarctic microalgae was around 10.6-11.5%, the copper content of the mesotherm *C. vulgaris* ARAS102 microalgae was measured as 3.1%. The copper content in psychrotolerant microalgae was 50 and 100 times higher than in whole wheat bread and gluten-free bread, respectively. Therefore, the addition of microalgae to bread showed remarkable increases in its content. In particular, the highest increase of 847.9% was measured in gluten-free bread to which *M. simplicissimum* ASYA46 was added.

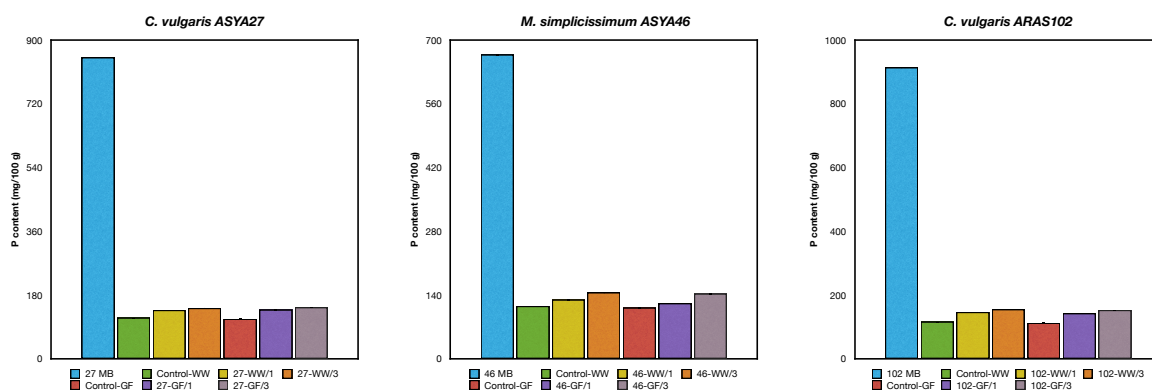


Figure 24. P content

Phosphorus content was measured as 850-912 mg/100 g in microalgae. As expected, the percentage of phosphorus increased as the percentage of microalgae increased. In the bread to which *C. vulgaris* ASYA27 microalgae was added, the highest increase was seen in the gluten-free bread with 29.3%, the most remarkable increase in the group to which *M. simplicissimum* ASYA46 was added was seen in the gluten-free group with 27.7%, and the change for *C. vulgaris* ARAS102 was seen in the gluten-free group with 36.2%. The three microalgae contributed to the bread at similar rates.

3.4 Moisture

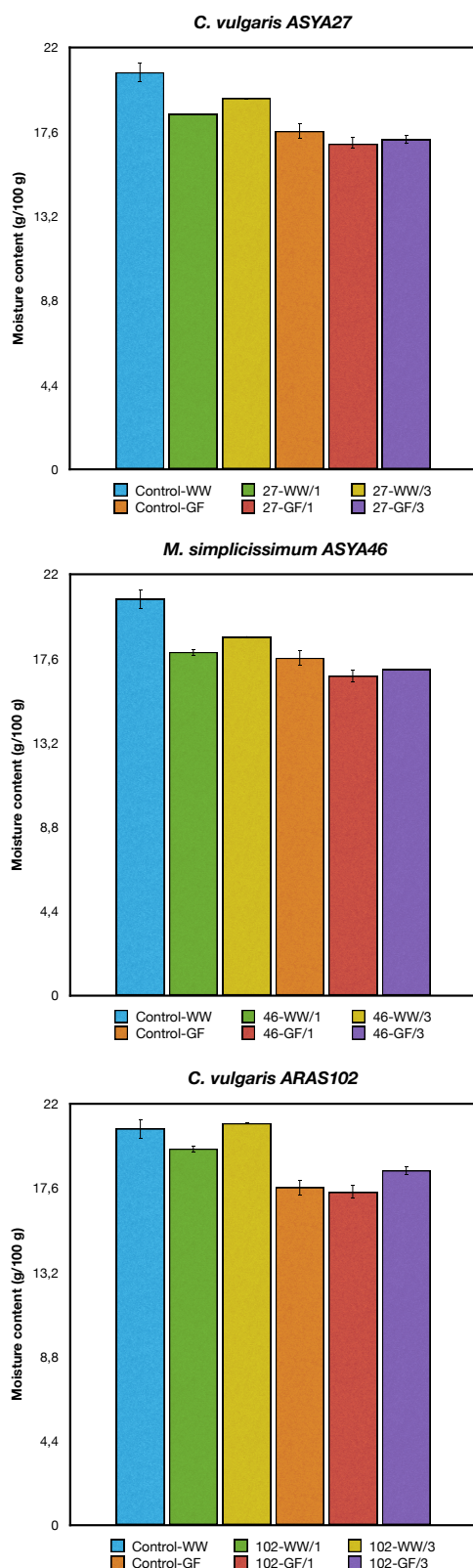


Figure 25. Moisture content

Although the moisture content of the breads decreased compared to the control group, the moisture content increased with increasing percentage among the breads supplemented with microalgae. The formulation with the highest moisture content in the group with *C. vulgaris* ASYA27 microalgae was whole wheat bread with 19.33%.

For *M. simplicissimum*, the leading formulation in terms of moisture content was whole wheat bread with 3% microalgae added with 18.7%. Moisture content increased with the percentage of microalgae.

The moisture content of breads containing *C. vulgaris* ARAS102 increased with the percentage of microalgae. The highest increase was seen in whole wheat flour bread formulation with 20.9%.

3.5 ASH

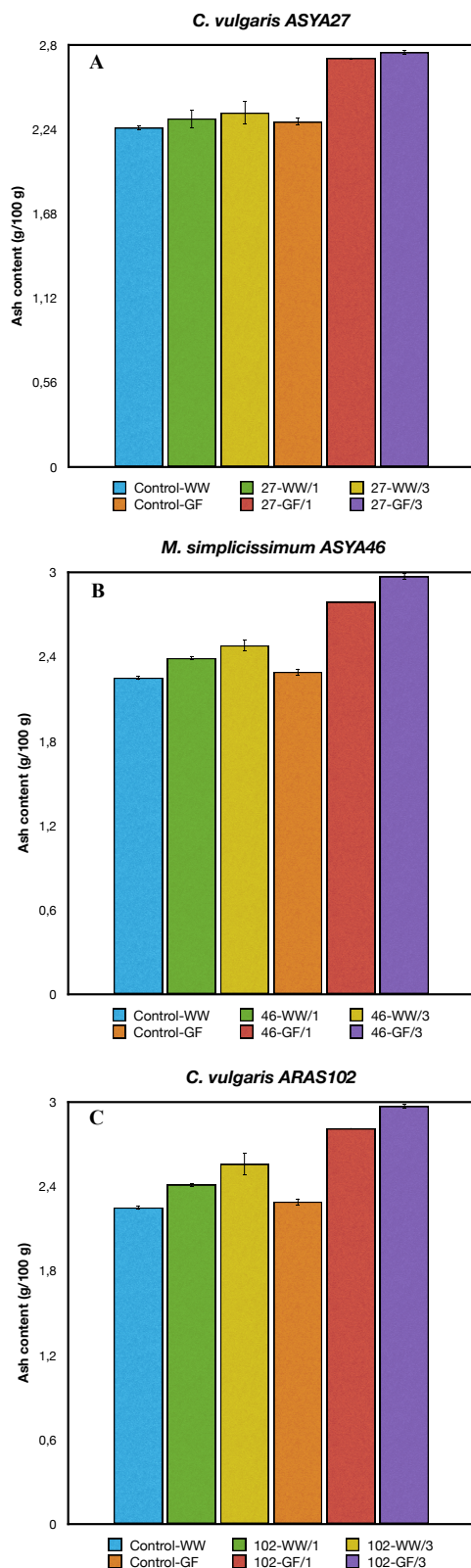


Figure 26. Ash content

The ash content of breads supplemented with *C. vulgaris* ASYA27 increased with the percentage of microalgae. The highest increase was 20.2% in the gluten-free dough formulation with 3% *M. simplicissimum*. The lowest increase was seen in the group with whole wheat flour and 1% microalgae with 2.7%.

The gluten-free flour dough formulation enriched with *M. simplicissimum* ASYA46 at 3% showed an ash increase of 29.9%. The lowest increase was seen in whole wheat flour with 1% microalgae addition with 6%.

The ash content of bread with *C. vulgaris* ARAS102 increased with the percentage of seaweed. The highest increase was seen in gluten-free flour bread with 29.6%. The lowest increase was seen in whole wheat flour with 1% microalgae addition with 6.7%.

