Turk. J. Fish.& Aquat. Sci. 26(6), TRJFAS27969
https://doi.org/10.4194/TRJFAS27969

RESEARCH PAPER

Turkish Journal of FISHERIES and AQUATIC SCIENCES

Investigation of Lipid Production of Microalgae Species in Fruit Juice-based Nutrient Medium

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How to Cite

Can, F., Yarkent, Ç., Öncel, D.Ş., Öncel, S.S. (2026). Investigation of Lipid Production of Microalgae Species in Fruit Juice-based Nutrient Medium. *Turkish Journal of Fisheries and Aquatic Sciences*, *26(6)*, *TRJFAS27969.* https://doi.org/10.4194/TRJFAS27969

Article History
Received 21 February 2025
Accepted 17 November 2025
First Online 10 December 2025

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Keywords
Schizochytrium limacinum;
Crypthecodinium cohnii,
Microalgae Biomass
Microalgal Lipid
Fruit Juice-based Medium

Abstract

In this study, *Schizochytrium limacinum* PA-968 and *Crypthecodinium cohnii* CCMP-316 were produced in the media including apple (AJ) and grape juices (GJ), which contain carbon sources that are alternatives to standard carbon containing media (SM). The *S. limacinum* biomass productions were 9.52±0.08 g L^{-1} (AJ), 8.70± 0.05 g L^{-1} (GJ), and 8.58±0.02 g L^{-1} (SM). *C. cohnii* produced biomass as 3.45±0.06 g L^{-1} (GJ), 1.52±0.04 g L^{-1} (AJ), and 1.35±0.02 g L^{-1} (SM). The fruit juice-based media enhanced biomass production. It was observed that the lipid production of *S. limacinum* increased by 17.6% in the medium with apple juice (2.54±0.02 g L^{-1}), while this increase was 65.3% in the grape juice-based medium (3.57±0.02 g L^{-1}). The addition of apple juice caused an increase in the lipid amount 1.9 times higher (0.23±0.02 g L^{-1}), while grape juice induced 5.3 times more lipid production in *C. cohnii* culture (0.50±0.03 g L^{-1}). The study emphasized that these wastes or by-products can be considered as sustainable and financially supportive solutions to be alternatives to carbon sources in production with *S. limacinum* and *C. cohnii* cultures.

Introduction

Microalgae have existed for billions of years and have been consumed as nutrients by humans worldwide (Gantar & Svirčev, 2008). These microorganisms play a crucial role in the global food chain while contributing to the sustainability of life (Gurlek et al., 2019; Molino et al., 2020). Microalgae can grow rapidly with high efficiency for biomass production while utilizing a low amount of water in the cultivation area. Due to these properties, they are considered as more ideal sources for biocompounds compared to macroalgae and plants (Suparmaniam et al., 2019; Yarkent & Öncel, 2023). Microalgae naturally synthesize valuable compounds such as carbohydrates, lipids, proteins, pigments, minerals, and others (Fernández et al., 2021; Tokgöz et al., 2023). The need for sustainable sources has induced us to put more effort into underscoring the capacity of microalgae to be included in unrealized applications. Research has proven that more than 75% of microalgal products can be evaluated as ingredients in food, nutraceuticals, and pharmaceutical products (Béligon et al., 2016; Guschina & Harwood, 2013; Pulz & Gross, 2004; Torres-Tiji et al., 2020). The increase in awareness among consumers about healthy eating and their attention to natural products led to an enhancement in demand for these products (Gouveia et al., 2008; Yarkent et al., 2020). The global market value of microalgae products was 33 billion $ in 2017, and it is expected that this value will reach 53 billion $ in 2026. Microalgal lipids in particular have gained global attention from the renewable energy industry in addition to the biopharmaceutical and nutraceutical industries (Calder & Yaqoob, 2009; Mittal & Ghosh, 2023; Yarkent & Oncel, 2022).