3.6 PROTEIN

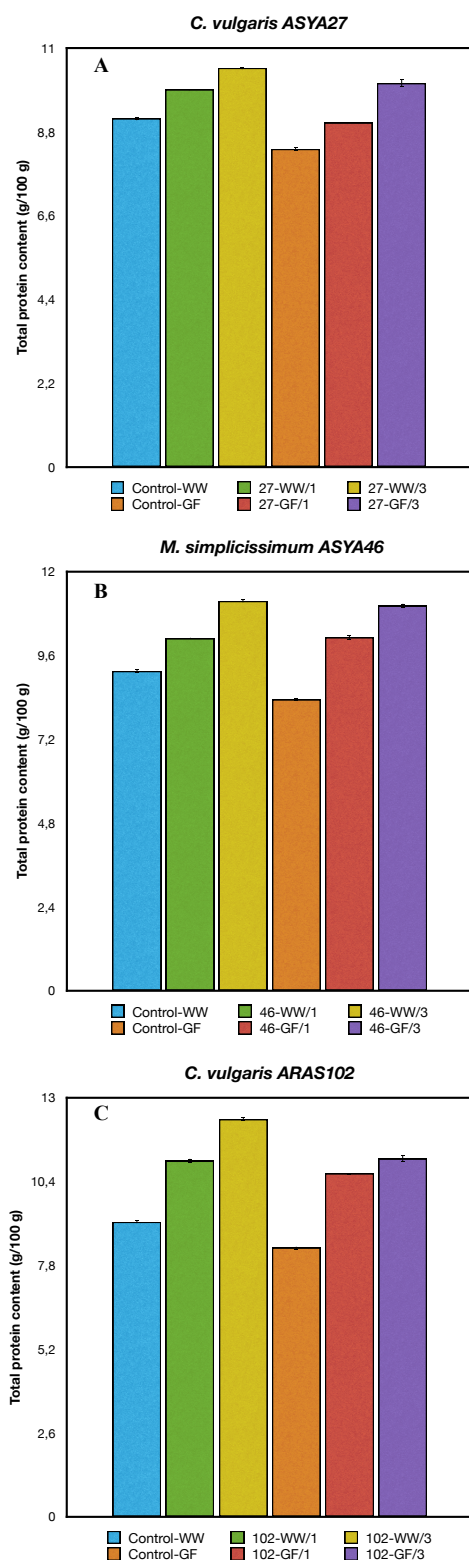


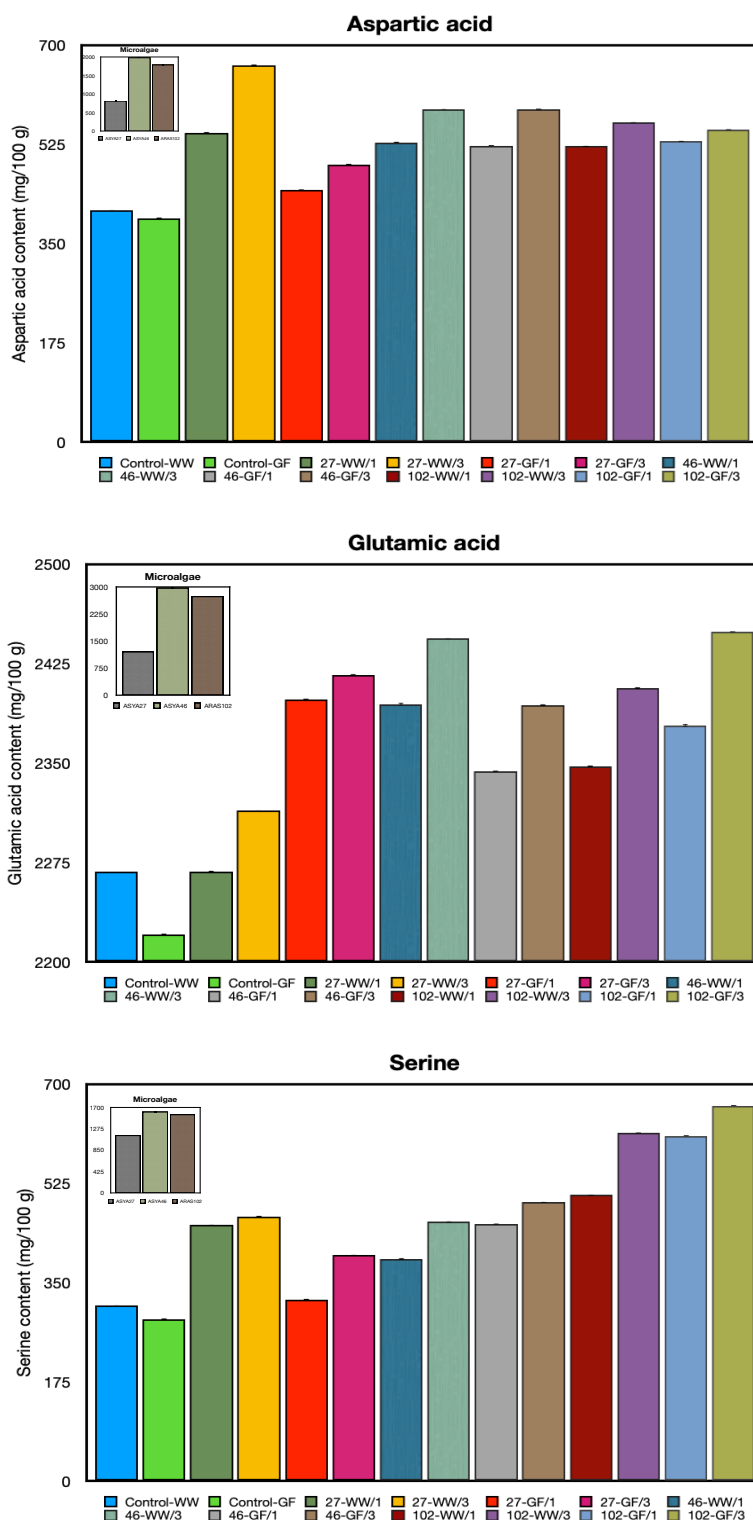
Figure 27. Protein content

The protein content of bread with *C. vulgaris* ASYA27 supplementation increased with the percentage of microalgae. The highest increase was seen in gluten-free flour bread with 20.7%. The lowest increase was seen in whole wheat flour with 1% microalgae addition with 8.2%.

The addition of microalgae into the dough formulations caused significant increments in the total protein content of the bread. The total protein content of whole-wheat bread increased by 10.2% and 21.9% while a 21.2% and 32.1% increase was recorded when *M. simplicissimum* was added with 1% or 3% ratios, respectively.

Likewise, *C. vulgaris* ARAS102 increased the total protein content of whole wheat and gluten-free breads. 1% or 3% addition of *C. vulgaris* ARAS102 caused 20.5% and 34.7% increase in whole wheat, 27.5% and 33% increase in gluten-free bread.

3.7 AMINO ACIDS

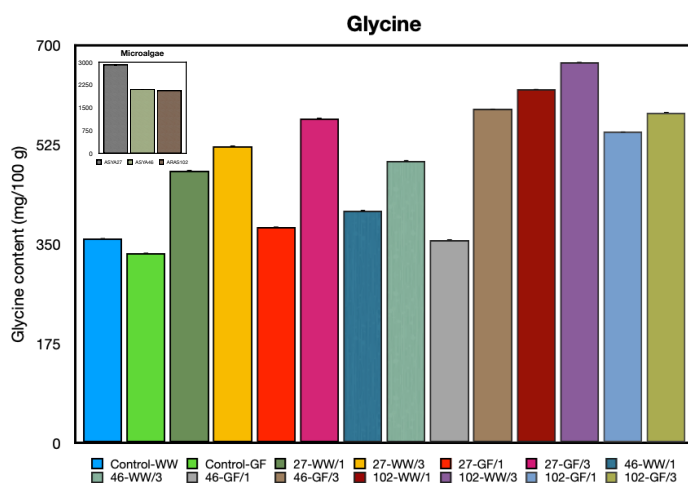


The whole wheat bread enhanced with *C. vulgaris* ASYA27 and 3 percent with 62.7 percent showed the most rise in aspartic acid values when compared to the control group. An increase was noted in tandem with the microalgae percentage increase.

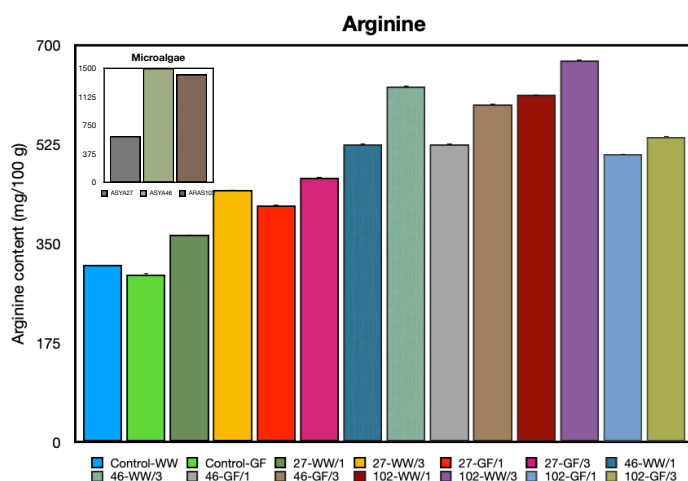
The gluten-free bread supplemented with *C. vulgaris* ARAS102 and 3 percent and 10.3 percent showed the highest increase in glutamic acid values compared to the control group. In general, the percentage of microalgae increased in parallel with the increase in glutamic acid levels.

For serine amino acid, the greatest increase was observed in ARAS102 with 131.7% and in the enriched gluten-free bread with 3%. *M. simplicissimum* ASYA46 also exhibited higher serine content than other microalgae.

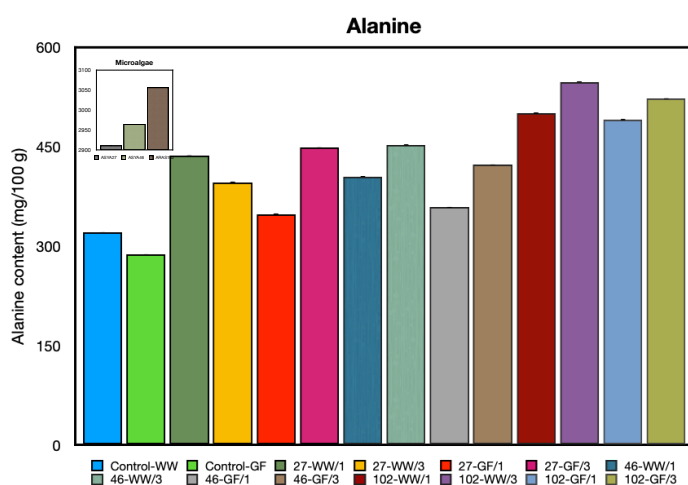
Figure 28. Aspartic acid, glutamic acid, and serine content



When glycine values were compared with the control group, the greatest increase was observed in the gluten-free bread enriched with ARAS102 and 3% with 74.1%. In general, an increase in glutamic acid level was observed parallel to the increase in microalgae percentage.

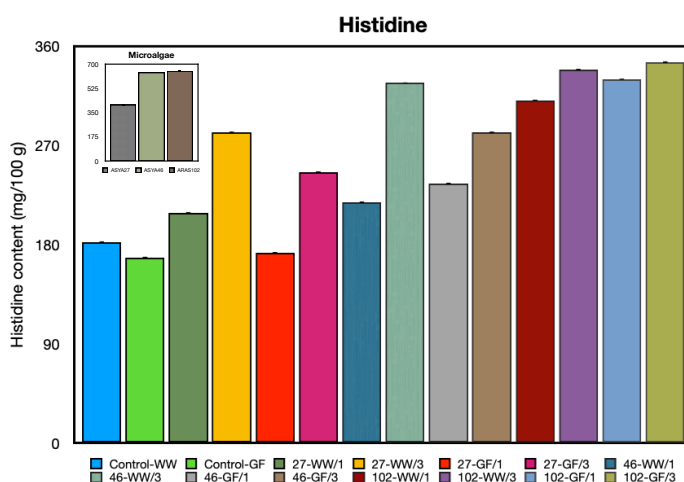


When the arginine values were compared with the control group, the greatest increase was observed in the gluten-free bread enriched with ARAS102 and 3% with 116.0%. In general, an increase in glutamic acid level was observed parallel to the increase in microalgae percentage.

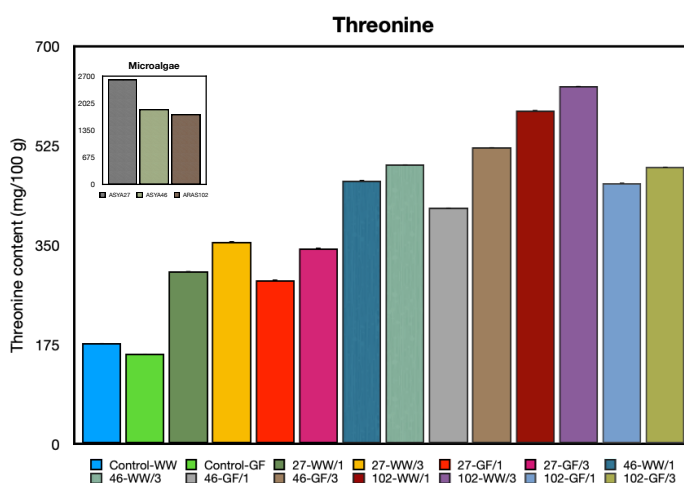


When the alanine values were compared with the control group, the greatest increase was observed in the gluten-free bread enriched with 3% ARAS102 with 81.7%. In general, an increase in glutamic acid level was observed parallel to the increase in microalgae percentage.

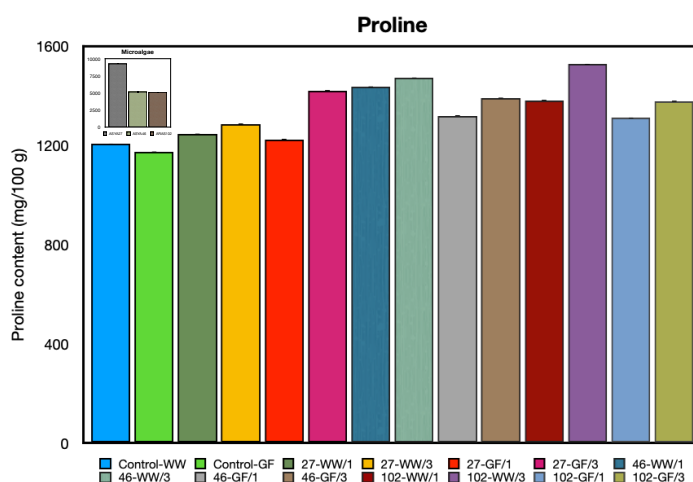
Figure 29. Glycine, arginine, and alanine content



When histidine values were compared with the control group, the greatest increase was observed in the gluten-free bread enriched with ARAS102 and 3% with 105.1%. In general, an increase in glutamic acid level was observed parallel to the increase in microalgae percentage.

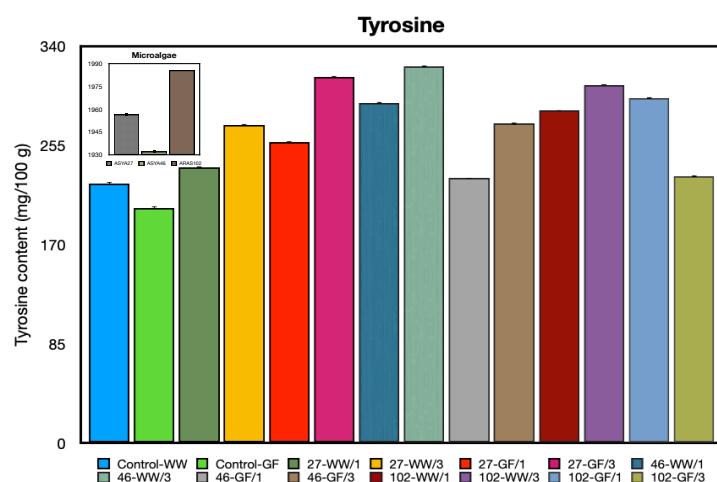


When threonine values were compared with the control group, the greatest increase was observed in whole wheat flour bread enriched with 3% ARAS102 with 254.6%. In general, an increase in glutamic acid level was observed parallel to the increase in microalgae percentage.

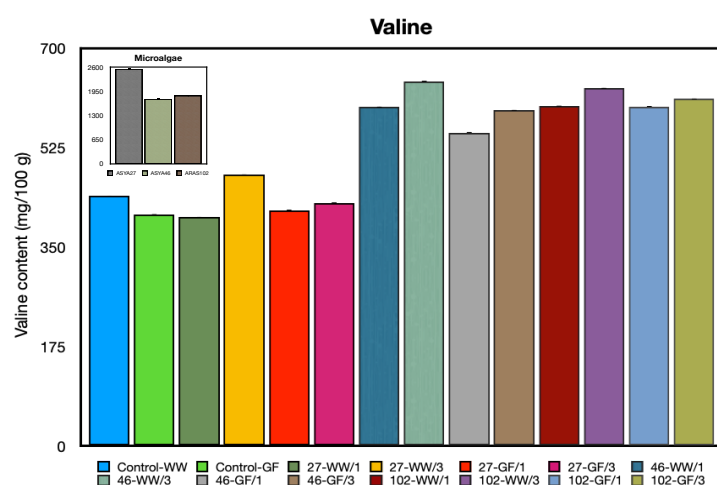


When the proline values were compared with the control group, the highest increase was observed in whole wheat flour bread enriched with 3% ARAS102 with 26.7%. In general, an increase in glutamic acid level was observed parallel to the increase in microalgae percentage.

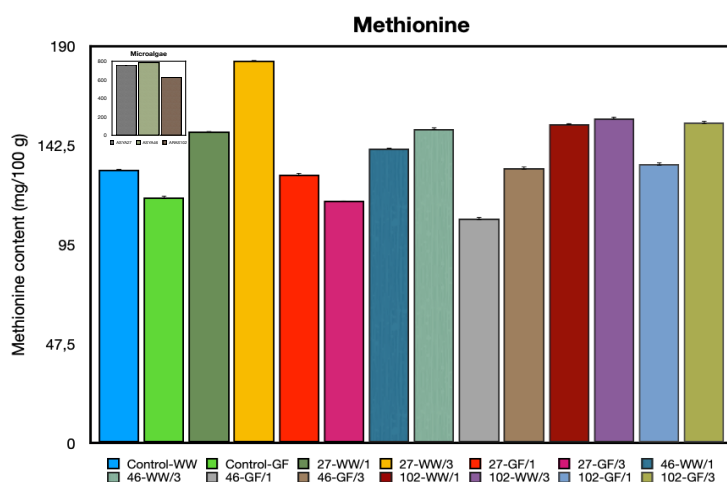
Figure 30. Histidine, threonine, and proline content



When the tyrosine values were compared with the control group, the greatest increase was observed in gluten-free flour bread enriched with ASYA27 and 3% with 55.5%. In general, an increase in glutamic acid level was observed parallel to the increase in microalgae percentage.

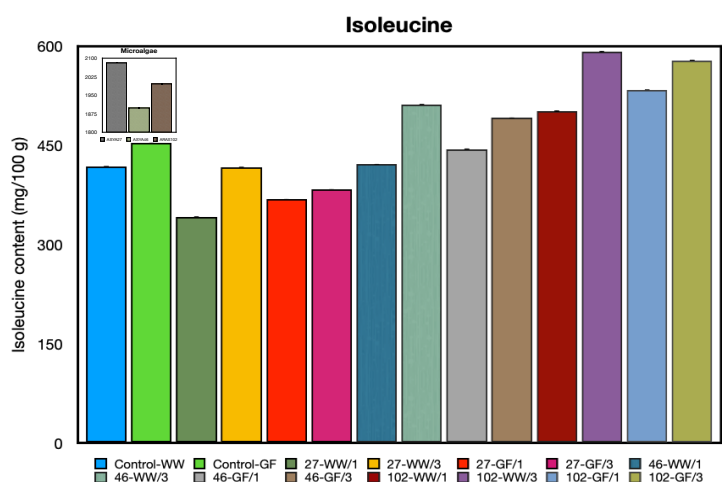


When the valine values were compared with the control group, the greatest increase was observed in the gluten-free flour bread enriched with 3% ARAS102 with 50.0%. In general, an increase in glutamic acid level was observed parallel to the increase in microalgae percentage.

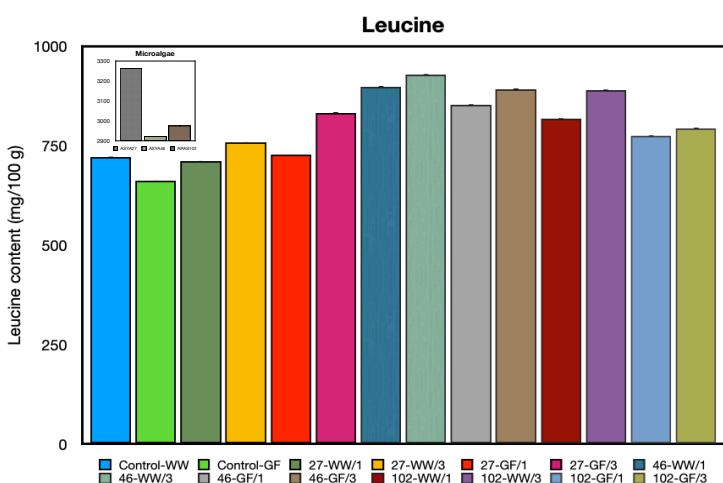


When methionine values were compared with the control group, the greatest increase was in whole wheat flour bread enriched with 3% ASYA27 with 39.7%. In general, an increase in glutamic acid level was observed parallel to the increase in microalgae percentage.

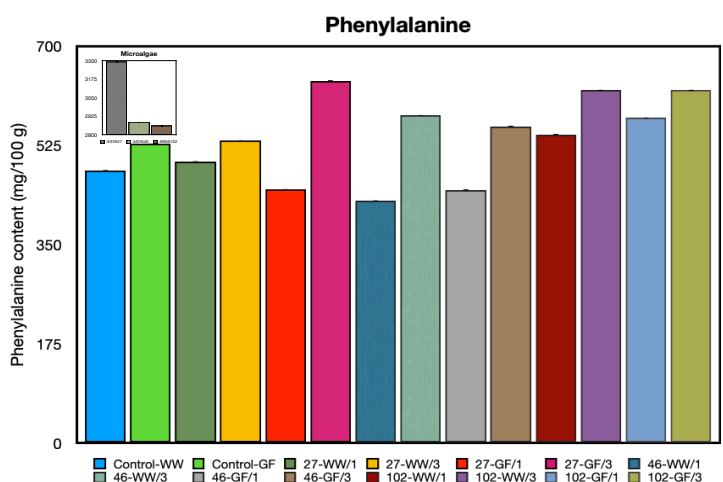
Figure 31. Tyrosine, valine, and methionine content



When isoleucine values were compared with the control group, the greatest increase was observed in whole wheat bread enriched with 3% ASYA27 with 41.5%. In general, an increase in glutamic acid level was observed parallel to the increase in microalgae percentage.

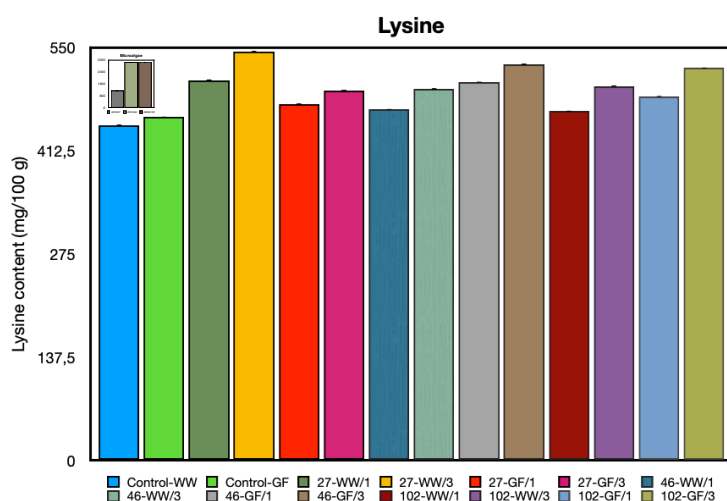


When leucine values were compared with the control group, the greatest increase was observed in gluten-free flour bread enriched with ASYA46 and 3% with 34.9%. In general, an increase in glutamic acid level was observed parallel to the increase in microalgae percentage.

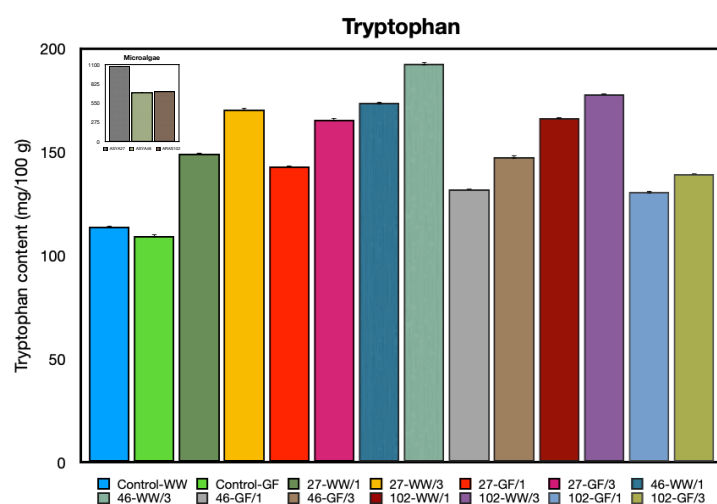


When phenylalanine values were compared with the control group, the greatest increase was observed in whole wheat flour bread enriched with 3% ARAS102 with 29.5%. In general, an increase in glutamic acid level was observed parallel to the increase in microalgae percentage.

Figure 32. Isoleucine, leucine, and phenylalanine content



When the lysine values were compared with the control group, the greatest increase was observed in whole wheat flour bread enriched with ASYA27 and 3% with 21.9%. In general, an increase in glutamic acid level was observed parallel to the increase in microalgae percentage.

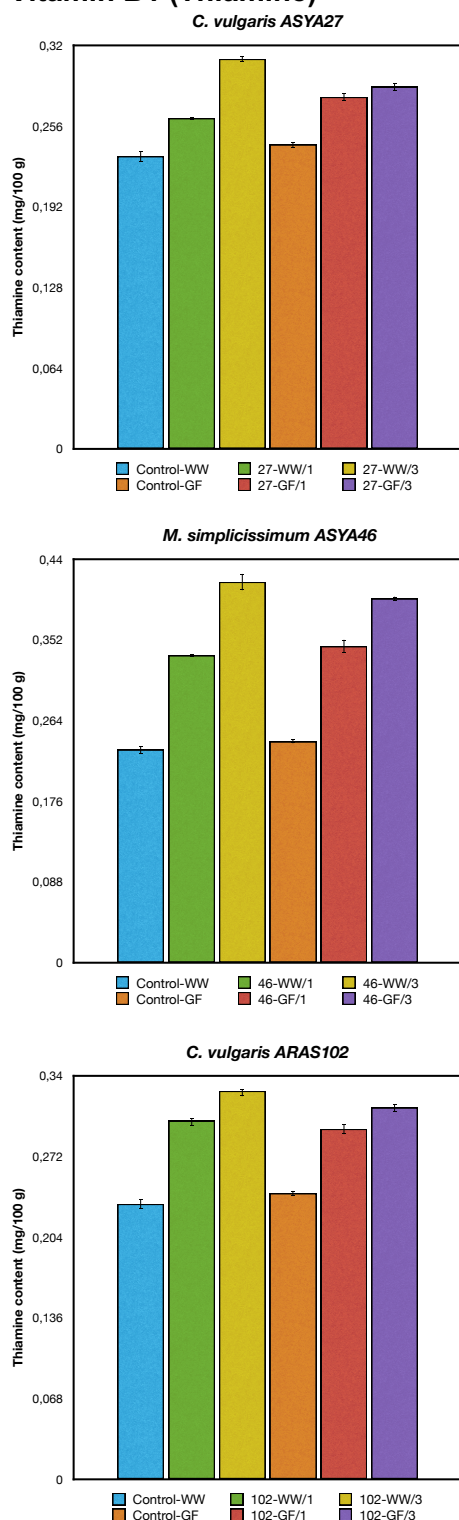


When tryptophan values were compared with the control group, the highest increase was observed in whole wheat flour bread enriched with 3% ASYA46 with 69.1%. In general, an increase in glutamic acid level was observed parallel to the increase in microalgae percentage.