Microalgae can grow autotrophically, heterotrophically, and mixotrophically. In autotrophic cultivation, microalgae absorb energy from sunlight or artificial light sources and produce organic metabolites through photosynthesis (Chew et al., 2018). Some microalgae species can consume organic carbon substrates as carbon sources to gain energy and grow heterotrophically in dark conditions (Cheirsilp & Torpee, 2012). That allows for the establishment of biomass production in high concentrations, as these microalgae do not require a light source and there is no self-shading issue between the cells (Fernández et al., 2021). Also, the energy consumption caused by the illumination system causes a rise in overall production costs, which limits scaling-up the production platform. Thus, the cultivation of heterotrophic cultures increases the possibility of microalgae production on a commercial scale (Yilmaz et al., 2023; Yin et al., 2020). Additionally, their high growth rate and high lipid concentration make them proper creatures for lipid production (Chew et al., 2018). In heterotrophic microalgae production, the carbon content of the medium has an impact on biomass production and the biochemical composition of biomass (Brennan & Owende, 2010). Among carbon sources, glucose, fructose, and glycerol are the most preferred carbon sources (Verma et al., 2019). These carbons are utilized and formed lipids. There are two important stages for lipid biosynthesis. The first one is the acetyl-CoA production phase, while the second is the conversion of acetyl-CoA into lipids. Acetyl-CoA formed in mitochondria is converted to palmitic acid via lipogenesis (Ren et al., 2009). After the lipogenesis stage, palmitic acid is converted into unsaturated fatty acids or polyunsaturated fatty acids (PUFAs) by desaturases and elongases (Béligon et al., 2016). Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) are polyunsaturated fatty acids synthesized from α-linolenic acid formed at this stage (Adarme-Vega et al., 2012; Ratledge, 2004, 2008). EPA and DHA have gained attention because they cannot be produced in the human and animal bodies and therefore, they must be supplied externally through nutrition (Yen et al., 2013). This situation reveals a demand for lipid production through natural, sustainable and renewable approaches and that caused an increase in experiments on understanding the lipid production capacity of microalgae species.

As heterotrophic microalgae consume organic carbon sources, the cost of medium constitutes a huge part of production costs (80%); therefore, cheaper carbon sources should be used as alternatives to costly ones. On the other hand, nitrogen is required at the initial stage of fermentation, where cell growth and development occur through the synthesis of amino acids and proteins (Perez-Garcia et al., 2011). Industrial wastes and by-products appear as complex nitrogen sources in lipid production from microalgae and the studies have proven that these mixtures have a high potential to be utilized in microalgal productions (Perez-Garcia et al., 2011; Yadavalli et al., 2014; Yokochi et al., 1998). Therefore, it may be a great strategy to obtain the required nutrient sources from a production platform created through the biorefinery approach. For example, corn steep liquor, whey, molasses, wheat straw, liquid waste from the brewing and potato industries, sweet sorghum juice, food waste, and industrial waste can be utilized as carbon sources (Abdel-Wahab et al., 2021; Chi et al., 2007; Liang et al., 2010; Patil & Gogate, 2015; Quilodrán et al., 2009; Unagul et al., 2007). Then, the biomass of microalgae can be considered as a potential candidate for biorefinery applications due to their biocompounds, which can be involved in a wide range of production systems such as pharmaceutical, nutraceutical, and biodiesel applications (Gürlek, et al., 2020a; Jacob-Lopes et al., 2015; Senturk, 2024; Yarkent et al., 2021; Yarkent et al., 2024).

In Türkiye, the fruit production and processing industry is one of the most important agricultural sectors. According to the report published in Statista, Türkiye has increased its capacity for fruit production each year and ranked 4th in the world market with 25.68 million tons of fruit produced in 2022 (Statista, 2022). The amount of fruit processed into fruit juice is over 1000 tons annually (FAO, 2019). As the amount of processed fruit increases, the amount of these wastes containing organic compounds prone to microbial degradation also increases and becomes a growing problem. Therefore, the utilization of wastes obtained from the fruit juice industry is crucial for creating a process through economic, sustainable, and eco-friendly approaches. The fruit pulp components, which constitute 25–35% of fruits, have high amounts of carbon, minerals, proteins, organic acids, and vitamins (Sülük et al., 2018). Both the high nutritional value of fruit pulp and the possibility of recycling it, have revealed the opportunity of using these natural compounds as nutrient sources in microbial production. Pulps or by-products, which are derived from fruit juice production, have a great untapped potential as substrates for supporting microalgae production with their high nutritional value.

The current study aims to examine the potential of fruit-juice based media as an alternative to high-cost carbon sources in microalgae production (Figure 1). *Schizochytrium limacinum* PA-968 and *Crypthecodinium cohnii* CCMP-316 were produced heterotrophically in apple and grape juices, which represent fruit juice industry wastes. Fruit juices were added to the carbon free standard media in appropriate quantities to maintain the C/N ratio present in the standard media. Since C/N ratio is one of the main factors in microalgae production, especially for lipid production, the maintenance of this ratio is centred at the heart of the study. Therefore, based on this principle, the main objective is to observe the effects of the determined amounts of fruit juice on the cultures. Each culture in juice-based medium and its standard medium was compared with each other to represent the effect of

juice-based medium on biomass production and lipid accumulation. The results proved that the utilization of wastes or by-products obtained from industrial fruit juice production can be a sustainable, renewable, and beneficial strategy in terms of evaluating waste as a substrate and decreasing overall cost in microalgae production while growing microalgae with valuable lipid components that have gained great attention from renewable energy, biopharmaceutical, and nutraceutical industries. This is a preliminary study highlighting the potential use of fruit juices in microalgal biomass and lipid production and has the potential to serve as a guide for future experiments.

Materials and Methods

The Stock Cultures

Schizochytrium limacinum PA-968 and *Crypthecodinium cohnii* CCMP-316 were grown in their specific standard media containing glucose and glycerol, respectively (Can, 2021). The medium was autoclaved at 121°C for 20 min. The cultures were transferred into 250 mL Erlenmeyers with 100 mL working volume (10% inoculation volume) in a class II biological safety cabinet to maintain the axenicity of the cultures. The cultivations were carried out at 120 rpm and 22°C under dark conditions in triplicate (Gürlek, et al., 2020b). 3 days old cultures were used as stock cultures for microalgae productions.

The Culture Production

Firstly, 100% apple juice and 100% grape juice (Dimes, Türkiye) were filtered to separate their pulps. The total carbohydrate content of the standard media and the fruit juices were determined. The carbohydrate concentrations of apple juice and grape juice were 103 g L^{-1} and 83 g L^{-1}, respectively. In order to maintain the same C/N ratio in each juice-based media, different amounts of juices were added on the carbon source deficient standard media (Table 1). For this step, firstly the fruit juice-less media and standard media were autoclaved at 121°C for 20 min. Then, fruit juices were sterilized using a 0.22 µm sterile filter (Sartorius, Germany) and added into the sterilized media. The stock cultures were transferred to their standard media and the juice-based media. The cultures were inoculated to