Figure 33. Lysine and tryptophan content

3.8 Vitamin

Vitamin B1 (Thiamine)



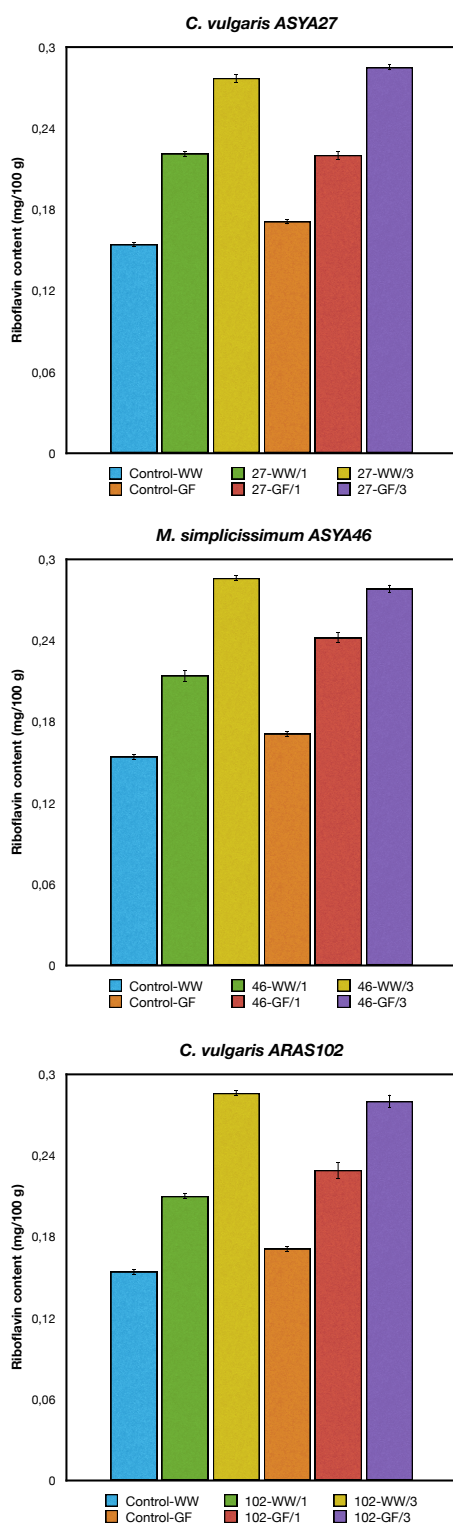
The thiamine content of bread with and without ASYA27 supplementation increased steadily with the percentage of seaweed. The highest increase with 0.077% was observed in the 3% microalgae supplemented group of whole wheat flour bread. The lowest increase was 0.030% in whole wheat flour bread with 1% microalgae supplementation.

The thiamine content of bread with and without ASYA46 supplementation increased steadily with the percentage of algae. The highest increase was 0.183% in whole wheat flour bread with 3% microalgae supplementation. The lowest increase was 0.103% in whole wheat flour bread with 1% microalgae supplementation.

The thiamine content of bread with and without ARAS102 supplementation increased steadily with the percentage of algae. The highest increase with 0.094% was observed in the 3% microalgae-supplemented group of whole wheat flour bread. The lowest increase was 0.054% in the 1% microalgae-supplemented group with gluten-free flour.

Figure 34. Vitamin B1 content

3.9 Vitamin B2 (Riboflavin)



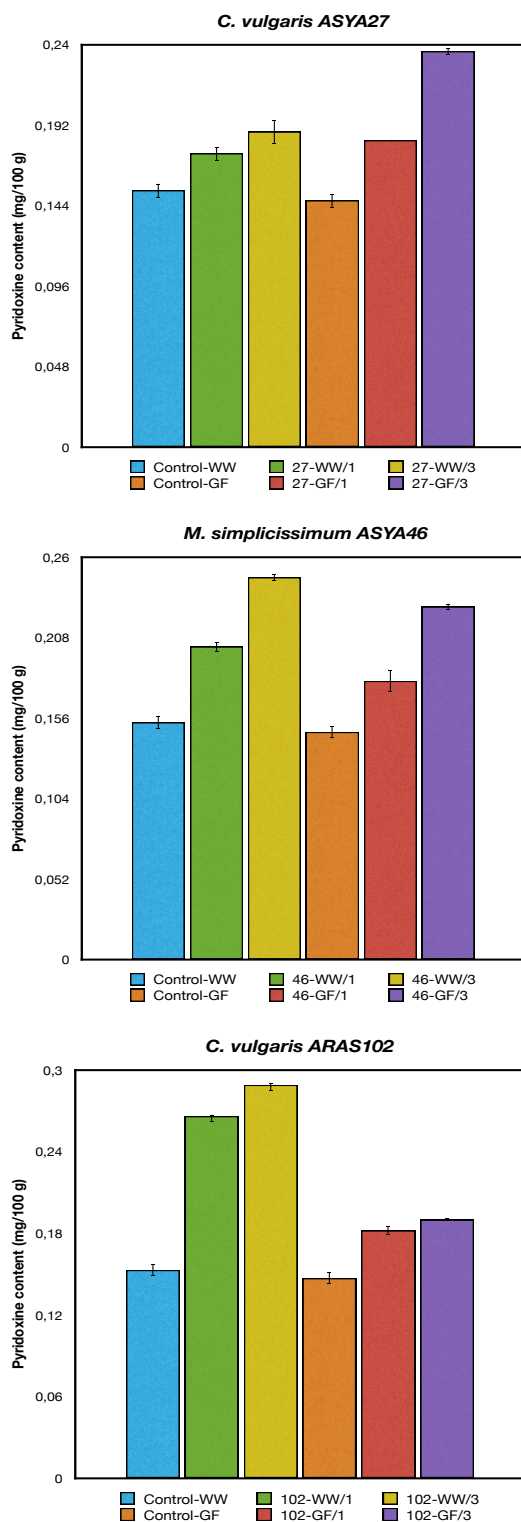
The riboflavin content of bread with and without ASYA27 supplementation increased steadily with the percentage of algae. The highest increase was 0.123% in the 3% microalgae-supplemented group of whole wheat flour bread. The lowest increase was 0.050% in the 1% microalgae-supplemented group with gluten-free flour.

The riboflavin content of bread with and without ASYA46 supplementation increased steadily with the percentage of algae. The highest increase with 0.131% was observed in the 3% microalgae supplemented group of whole wheat flour bread. The lowest increase was 0.060% in the 1% group with gluten-free flour.

The riboflavin content of bread with and without ARAS102 supplementation increased steadily with the percentage of algae. The highest increase of 0.131% was observed in the 3% microalgae-supplemented group of whole wheat flour bread. The lowest increase was 0.056% in the 1% microalgae-supplemented group of whole wheat flour bread.

Figure 35. Vitamin B2 content

3.10 Vitamin B6



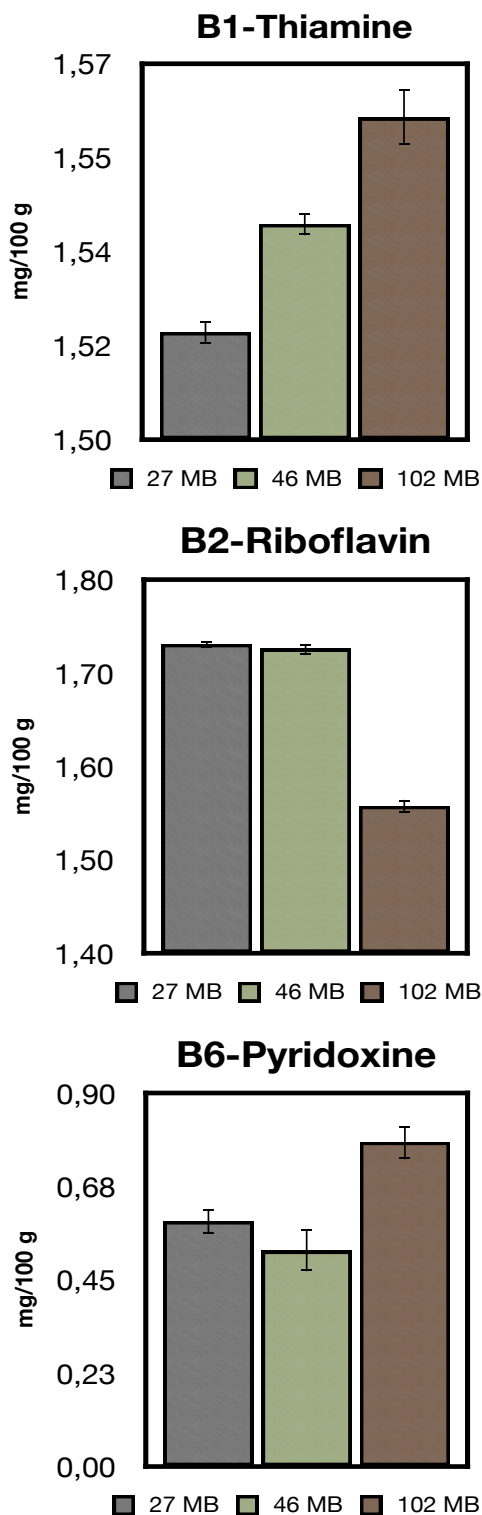
The pyridoxine content of bread with and without ASYA27 supplementation increased steadily with the percentage of algae. The highest increase was 0.089% in the 3% microalgae-supplemented group of gluten-free flour bread. The lowest increase was 0.022% in the 1% microalgae-supplemented group with whole wheat flour.

The pyridoxine content of bread with and without ASYA46 supplementation increased steadily with the percentage of algae. The highest increase was 0.094% in whole wheat flour bread with 3% microalgae supplementation. The lowest increase was 0.033% in whole wheat flour bread with 1% microalgae supplementation.

The pyridoxine content of bread with and without ARAS102 supplementation increased steadily with the percentage of algae. The highest increase was 0.136% in the 3% microalgae-supplemented group of whole wheat flour bread. The lowest increase was 0.035% in the 1% microalgae-supplemented group with gluten-free flour.

Figure 36. Vitamin B6 content

3.11 Vitamin Content



As a result of thiamine analysis in the biomass of *C. vulgaris* ASYA27, *M. simplicissimum* ASYA46, and ARAS102 microalgae, the highest value was found in *C. vulgaris* ARAS102 microalga with 1.56 mg/100 g.

Riboflavin level of *C. vulgaris* ASYA27 and *M. simplicissimum* ASYA46 was around 1.7mg/100 g while it was measured as 1.55mg/100g in *C. vulgaris* ARAS102.

Pyridoxine levels of psychrotolerant strains were similar while mesophilic *C. vulgaris* ARAS102 includes higher level of pyridoxine with a value of 0.78 mg/100g biomass.

Figure 37. Vitamin content of microalgae

3.12 Total Dietary Fiber

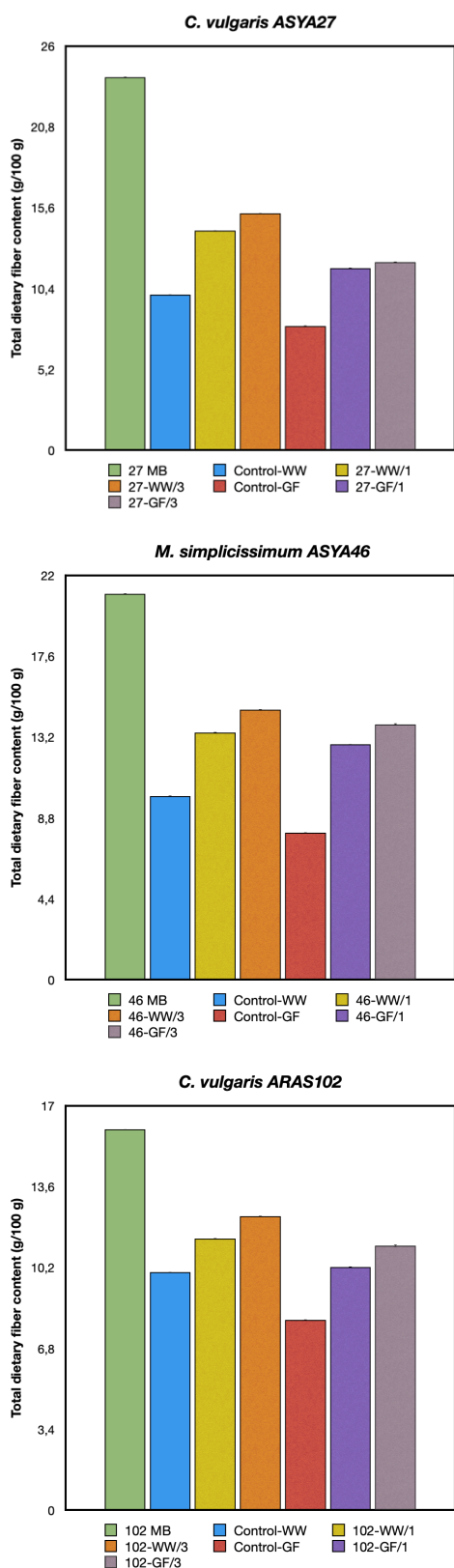


Figure 38. Total dietary fiber content

Enrichment of bread with *C. vulgaris* ASYA27 increased the fiber content. The biggest change was seen in whole wheat breads when *C. vulgaris* ASYA27 was added at 3% with an increase of 52.3%.

M. simplicissimum ASYA46 also increased the fiber content of the bread to which it was added. The biggest change was seen in gluten-free breads when ASYA46 was added at 3% with a change of 73.8%.

As expected, there was an increase in the fiber content of the bread to which *C. vulgaris* ARAS102 was added. The biggest change was seen in gluten-free breads when ARAS102 was added at 3% with a change of 39%.

3.13 Starch Content

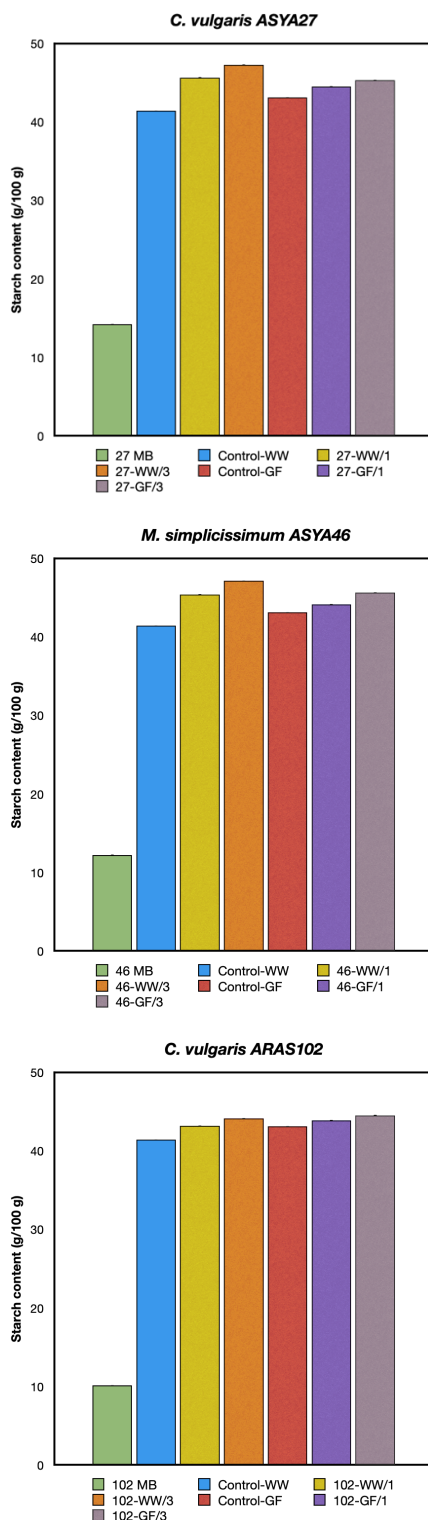


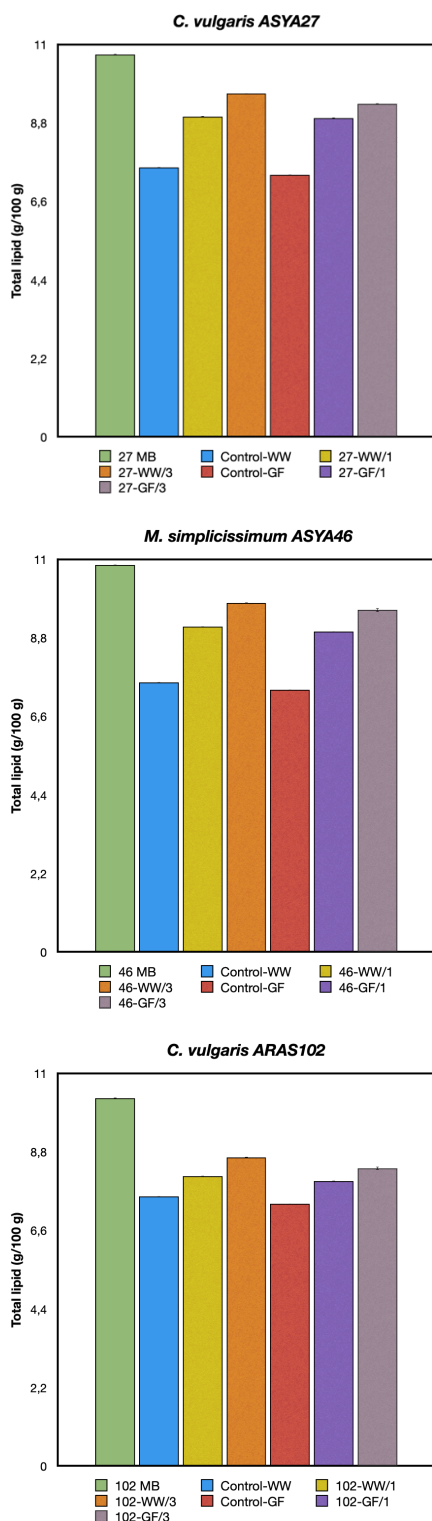
Figure 39. Starch content

An increase of 3.3-14.3% was observed in the starch content of the bread to which ASYA27 was added. The most striking change occurred in the whole wheat bread group with 3% microalgae addition with 14.3%.

The starch content of the bread to which ASYA46 was added changed between 2.4-13.8%. The most striking increase occurred in the whole wheat bread group with 3% microalgae addition with 13.8%.

There was also a change between 1.8-6.6% in the bread to which ARAS102 was added. The most striking increase occurred in the whole wheat bread group with 3% microalgae addition with 6.6%.

3.14 Total Lipid



The total lipid content of the bread supplemented with ASYA27 varied between 18.9-27.4%. The most striking increase was seen in the whole wheat bread group with 3% microalgae supplementation with 27.4%.

The total lipid content of the ASYA46 bread also varied between 20.7-30.5%. The highest change was observed in the gluten-free bread group with 3% microalgae addition with 30.5%.

The total lipid content of ARAS102-supplemented breads varied between 7.5-14.6%. The highest change was observed in the whole wheat bread group with 3% microalgae addition with 7.5%.

Figure 40. Total lipid content

3.15 Fatty Acids

Table 5. Fatty acid profile of breads with/without ASYA27

Formula	% Fatty Acids	ASYA27	Control- WW	27- WW/1	27- WW/3	Control- GF	27- GF/1	27- GF/3
C4:0	Butyric acid	0	0,56	0,14	0,13	0,67	0,21	0,18
C6:0	Caproic acid	0	0,45	0,15	0,15	0,69	0,29	0,22
C8:0	Caprylic acid	0,23	0,1	0,09	0,1	0,05	0,19	0,12
C10:0	Capric acid	0,58	0,44	0,22	0,22	0,63	0,37	0,31
C12:0	Lauric acid	0,03	0,75	0,27	0,27	0,16	0,45	0,4
C14:0	Myristic acid	0,47	0,92	1,05	1,08	0,94	2,83	1,67
C15:0	Pentadecanoic acid	0,21	0,34	0,1	0,1	0,07	0,15	0,14
C16:0	Palmitic acid	25,56	14,83	13,81	13,2	14,15	18,03	15,49
C17:0	Heptadecanoic acid	6,11	0,28	0,16	0,17	0,16	0,21	0,18
C18:0	Stearic acid	1,01	4,56	3,75	3,73	3,91	4,74	4,12
C20:0	Arachidic acid	0	0,37	0,35	0,32	0,39	0,42	0,34
C21:0	Henicosanoic acid	0	0,02	0,03	0,03	0,03	0,45	0
C22:0	Docosanoic acid	0	0,31	0,09	0,09	0,1	0,13	0,08
C23:0	Tricosanoic acid	0	0,03	0,01	0,02	0,21	0,03	0,02
C24:0	Lignoceric acid	0,8	0,05	0,04	0,04	0,04	0,07	0,05
	ΣSFA	35	23,45	20,12	19,52	21,53	28,36	23,14
C14:1	Myristoleic acid	0	0,23	0,07	0,07	0,04	0,1	0,1
C16:1	Palmitoleic acid	7,64	0,9	0,84	0,87	0,79	0,8	0,82
C18:1,trans n9	Elaidic acid	0,09	1,16	0,01	0,16	0,1	0,24	0,26
C18:1, cis (n9)	Oleic acid	26,22	62,08	64,08	64,19	65,91	58,45	59,37
C20:1,Cis-11 n9	Eicosenoic acid	0,95	0,25	0,25	0,26	0,26	0,31	0,32
C22:1 (n9)	Erucic acid	0	0,32	0,14	0	0,33	0,06	0,08
C24:1 (n9)	Nervonic acid	0,06	0	0	0	0	0	0,01
	ΣMUFA	34,96	64,94	65,39	65,55	67,43	59,96	60,96
C18:2,cis (n6)	Linoleic acid	29,05	10,96	13,12	13,4	10,40	11,02	14,76
C18:3 (n3)	α-Linolenic acid	0,75	0,65	0,87	1	0,64	0,66	1,14
C20:2,Cis-11,14	Eicosadienoic acid	0,21	0	0	0	0	0	0
C20:3,Cis-8,11	Eicosatrienoic acid	0,03	0	0,5	0,53	0	0	0
	ΣPUFA	30,04	11,61	14,49	14,93	11,04	11,68	15,9
	TOTAL	100	100	100	100	100	100	100

In the whole wheat bread group, addition of the microalga in dough formulations increased the unsaturation ratio of the breads. saturated fatty acid content decreased from 23.5% in the control group to 19.52% with the addition of 3% *C. vulgaris* ASYA27 biomass. On the other hand, PUFA content increased from 11.61% to 14.93%. When it comes to gluten-free bread, the PUFA content increased from 11.04% to 15.9% while monounsaturated fatty acid levels decreased from 67.43% to 60.96% because of 3% microalgal biomass addition.

Table 6. Fatty acid profile of breads with/without ASYA46

Formula	% Fatty Acids	ASYA46	Control- WW	46- WW/1	46- WW/3	Control- GF	46- GF/1	46- GF/3
C4:0	Butyric acid	0	0,56	0,54	0,19	0,67	0,16	0,14
C6:0	Caproic acid	0	0,45	0,2	0,2	0,69	0,69	0,2
C8:0	Caprylic acid	0,14	0,1	0,14	0,13	0,05	0,11	0,11
C10:0	Capric acid	0,87	0,44	0,28	0,31	0,63	0,27	0,27
C12:0	Lauric acid	0,04	0,75	0,37	0,37	0,16	0,34	0,35
C14:0	Myristic acid	0,43	0,92	1,41	1,46	0,94	1,36	1,44
C15:0	Pentadecanoic acid	0,13	0,34	0,12	0,14	0,07	0,11	0,13
C16:0	Palmitic acid	26,79	14,83	15,86	15,3	14,15	15,04	16,01
C17:0	Heptadecanoic acid	0,06	0,28	0,19	0,18	0,16	0,77	0,18
C18:0	Stearic acid	0,56	4,56	4,22	4,13	3,91	4,15	4,14
C20:0	Arachidic acid	0	0,37	0,37	0,33	0,39	0,54	0,3
C21:0	Henicosanoic acid	0	0,02	0	0	0,03	0,03	0
C22:0	Docosanoic acid	0	0,31	3,4	0,09	0,1	0,11	0,11
C23:0	Tricosanoic acid	0	0,03	0,04	0,01	0,21	0,02	0,06
C24:0	Lignoceric acid	1,45	0,05	0,05	0,04	0,04	0,05	0,06
	ΣSFA	30,47	23,45	26,65	22,69	21,53	23,59	23,36
C14:1	Myristoleic acid	0	0,23	0,08	0,1	0,04	0,08	0,09
C16:1	Palmitoleic acid	6,3	0,9	0,81	0,88	0,79	0,89	0,82
C18:1,trans n9	Elaidic acid	0,09	1,16	0,21	0,48	0,1	0,21	0,23
C18:1, cis (n9)	Oleic acid	19,76	62,08	57,79	59,49	65,91	58,91	60,75
C20:1,Cis-11 n9	Eicosenoic acid	0	0,25	0,26	0,28	0,26	0,31	0,28
C22:1 (n9)	Erucic acid	0	0,32	0,81	0	0,33	0,06	0
C24:1 (n9)	Nervonic acid	0	0	0,17	0,57	0	0,89	0
	ΣMUFA	26,15	64,94	60,13	61,8	67,43	61,35	62,17
C18:2,cis (n6)	Linoleic acid	24,17	10,96	12,35	13,96	10,40	13,83	13,21
C18:3 (n3)	α-Linolenic acid	18,99	0,65	0,87	1,55	0,64	1,23	1,26
C20:2,Cis-11,14	Eicosadienoic acid	0,22	0	0	0	0	0	0
C20:3,Cis-8,11	Eicosatrienoic acid	0	0	0	0	0	0	0
	ΣPUFA	43,38	11,61	13,22	15,51	11,04	15,06	14,47
	TOTAL	100	100	100	100	100	100	100

Addition of *M. simplicissimum* ASYA46 into the dough formulations caused significant changes in the unsaturation level of the breads. The saturated fatty acid levels did not show remarkable variations while PUFA levels increased from 11.61% to 15.51% in whole wheat breads, from 11.04% to 14.47% in gluten free breads when the microalga was added in 3% ratio.