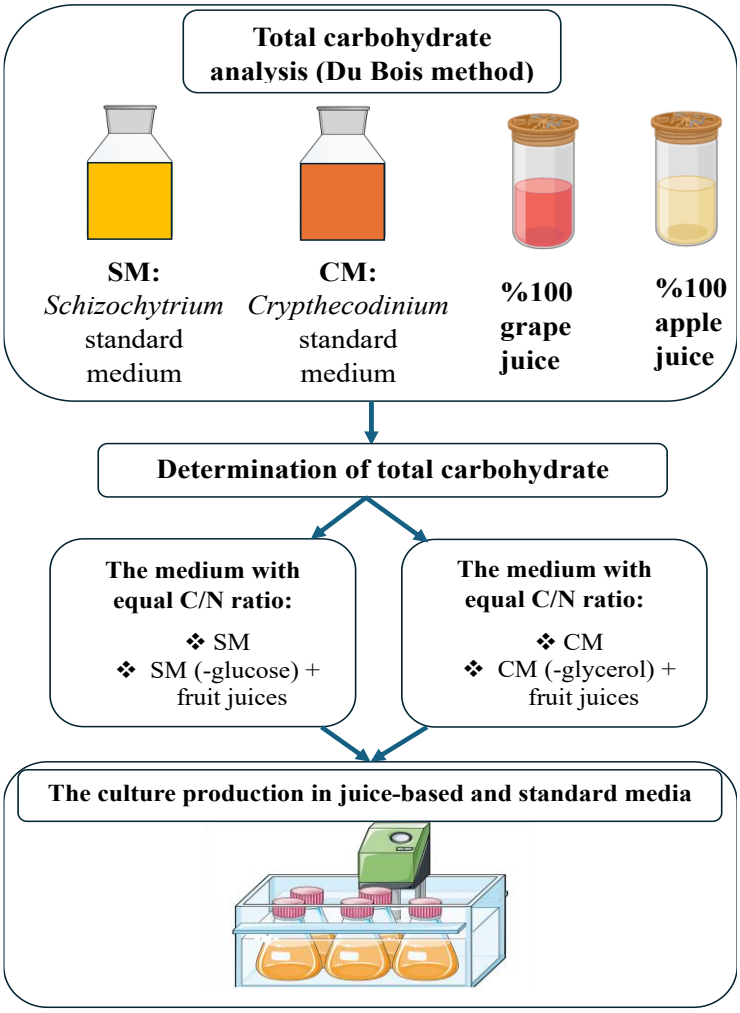

Figure 1. The flowchart of the presented paper, showing that cultures cultivated after determining the carbohydrate content of different media to maintain the same C/N ratio.

attain %10 inoculation volume to 250 mL Erlenmeyers with 150 mL working volume. These steps were carried out in a class II biological safety cabinet to maintain the axenicity of the cultures. The cultivations were performed heterotrophically at 120 rpm and 22°C in triplicate. After 11 days, the cultures were harvested at 3500 rpm for 8 min, and this step was repeated 2 times with distilled water. The cell pellets were freeze-dried and kept at -20°C for further experiments.

The Lipid Extraction

The lipid extraction process was carried out according to the modified Bligh and Dyer method (Bligh & Dyer, 1959). 4 mL of chloroform: methanol (2:1, v/v) containing 0.25 mg mL^{-1} nonadecanoic acid and 0.5 mg mL^{-1} butyl hydroxytoluene was added on 100 mg dry biomass. The sample was sonicated by using Bandelin Sonopuls HD2070 sonicator (9 cycle and %55 power) for 1 min (Kaya et al., 2011; Panchal et al., 2016) and kept in an orbital shaker at 120 rpm and 22°C for 24 h. After that, the samples were centrifuged at 3500 rpm for 8 min. The supernatant was transferred into a new centrifuge tube, then centrifuged again. 1 mL of distilled water was added on the supernatant part to separate the lipid part. The lipid part was carefully filtered through 0.22 µm syringe type polytetrafluoroethylene (PTFE) filters (Sartorious Stedim, Germany) and collected in the pre-weighted glass vials. After the solvent evaporation at 36°C (Stuart RE300DB, UK), the lipid content of biomass (% in biomass) was measured gravimetrically. The process was performed in triplicate.

Analytical Methods

The culture suspension was daily sampled to follow the culture growth, and the analysis were carried out in triplicate. The optical density was detected using a UV/VIS spectrophotometer (Optizen, POP, MECASYS) at 660 nm (Gürlek, et al., 2020b). The pre-weighted GF/C Whatman filter paper was washed with 1 mL of distilled water, 2 mL of culture suspension, and 1 mL of distilled water, respectively. The process was carried on using the vacuum pump (Knf, Germany). After keeping at 60 °C for 24 h, the paper was weighted to determine dry weight of the sample (Oncel & Sukan, 2008). The cell counting was done using Thoma lam under the light microscope (Micros, Austria) and the cell concentration was calculated according to Formula 1.

$$Total\ number\ of\ cells\ (cells\ mL^{-1}) = The\ counted\ cells \times Dilution\ factor \times 10^4 \quad (1)$$

The total carbohydrate content of the standard media and fruit juices were determined according to DuBois method (Dubois et al., 1956). Firstly, glucose solutions are prepared in different concentrations at 0, 20, 40, 60, 80, and 100 µg mL^{-1}. Respectively, 0.5 mL of 5% phenol solution and 2.5 mL of sulfuric acid (98%, Merck) were added into 0.5 mL of each glucose solution. The mixtures were vortexed and incubated for 15 min at room temperature, then vortexed again. The calibration curve was obtained by measuring the samples at 490 nm using a UV/VIS spectrophotometer (Quero-Jiménez et al., 2019). Distilled water was used as a blank. The carbohydrate concentration of the sample was determined based on the calibration curve. The process was carried out in triplicate. Since the total carbohydrate amount of each media is higher than the concentration evaluated for the calibration curve, necessary dilution processes were carried out using distilled water to reach the total carbohydrate value of the samples to the values in the calibration curve. Then the total carbohydrate amount obtained from the calibration curve was multiplied by this dilution factor and the total carbohydrate amount of the sample was calculated and reported.