Table 7. Fatty acid profile of breads with/without ARAS102

Formula	% Fatty Acids	ARAS102	Control- WW	102- WW/1	102- WW/3	Control- GF	102- GF/1	102- GF/3
C4:0	Butyric acid	0	0,56	0,16	0,17	0,67	0,26	0,24
C6:0	Caproic acid	0	0,45	0,59	0,18	0,69	0,87	0,25
C8:0	Caprylic acid	2,7	0,1	0,14	0,11	0,05	0,16	0,14
C10:0	Capric acid	3,1	0,44	0,28	0,26	0,63	0,39	0,36
C12:0	Lauric acid	0,45	0,75	0,33	0,32	0,16	0,48	0,44
C14:0	Myristic acid	1,12	0,92	1,69	1,28	0,94	1,8	1,71
C15:0	Pentadecanoic acid	0,4	0,34	0,12	0,12	0,07	0,16	0,15
C16:0	Palmitic acid	28,12	14,83	17,9	13,86	14,15	18,65	15,04
C17:0	Heptadecanoic acid	5,7	0,28	0,17	0,16	0,16	0,18	0,18
C18:0	Stearic acid	7,6	4,56	4,07	3,67	3,91	4,24	4,14
C20:0	Arachidic acid	0,7	0,37	0,38	0,29	0,39	0,34	0,32
C21:0	Henicosanoic acid	0	0,02	0	0	0,03	0,14	0
C22:0	Docosanoic acid	0	0,31	0,13	0,09	0,1	0,08	0,1
C23:0	Tricosanoic acid	1,61	0,03	0,04	0,01	0,21	0,41	0
C24:0	Lignoceric acid	4,5	0,05	0,06	0,02	0,04	0,04	0,02
	ΣSFA	56	23,45	25,9	20,37	21,53	27,94	22,85
C14:1	Myristoleic acid	0	0,23	0,08	0,09	0,04	0,12	0,11
C16:1	Palmitoleic acid	6,7	0,9	0,75	0,81	0,79	0,84	0,86
C18:1,trans n9	Elaidic acid	0	1,16	0,21	0,21	0,1	0,23	0,28
C18:1, cis (n9)	Oleic acid	14,1	62,08	58,17	60,65	65,91	54,84	58,13
C20:1,Cis-11 n9	Eicosenoic acid	0	0,25	0,27	0,25	0,26	0,31	0,27
C22:1 (n9)	Erucic acid	0	0,32	0	0	0,33	0	0
C24:1 (n9)	Nervonic acid	0,1	0	0	0	0	0	0
	ΣMUFA	20,09	64,94	59,48	62,01	67,43	56,34	59,65
C18:2,cis (n6)	Linoleic acid	20,9	10,96	13,72	16,34	10,40	14,62	16,43
C18:3 (n3)	α-Linolenic acid	2,2	0,65	0,9	1,28	0,64	1,1	1,07
C20:2,Cis-11,14	Eicosadienoic acid	0	0	0	0	0	0	0
C20:3,Cis-8,11	Eicosatrienoic acid	0	0	0	0	0	0	0
	ΣPUFA	23,1	11,61	14,62	17,62	11,04	15,72	17,5
	TOTAL	100	100	100	100	100	100	100

The saturation level of the whole wheat bread decreased from 23.45% to 20.37% when 3% *C. vulgaris* ARAS102 was added as an ingredient. The addition of *C. vulgaris* improved the PUFA content of the bread from 11.61% to 17.62%.

4. DISCUSSION

4.1 Structural View

In general, when compared to the control group. As the percentage of microalgae increases, the bread turns a darker green color. The attractive green tones also varied according to the type of microalgae used. The most pronounced and darkest green was observed in bread supplemented with ARAS102. However, there is a difference between gluten-free and whole-wheat bread in terms of puffiness. The main reason for this is the tendency of gluten to retain water and air (Don et al., 2005). Namely, the protein added due to gluten deficiency reduces the volume. This is because the added protein disrupts the disulfide bonds between the gluten, causing the gluten network to retain a lower amount of air (Abedi & Pourmohammadi, 2021).

4.2 Moisture

Moisture refers to the amount of water present in a food sample. Accurate measurement of moisture in food is critical as it affects the product's overall quality, stability, and shelf life. Moisture absorption properties in agricultural and food products play a vital role in various technological processes (Dadmohammadi & Datta, 2020). These processes include drying, transportation, packaging, storage, mixing, freeze-drying, and other operations that require prediction of food stability, shelf life, glass transition temperature, and drying time and texture. Knowledge of moisture absorption isotherms and models helps calculate the thermodynamic energies associated with moisture in products, leading to increased efficiency in food processing. Furthermore, the equilibrium moisture content of a food system is influenced by the interaction between the material and the environment. This is particularly important in the storage of food products, as higher moisture content can lead to spoilage through physical, chemical, and microbiological processes. The moisture content of bread plays a crucial role in the quality and shelf life of wheat-based products. Moisture content affects the texture, taste, and overall freshness of bread. The ideal moisture content for bread is usually around 30-

35% (Różyło et al., 2015). Too high a moisture content can result in a wet and dense texture and encourage the growth of mold and bacteria (Rokey et al., 2010). On the other hand, if the moisture content is too low, the bread can become dry, crumbly, and stale (Baik & Chinachoti, 2000). Furthermore, the moisture content of bread can be influenced by factors such as the type of flour used, the fermentation process, and storage conditions. It is important to note that enzymes such as lipases and proteases can indirectly affect the moisture content of bread. Lipases and proteases can break down lipids and proteins, affecting the structure and moisture-holding capacity of bread dough.

When the results were examined, 20.69% moisture content was determined in the control bread prepared with whole wheat flour, while 17.63% moisture content was determined in the control bread prepared with gluten-free. The high-water holding capacity of gluten may explain this difference. In the groups where microalgae were added, moisture content increased with the percentage of microalgae in general. Microalgae protein content explains the increase in moisture content. When water is structurally evaluated, it contributes to the hydrogen bonding of proteins in complex formation. In addition, water-protein interaction is effective in protein dynamics (P. Dutta et al., 2014). That is, proteins in general tend to hydrate and compete for water in the environment. This leads to an increase in the amount of moisture in the environment. When the literature is examined, it is seen that the percentage of moisture increases with the percentage of microalgae in many studies (Bellissent-Funel et al., 2016). The reason why the moisture content is lower than the ratio for bread is that the analysis was performed on lyophilized bread samples. Moisture is the most important parameter for shelf life and functional breads with microalgae content recommended for daily consumption do not require a very long shelf life (Escobedo-Avellaneda et al., 2012)

4.1 Ash

Ash is the residue left after the complete combustion of a food sample at high temperatures (Marshall, 2010). It represents the inorganic mineral content of the

food, including elements such as calcium, magnesium, potassium, zinc, iron, copper, and phosphorus (Liu, 2017). The main elements that make up ash are approximately 3% calcium, 13% magnesium, 38% potassium, and 45% phosphorus, with other elements making up the rest (Kmiecik et al., 2000).

These minerals are essential for various physiological processes in the human body and play an important role in maintaining overall health. As ash parameters provide valuable information about the composition and quality of food, they can also indicate the presence of contaminants or additives in food.

The grain from which the flour is made also affects the amount of ash. The mineral content of cereals can vary according to both season and growing conditions. Especially minerals are mostly found in the embryo, husk, or bran of wheat (Brier et al., 2015). Therefore, the ash content of whole wheat is higher. The ash content of whole wheat and oat flour used in the study is higher than other cereals.

Another indicator for ash is the color of bread (Samilyk et al., 2022). As the bran part, which is effective in giving color to the bread, increases, the color of the bread becomes darker. In other words, as the whiteness of the bread decreases, the ash content increases, and as the whiteness increases, it decreases. When the results are analyzed, as expected, the ash content of the bread supplemented with microalgae is higher than the control group. Likewise, there is a parallelism between the ratio of microalgae content and ash content. When the literature is examined, the ash content of bread supplemented with microalgae is higher than that of bread not supplemented with microalgae. The rich mineral profile of microalgae is the most effective reason for the increase in ash.

4.2 Protein

Protein is a key indicator of the nutritional value and quality of food (Bohrer, 2017). The amount and type of proteins present in a food product provide information about the food. Proteins are essential macronutrients that play a critical role in building and repairing body tissues, supporting immune function, and serving as a source of energy (Kumar et al., 2017). Protein parameters in food help determine the amino acid profile, which is crucial for assessing the bioavailability

and nutritional adequacy of proteins in the diet.

Protein levels in bread are very important for its quality and nutritional value. Composed mainly of glutenins and gliadins, protein in bread contributes to the structure and texture of the final product (Chaudhary et al., 2022). The protein content of bread can vary depending on the type of flour used and the processing techniques employed. Studies have shown that protein content has a significant impact on the rheological properties of bread dough and the volume and texture of the final baked bread (Ziobro et al., 2013). High-protein bread, such as bread made from flour with a higher protein content, tends to rise better and have a more desirable texture compared to low-protein bread. The protein content of bread is also important for its nutritional value. Proteins in bread contribute to the body's supply of amino acids and are important for the growth, repair, and maintenance of tissues. They are also involved in enzyme production, immune function, and hormone regulation (Kourkouta, 2017).

With the spread of celiac disease, the demand for gluten-free products has increased. Consumption of wheat, rye, and barley varieties by people with this disease causes health problems. The most effective method to prevent and/or reduce this condition is to eliminate gluten from the diet (Leonard et al., 2017). In addition to celiac patients, the gluten-free diet has attracted interest from other consumer groups in recent years due to individual consumption preferences and popular diets.

Gluten is a storage protein composed of gliadin and glutenin fractions (P. Wang et al., 2014). While the gliadin fraction provides extensibility and viscosity to the dough, the glutenin fraction is responsible for elasticity and stickiness and therefore has an impact on the appearance, internal structure, baking, and rheological properties of bakery products (Uthayakumaran et al., 2001). Considering these problems, it has become necessary to add different protein sources to gluten-free product formulations. With the use of many alternative protein sources, it is now possible to both increase the protein content and add functional properties to the product. Microalgae is an ideal solution for this situation.

The results are examined, an increase of 10-30% in protein content was observed in all groups with microalgae added compared to the control group. The protein value, which was 8.34% in gluten-free bread, increased up to 11.10% after the addition of microalgae. The protein content increased in parallel with the microalgae percentage. When the literature is examined, it is seen that the addition of microalgae increases the protein content in bread (Qazi et al., 2021). When the literature is examined, the protein content of whole wheat bread varies between 8-10%, while the protein content of gluten-free bread is between 7-8%. In other words, supplementation of bread with microalgae increased the protein content in both flour types.

4.3 Mineral

Minerals play a vital role in human health as they are involved in numerous physiological processes. These processes include bone formation and maintenance, nerve conduction, muscle contraction, hormone synthesis, enzyme activity, and maintaining proper fluid balance. Minerals are also essential for the proper functioning of the immune system and oxidative processes in the body. Inadequate intake of minerals can lead to deficiencies that can have detrimental health effects. These effects can include weakened bone structure, impaired cognitive function, reduced energy levels, impaired immune response, and increased risk of chronic diseases (Gharibzahedi & Jafari, 2017). It is therefore very important to ensure adequate mineral intake through a balanced diet that includes mineral-rich foods such as bread (Sales et al., 2017).

Wheat-based products, especially bread and biscuits, have long been used as a means of enriching flour with micronutrients such as vitamins and minerals to increase their nutritional value. The mineral content of bread can vary depending on factors such as the type of grain used, processing methods, and fortification practices (Wilder, 1956). Some minerals commonly found in bread include sodium, potassium, calcium, magnesium, phosphorus, iron, copper, and zinc (Iskander & Davis, 1992). These minerals play important roles in supporting various body functions such as nerve conduction, muscle contractions, and enzyme

activity (Godswill et al., 2020). Bread can also provide minerals such as potassium and phosphorus, which are essential for maintaining fluid balance and energy metabolism in the body (Jiaying et al., 2022). By including bread in one's diet, individuals can increase their mineral intake and support the overall health and functioning of their body. Furthermore, bread enriched with additional minerals can provide a higher concentration of essential nutrients.

The mineral analyses performed within the scope of the study are as follows: Sodium (Na), magnesium (Mg), potassium (K), calcium (Ca), and phosphorus (P) for macro elements; copper (Cu), manganese (Mn), iron (Fe) and zinc (Zn) for microelements.

When the sodium results were analyzed, it was observed that sodium content increased with the addition of microalgae compared to the control group. The increase in the percentage of microalgae also increased the sodium content. Sodium is an important mineral involved in various physiological processes including maintaining fluid balance, transmission of nerve impulses, and regulation of blood pressure, especially as a major cation in extracellular fluid (Humalda et al., 2020). The recommended daily intake is 2300 mg (Bossola et al., 2020). The recommended amount of sodium for bread varies between 400-500 mg/100 g (Grimes et al., 2008). In short, the addition of microalgae increased the sodium content to the ideal range for bread. In the literature, similar results were found in the sodium content of bread with microalgae addition (Batista et al., 2019).

The addition of microalgae increased the magnesium content in bread. Magnesium participates in more than 300 biochemical reactions in the body and plays a vital role in energy metabolism, muscle function, nerve conduction, and maintenance of normal heart rhythm. It is responsible for catalyzing enzymatic reactions related to energy transfer, storage, and utilization. It also reduces the hypersensitivity of the nervous system (Fiorentini et al., 2021). When we examine the importance of magnesium for microalgae, it is an essential element in microalgae growth because it is important in the chlorophyll molecule and plays a role in the activity of photosynthetic enzymes (da Silva Ferreira & Sant'Anna, 2017).

The recommended daily intake for magnesium is 350 mg, and the amount of magnesium common for bread varies between 50-80 mg depending on the flour (Agostoni et al., 2015). Enriching bread with microalgae makes the necessary magnesium intake accessible. In addition, hazelnuts, one of the richest foods in magnesium, contain 244 mg/100 g of magnesium, while the magnesium content of ARAS102 is 438.5 mg/100 g (Amini-Noori & Ziarati, 2015). This makes it a very rich alternative.

Potassium results in an increase with the addition of microalgae as expected. Potassium is another important mineral that helps maintain fluid balance, supports proper muscle and nerve function, and regulates heart rhythm (He & MacGregor, 2008). While the recommended amount of potassium for bread is 115 mg, the recommended daily intake varies between 2600-3400 mg depending on gender and age. In the literature, it has been observed that the addition of microalgae enriches the potassium content of bread (Calderón Bravo et al., 2021; Reißner et al., 2019). Bananas, which are one of the richest sources of potassium, have a content of 358 mg/100 g, while ARAS102 shows a good competitor potential with a magnesium content of 425.7 mg/100 g (Ranjha et al., 2022).

As expected, the zinc, manganese, and calcium contents also increased because of microalgae addition. Zinc is an important mineral that plays a key role in enzyme function, immune system support, DNA synthesis, and wound healing (Brandão-Neto et al., 1995). Daily intake varies between 8-11 mg depending on gender (Gibson et al., 2016). The common amount for bread varies between 1.2-1.8 mg/100g (Sandstrom et al., 1980). The most ideal sources of zinc are meat, seafood, and seeds. The ideal source of zinc in meat is 8mg/100g and ASYA27 is a good alternative with a zinc content of 7.4 mg/100 g (Ayodele & Ajibola, 2012). Manganese is essential for the activity of some enzymes and is involved in glycoprotein synthesis and the formation of proteoglycans (Lawrence & Sawyer, 1978). It also plays a role in the regulation of lipid metabolism and the prevention of atherosclerosis (L. Li & Yang, 2018). The recommended daily intake is between 1.8-2.3 mg depending on gender (Tripathi et al., 2000). Common amounts for bread range from 0.06-2.55 mg/100 g (Aghalari et al., 2022). Mussels are the

highest source of manganese at 5.8 mg/100 g, but ASYA27 may be an alternative source with twice the manganese content at 11.7 mg/100 g (Langlet et al., 2007). Calcium is essential for maintaining strong bones and teeth, as well as supporting muscle function, nerve communication, and blood clotting (Theobald, 2005). The recommended daily intake is 1200 mg (Cormick & Belizán, 2019). The amount of calcium in bread is between 200-240 mg/100 g (Prouty & Cathcart, 1939). Although the calcium content of bread is increased with the addition of microalgae, it is not as high as the calcium content of bread in the literature (Sugiati et al., 2020). However, while the content of tofu cheese, which is one of the most important sources of calcium, is 683 mg /100 g, the content of ARAS102 at 512.7 mg /100 g may be a good alternative for calcium (Choi et al., 2020).

Phosphorus is an essential component of DNA, RNA, and ATP (adenosine triphosphate), which are crucial for cellular energy production, bone and tooth formation, and maintenance of acid-base balance (Maciá, 2005). It plays a role with calcium in the formation of bones and teeth (Ciosek et al., 2021). Daily phosphorus intake is recommended at 700 mg (Calvo & Uribarri, 2013). The amount of phosphorus common in bread varies between 106-120 mg/100 g eggs, the richest source of phosphorus, contain 198 mg/198 g (Miles et al., 1983; Ozturk et al., 2005). The phosphorus content of ARAS102 was measured to be 912.4 mg/100 g, making it one of the ideal sources of phosphorus.

The amount of iron, copper, and phosphorus increased with the addition of microalgae. Iron is necessary to produce hemoglobin, a protein found in red blood cells that carries oxygen throughout the body (Abbaspour et al., 2014). The recommended daily intake of iron is between 8-18 mg (Schümann et al., 2007). The liver, the richest source of iron, contains 17.9 mg/100 g, while ASYA27 is a much richer source of iron with a content of 65.6 mg/100 g (Tziomalos & Perifanis, 2010). The iron content of bread is generally between 1.76 and 2.6 mg/100 g (Ranjbar et al., 2019). When the study was examined, a significant increase was observed in the iron profile of the breads supplemented with microalgae and it has a richer iron content compared to the breads in the market. Again, when the literature is examined, the enrichment of bread with microalgae led to increases

in iron content (Khemiri et al., 2020).

Copper is important for hemoglobin synthesis and acts as a cofactor for various enzymes involved in redox processes and is also effective in the absorption of iron from the intestines and mobilization from tissues to plasma (Stern et al., 2007). The recommended daily intake is 900 µg (Bost et al., 2016). The copper content of bread is also commonly expressed as 0.15-0.2 mg/100 g (Ozbek & Akman, 2016). With a high copper content of 15 mg/100 g, ASYA46 with 11.5 mg/100 g copper content can be a good alternative for the liver (Sternlieb, 1980).

4.4 Vitamins B1, B2 and B6

Understanding vitamin B parameters in food is important to assess its nutritional value and impact on human health. Vitamin B is a group of water-soluble vitamins that play important roles in various body functions, including metabolism, energy production, and nervous system functioning (Yaman et al., 2021). Each B vitamin has specific parameters that can be measured to determine its concentration and bioavailability in foods. These parameters include factors such as vitamin B1 (thiamine), B2 (riboflavin), and B3 (niacin). Within the scope of the study, vitamins B1, B2, and B3 of the breads were measured.

Vitamin B is an essential nutrient found in bread that plays an important role in supporting various body functions (Suter, 2020). It is responsible for converting food into energy, helping the nervous system to function properly, and supporting the production of red blood cells (Shabbir et al., 2020). It is important to note that the amount of vitamin B in bread can vary depending on the type of flour used and any supplements that may have occurred. Including vitamin B in bread can provide individuals with a convenient and readily available source of this essential nutrient and contribute to their overall health and well-being. The vitamin results show that there was vitamin enrichment in the bread supplemented with microalgae.

Vitamin B1 is necessary to produce different enzymes that break down blood sugar. It acts as a coenzyme in fat, carbohydrate, and protein metabolism (Fitzpatrick & Chapman, 2020). Its deficiency leads to beriberi (Wilson, 2020). The

recommended daily intake is between 1.1-1.2 mg (Y. Wu et al., 2022). Vitamin B1 in bread varies between 0.148-0.164 mg/100 g depending on the flour (Çatak & Gizlici, 2017). Considering the literature, it was observed that vitamin B1 levels were high in the control group (Ghali Habib et al., 2021). In addition, microalgae can be a good alternative to wheat germ, which is the best source of vitamin B1 with a content of 2 mg/100 g, with a vitamin content ranging between 1.52-1.56 mg/100 g in general.

Vitamin B2 is essential for the growth and development of cells. It also helps metabolize food. In cases where it is deficient, inflammation of the lips and cracks in the mouth occur (Biagi et al., 2020). The recommended daily intake is between 1.1-1.3 mg (H. Li et al., 2023). The amount in bread varies between 0.060-0.150 mg/100 g (Capozzi et al., 2011). In bread supplemented with microalgae, this amount increases to 0.286 mg/100 g. In other words, the addition of microalgae made the bread more ideal in terms of vitamin B2 and close to the amount that should be taken. In addition, almonds are a prominent plant source of vitamin B2 with a content of 0.920 mg/100 g, while ASYA27 with 1.731 mg/100 g may be an ideal alternative (Yada et al., 2011).

Vitamin B6 can be found in foods in three different forms: pyridoxine, pyridoxamine, and pyridoxal (Çatak & Çaman, 2020). The main difference between pyridoxal and pyridoxamine is that pyridoxal is an aldehyde and pyridoxamine is an amine (Xiao & Zhao, 2023). They can change into each other because of chemical reactions. Plant foods are rich in pyridoxine, while animal foods are rich in pyridoxamine and pyridoxal (Makalesi & Yaman, 2019). Pyridoxine is the passive form and requires several cofactors, such as magnesium and vitamin B2, to be converted into the active form, pyridoxal phosphate (PLP), which has the actual ability to function in the body (Gürbüz et al., 2023). Therefore, even if vitamin B6 is taken in sufficient amounts, some cofactors must also be present for it to be used functionally in the human body (Stach et al., 2021).

Vitamin B6 is vital for the formation of red blood cells, the reaction of many enzymes, and the metabolism of macronutrients (V. R. da Silva & Gregory, 2020). Deficiency can lead to anemia and neuropathy (Searcy et al., 2021). The

recommended daily intake is 1.3 mg (Jungert et al., 2020). In general, its value in bread can vary between 0.061-0.1 mg/100 g (Nurit et al., 2021). According to our analysis, this value can reach up to 0.289 mg/100 g with the addition of microalgae. In other words, enriching bread with microalgae makes it more efficient in terms of vitamin B6. In addition, garlic, which is one of the plant sources with the richest content in terms of vitamin B6, with a content of 1.10 mg/100 g, is not far from ARAS102 with 0.78 mg/100 g of vitamin B6 (Eze-Steven & Nnadi, 2020).

4.5 Amino acids

Deciphering the functions and benefits of amino acids is key to understanding their importance to our overall health and well-being. Proteins are the result of synthesizing 20 amino acids (Lopez & Mohiuddin, 2020). There are about 300 amino acids in nature, but 20 amino acids are standard in proteins (Weber & Miller, 1981). The rest are called modified amino acids and non-proteins. These 20 common amino acids are alpha amino acids (Gorman et al., 1992). They all have a carboxyl group amino group and a side chain (R group) attached to the α -carbon. Amino acids are classified according to diet as given in Table 8. Non-essentials are synthesized by the body and do not have to be taken from the outside, but essentials must be taken from the outside (Mirmiran et al., 2017).

Table 8. Classification of amino acids according to aging

Essentials	Conditionally Non-Essential	Non-Essential
Histidine	Arginine	Alanine
Isoleucine	Cystine	Asparagine
Leucine	Glutamine	Aspartate
Lysine	Glycine	Glutamate
Methionine	Proline	Serine
Phenylalanine	Tyrosine	
Threonine		
Tryptophan		
Valine		

Amino acids play vital roles in various physiological processes. They are involved in the synthesis of enzymes, hormones, and neurotransmitters, as well as in the growth, repair, and maintenance of tissues (G. Wu, 2009). Amino acids also contribute to immune function, nutrient absorption, and energy production (P. Li & Wu, 2022). Bread contains a variety of amino acids that contribute to its nutritional content (Kowalski et al., 2022). Some of the most common amino acids found in bread include lysine, tryptophan, and methionine (Meybodi et al., 2019). Lysine is an essential amino acid required for the synthesis of proteins in the body. Lysine in bread is important for its role in supporting healthy growth and development and the immune system (Abimorad et al., 2010).

Tryptophan is another important amino acid found in bread. It is a precursor to the neurotransmitter serotonin, which regulates mood and sleep (L. Wang et al., 2002). Tryptophan in bread contributes to supporting a sense of well-being and relaxation (Kałużna-Czaplińska et al., 2019).

Methionine is an essential amino acid that plays a vital role in protein synthesis and the production of other important molecules in the body (Yin et al., 2016). It is worth noting that the presence and number of amino acids in bread can be influenced by various factors, including the type of flour used, processing methods, and the addition of other ingredients such as enzymes or additives.

Enzymes such as xylanases, lipases, oxidoreductases, and proteases are often used in bread-making to improve texture, shelf life, and nutrient availability. These enzymes can also affect the composition and availability of amino acids in bread (Dahiya et al., 2020).

In this study, the contents of aspartic acid, glutamic acid, serine, glycine, histidine, arginine, threonine, alanine, proline, tyrosine, valine, methionine, isoleucine, leucine, phenylalanine, lysine, and tryptophan were analyzed.