Statistical Analysis

The results were examined using GraphPad Prism Software (Version 8.3.0). The statistical analysis was performed using means, standard deviation, and standard errors. The statistical meaningful differences were mentioned when the *P* value was <0.05. The comparison was processed with a 95% confidence interval.

Results and Discussion

The Growth Kinetics of the Cultures

Microalgae species reveal different growth kinetics and biochemical composition as a response to the growth medium (C, N, C/N, etc.) (De Morais et al., 2015; Gupta et al., 2022; Rashid et al., 2014; Razzak et al., 2017; Singh & Singh, 2014; Tandon & Jin, 2017). Therefore, the growth phase of each culture in different media should be underlined by following some parameters such as optical density, dry weight, and cell concentration (Gouveia et al., 2017).

For *S. limacinum*, the time taken for each culture to complete the exponential phase was different due to variations between the media (Figure 2). The culture in apple juice-based medium reached its stationary phase on the 7th day in addition to performing the highest

Table 1. The amount of fruit juices added on the carbon source deficient standard media (mL L^{-1})

	Apple juice (mL L^{-1})	Grape juice (mL L^{-1})
Glucose deficient *S. limacinum* standard medium	291 mL L^{-1}	361 mL L^{-1}
Glycerol deficient *C. cohnii* standard medium	116 mL L^{-1}	144 mL L^{-1}

growth capacity compared to others. Grape juice-based medium presented more suitable expendable nutrients than those in standard medium. The exponential growth of the culture in grape juice-based medium was finished on the 8th day, while the culture in standard medium performed the exponential phase on the 9th day. Juice-based media were more suitable for *S. limacinum* production, with apple juice-based medium being utilized more to achieve the highest growth capability.

Figure 3 shows that *C. cohnii* cultures completed their growth phases at different times with respect to medium content. The culture in grape juice-based medium continued to grow and reached the stationary phase on the 7th day. The growth capacity of *C. cohnii* cells in apple juice-based medium was lower than that in grape juice-based medium, and they kept exhibiting exponential phase until the 10th day. The lowest culture growth was observed in the culture in standard medium and the culture passed to their stationary phase after 5 days. Juice-based media served as more favourable media for *C. cohnii*, and among them, grape juice-based medium was consumed more efficiently to reach the highest culture concentration.