The amino acid results of the control groups, microalgae, the highest group, and the recommended daily amino acid intake values to evaluate the amino acid results of the bread. When the results are analyzed in general, the amino acid value of the bread enriched with microalgae increased for each group compared

to the control group.

When the amino acid profile for whole wheat and gluten-free bread was compared, the amino acid content of gluten-free bread was generally lower.

Aspartic acid is a building block amino acid responsible for synthesizing proteins and regulating hormones. It makes up about 5% of proteins. Available in L and D forms, this amino acid promotes antibody production (Y. Wang et al., 2023). It is also involved in the synthesis of isoleucine, methionine, threonine, and lysine. Since it is not essential, it does not need to be taken as a supplement. When the results were analyzed, the highest increase compared to the control group was in the 27WW/3 group. The aspartic content of bread, which is generally 409 mg/100 g in the literature, has become richer because of supplementation with microalgae (Y. Wang et al., 2023).

Glutamic acid is one of the alpha-amino acids (Tavakoli et al., 2020). It is important in brain, heart, and metabolism functions. It is necessary for the biosynthesis of glutamate, an important neurotransmitter in the nervous system (S. Dutta et al., 2013). It also plays a role in maintaining the pH value of the skin. One of the issues that needs to be addressed at this point is monosodium glutamate (MSG), which has recently been frequently used to flavor foods. Unfortunately, the body cannot distinguish between naturally occurring glutamate and MSG from processed foods. This results in a severe glutamic acid buildup in the body and hyperglutamatergic neurotransmission, which can lead to depressive symptoms (Populin et al., 2007). It is therefore important to obtain glutamic acid from animal or plant sources. At this point, the enrichment of the glutamic acid profile of bread supplemented with microalgae may make microalgae an important alternative source.

Serine is important in improving cognitive abilities. It is involved in activating NDMA receptors, which are neurotransmitters. It is also used in Parkinson's and schizophrenia. It is also involved in the synthesis of glycine and sulfur amino acids (Holeček, 2022). Studies have shown that its deficiency causes serious damage to the nervous system (Tabatabaie et al., 2010). Eggs and soy are the strongest sources of serine. In the study, the addition of microalgae nearly doubled the

serine content in bread (De Campos Zani et al., 2018).

Glycine is one of the important amino acids that reduce oxidative stress (Ruiz-Ramírez et al., 2014). It is also involved in glutathione synthesis (Dickinson & Forman, 2002). It is also the primary amino acid in collagen supplements. In the literature, its value in bread is expressed as 351 mg/100 g (Fink et al., 2006). In bread enriched with microalgae, this value was almost doubled.

Histidine is an important amino acid in metal ion chelation, ROS scavenging, and proton buffering (Chikh et al., 2002). Since it is essential, it cannot be synthesized and must be taken from the diet. Breads supplemented with microalgae showed an increase in histidine content.

Arginine is useful for chest pain and high blood pressure (Dong et al., 2011). It is also involved in the release of nitric oxide and insulin into the blood (G. Wu & Meininger, 2009). It is especially common in foods such as fish and meat (Rahimnejad & Lee, 2014). The addition of microalgae also more than doubled the arginine content.

Threonine is converted to glycine in the body, preventing unwanted muscle contractions (Zeng et al., 2011). It is also used in the treatment of multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS) (Moszczynski et al., 2018). For skin health, it is effective in protecting elastin and collagen tissues (K. E. Silva et al., 2020). Enrichment with microalgae has also enriched the profile of the bread.

Since alanine is not essential, it does not need to be taken as a supplement. One of its most important effects is to regulate blood sugar. It is also effective in the treatment of diarrhea and prostate enlargement (Varanoske et al., 2019). Again, as expected from the results, the addition of microalgae increased the alanine content of the bread.

Proline is involved in wound healing and protection against oxidative damage. It is abundant in milk and collagen (Vettore et al., 2021). In general, it is the most needed amino acid for protein synthesis. Again, it was observed that the proline content of breads enriched with microalgae increased.

4.6 Fiber

Fiber parameters in foods are important for understanding the nutritional and functional properties of dietary fiber. Dietary fiber is a complex carbohydrate that cannot be fully digested by the human body. It consists of two types: soluble fiber and insoluble fiber. Soluble fiber parameters include viscosity and gel-forming ability, which are important for their role in increasing satiety, regulating blood glucose levels, and supporting healthy digestion. Insoluble fiber parameters include parameters such as water holding capacity and volume, which contribute to regulating bowel movements, preventing constipation, and maintaining a healthy intestinal environment (Kale et al., 2011). The fiber composition of bread refers to the amount and type of dietary fiber present in the final product. Bread contains two main types of dietary fiber: soluble fiber and insoluble fiber. Soluble fibers such as beta-glucan and pectin form a gel-like substance in the digestive tract, helping to lower cholesterol levels and regulate blood sugar levels. Insoluble fiber, on the other hand, adds bulk to stool and supports regular bowel movements (Mudgil, 2017). The fiber content of bread can vary depending on factors such as the type of flour used and additional ingredients such as bran or whole grains (Gębski et al., 2019). Fiber is often added to bread to increase its nutritional value and health benefits. In addition, the presence of fiber in bread is known to contribute to satiety, helping individuals feel fuller for longer and potentially aiding in weight management. Adding fiber to bread has also been shown to improve the texture and shelf life of bread (Kurek & Wyrwisz, 2015).

When the fiber results obtained within the scope of the study were examined, an increase in fiber content in bread enriched with microalgae was observed. *Chlorella* contains 14-17% dietary fiber according to the literature (Zarzycki et al., 2023). In general, microalgae are excellent sources of potential insoluble and soluble prebiotic fibers such as agar, carrageenan, sulfated rhamnoxyloglucan, xylan, alginate, cellulose, mannan, fucan, laminaran (Gotteland et al., 2020). This highlights microalgae as beneficial organisms for the human gut (de Medeiros et al., 2021). Based on this information, the fiber content of the microalgae used in the study was calculated between 16-24 g/100 g. The fiber content of Antarctic

microalgae was found to be higher than mesotherm microalgae. This may be due to cold stress. Carbohydrate metabolism is altered during acclimatization. This is because mitochondrial respiration decreases less than photosynthesis at low temperatures. This is because respiration is more resistant to freeze-thaw and dehydration. As a result of the decrease in temperature and changes in metabolism, there is an increase in carbohydrate accumulation in microalgae (Fernandes et al., 2012; Sharifani et al., 2012). Since dietary fiber is also a carbohydrate component, the dietary fiber content of Antarctic microalgae is likely higher (Walter de Gruyter et al., 2005). When the literature is examined, it is seen that the dietary fiber content of whole wheat bread varies between 6-10 g/100 g. This amount increased up to 15.23 g/100 g in breads enriched with microalgae. The fiber content of chia seed, which is shown as one of the most ideal sources of fiber with a recommended daily intake of 28-35 g, is 34 g/100 g and microalgae can be ideal sources of fiber with their high fiber content.

4.7 Starch

Starch is a complex carbohydrate that serves as an important source of energy in the human diet. It is also crucial for understanding the properties and functionality of starch in foods (Stephen, 1994). It is composed of glucose molecules and has unique properties such as gelatinization, retrogradation, and viscosity (Tako et al., 2014). These properties of starch determine its functionality in food products, such as thickening, binding, and providing texture (Mahmood et al., 2017). Starch parameters can also be used to determine the degree of degradation or cooking of starch, which is important for optimizing cooking times and processes in food preparation (Fan et al., 2023).

Starch is an important component of bread and an important source of energy (Behall & Howe, 1996). During the bread-making process, the starch in flour undergoes gelatinization (Lelievre, 1974). When heated, the starch granules absorb water and swell, leading to the formation of a gel-like structure. This gelatinization process is essential for the texture and structure of the bread (Garcia et al., 1997). It also contributes to the flavor and browning of the bread

during baking. It also plays a role in the shelf life of bread by absorbing excess moisture and preventing staling. Various methods are used to modify starch, such as etherification, esterification, crosslinking, grafting, decomposition, and physical treatment using heat or moisture.

These modifications cause changes in solution viscosity, gelatinization properties, paste properties, retrogradation behavior, coalescence behavior, and shelf-life stability in bread and other food products (L. Zhang et al., 2019). The use of these modified starches in bread making is also increasing. They contribute to the overall quality and consistency of bread, providing consumers with a pleasant eating experience. A common type of modified starch is acid-thinned starch, which is treated in an acid slurry to modify its properties for specific applications in the baking industry (Ahmed & Auras, 2011).

Within the scope of the study, an increase was observed in the starch content of the breads to which microalgae was added. Although the starch content for bread varies between 25-45%, the starch content increased up to 47% because of the addition of microalgae. The increase in starch content is expected due to the rich carbohydrate content of microalgae. In the literature studies, an increase in the starch content of foods to which microalgae was added was also observed.

4.8 Lipid

Lipid parameters in foods play an important role in determining the nutritional quality and sensory properties of food products. Lipids, also known as fats, are a type of macronutrient that provide energy, support cell function, and aid in the absorption and transport of fat-soluble vitamins (Calder, 2015; Ryan & Van Der Horst, 2003). Lipid parameters in foods, such as fatty acid composition and melting point, can provide valuable information about the type and quality of fats present in the food (Smuts & Wolmarans, 2013). These parameters can help assess the shelf life of food products, as well as their texture, flavor, and overall sensory appeal (Shahidi & Hossain, 2022). Lipids such as butter or oil play an important role in bread making. They contribute to the flavor, texture, and overall quality of bread. Lipids help to add moisture and softness to the breadcrumb. They also help to evenly distribute

heat during baking, helping to create a desirable crust (Zhong et al., 2014). In addition, lipids act as emollients by inhibiting the development of gluten, resulting in a softer and more delicate texture. They also help to extend the shelf life of bread by slowing staling and preventing moisture loss.

The fat content in breads generally varies between 2.5%-8%, and the fat content of whole wheat and gluten free breads analyzed in the study was calculated around 7%. This amount increased up to 9.78% with the addition of microalgae. The rich fat content of microalgae, which can vary between 10-10-32 g/100 g, increased the total fat content of the breads.

5. Conclusion

There is a trend towards non-traditional resources to ease the burden of population growth on traditional resources. The inefficient use of agricultural land due to climate crisis and water scarcity is one of the biggest factors. However, people's search for alternative foods is also increasing. Microalgae is one of these alternatives. For the 21st century, it is promising as a new product with a high potential to meet the need for feed and fuel, especially food. For the widespread use of microalgae, growth quality, organoleptic properties, and nutritional quality need to be improved. Social acceptance by the society is also a separate issue. The openness of microalgae to environmental manipulations enables more effective conditions to be applied to the desired product/extract.

The more studies on microalgae, the more their benefits are documented and their potential for use is diversifying. Due to their rich bioactive content, these organisms have many properties such as antioxidant, anti-cancer, anti-inflammatory, anti-bacterial, and anti-diabetic. The proteins, phenolic compounds, polyunsaturated fatty acids, vitamins, polysaccharides, minerals, and pigments of microalgae also increase the functional value of foods. Given the need to increase food production by about 70% in the next 40 years, the concept of microalgae culture seems highly attractive. They possess a variety of biomolecules with great potential as supplements, such as astaxanthin, phycobiliproteins, beta-carotene,

vitamins, and PUFAs, all of which have beneficial properties for the prevention and treatment of diseases. These compounds can be incorporated into food products to enhance their nutritional and health benefits.

In this study, psychrotolerant *C. vulgaris* ASYA27 *M. simplicissimum* ASYA46, and mesotherm *C. vulgaris* ARAS102 were used to improve the nutrient profile of whole wheat and oat flour (gluten-free) bread. Moisture, ash, total protein, amino acids, total lipids, fatty acid profile, total starch, total dietary fiber, mineral, and vitamin contents were analyzed, and the biochemical profile of the bread was evaluated in comparison with the control group.

As a result, an increase in each parameter was found with the addition of microalgae. The enrichment of bread formulations with microalgae, especially traditional, accessible to everyone and not requiring much shelf life, offers positives for the problem of access to healthy food. In addition, people on a gluten-free diet due to increasing gluten intolerance will be able to consume a product with a richer nutrient profile compared to regular bread. Heavy metals, which are considered a problem for microalgae, were also analyzed, and found to be below the threshold value.

The use of species from different climatic conditions led to different results, especially in terms of fatty acid profile. Microalgae that increased their fluid lipid (unsaturated fatty acid) content to adapt to the cold provided a higher increase in the PUFA content of the bread. However, the microalga *M. simplicissimum* ASYA46, which is not yet included in the GRAS and EFSA lists and has not been studied on human food consumption, gave similar results to *C. vulgaris*, the best known of the other two GRAS and EFSA lists, both as biomass and when used in bread. This makes *M. simplicissimum* ASYA46 microalgae a potential organism for human consumption as food.

6. REFERENCES

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