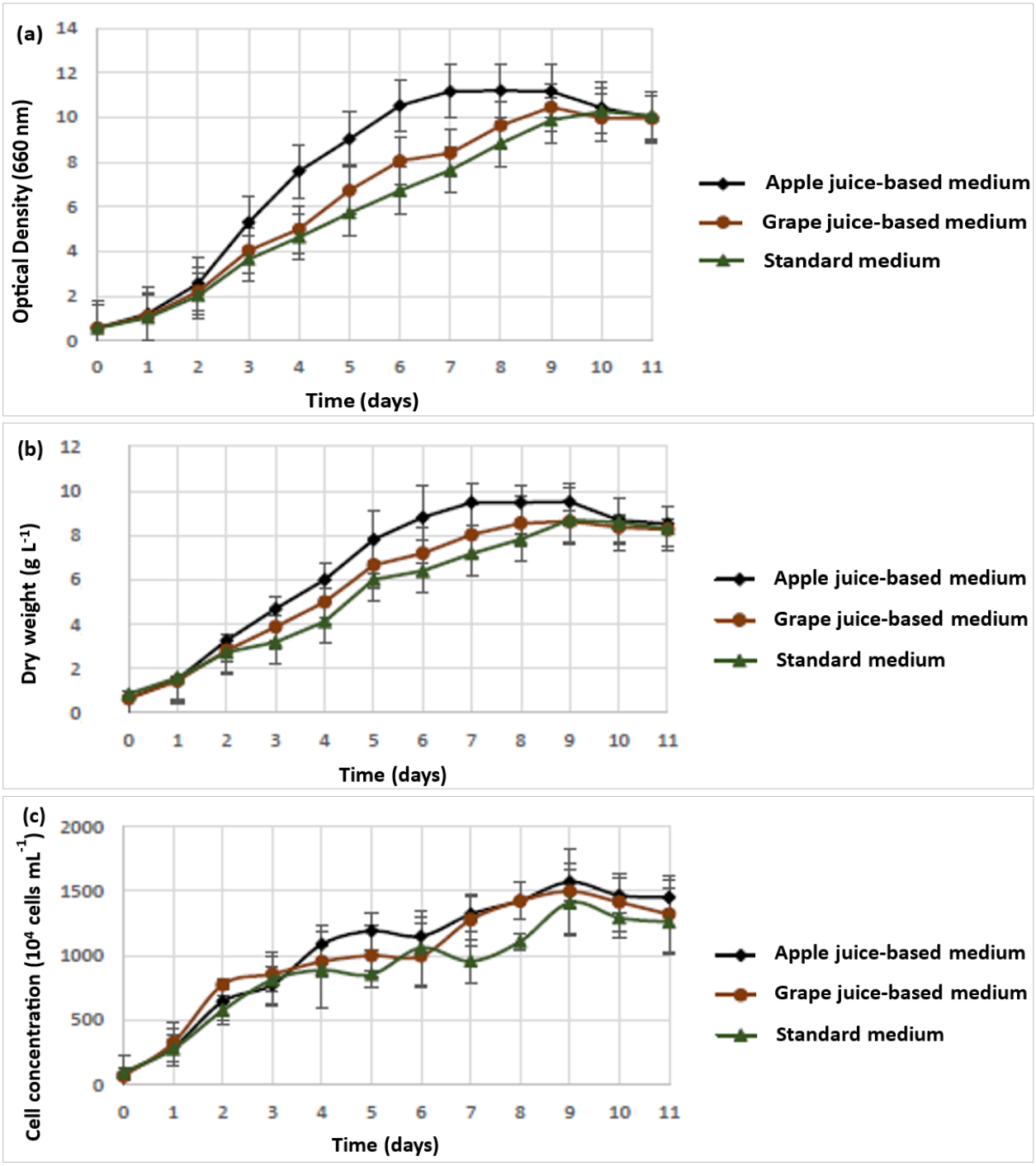

Figure 2. The growth kinetics of *Schizochytrium limacinum* cultures in juice-based media and standard medium observed by following (a) the optical density (OD) at 660 nm, (b) the dry weight, and (c) the cell concentration of the cultures.

The Microalgae Biomass Production

In this study, *S. limacinum* produced different amounts of biomass depending on the medium. The biomass concentrations of *S. limacinum* were 9.52±0.08 g L^{-1}, 8.70±0.05 g L^{-1}, and 8.58±0.02 g L^{-1} in apple juice-based medium, grape juice-based medium, and standard medium, respectively (Figure 4). It was observed that *S. limacinum* cells had a higher growth rate in nutrient medium containing apple juice, which was followed by the cultures grew in grape juice-based medium and standard medium. The research compared the utilization capacity of glucose and fructose by *S. limacinum* culture and proved that the culture consumed more fructose instead of glucose and produced higher biomass in fructose-containing medium (Nazir et al., 2020; Patil & Gogate, 2015). *S. limacinum* cells have a carbon metabolism, whose first reaction is the direct formation of fructose-1-phosphate with phosphoenolpyruvate (PEP). In fructose metabolism, there is a need for an extra step in which glucose is first converted to glucose-6-phosphate and then formed to fructose-6-phosphate by glucose-6-phosphate isomerase using an ATP molecule. As a result, using fructose as a carbon source requires less energy and steps than glucose; therefore, the cells consume more fructose than glucose. The fructose/glucose ratio of apple juice is higher than that of grape juice (Li et al., 2020). That explains why the culture produced more biomass in apple juice-based medium than in grape

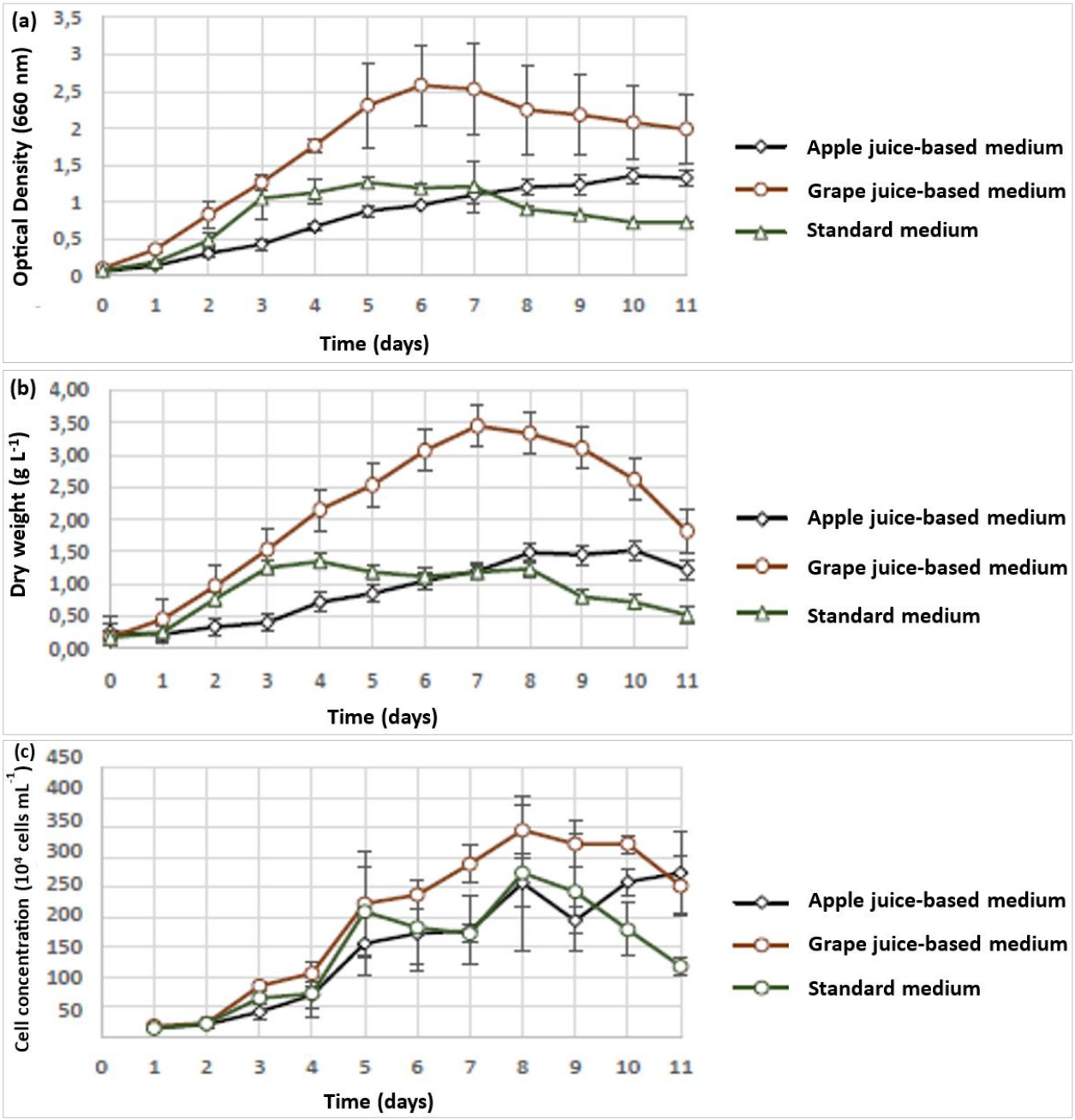

Figure 3. The change in *Crypthecodinium cohnii* cultures grown in juice-based media and standard medium observed by following (a) the optical density (OD) at 660 nm, (b) the dry weight, and (c) the cell concentration of the cultures.

juice-based medium. As its standard medium includes glucose as a carbon source and there is no fructose, the culture growth capacity was lower in standard medium compared to juice-based medium (Can, 2021).

Compared to *S. limacinum* cultures, *C. cohnii* in each of the media produced lower biomass. *C. cohnii* produced biomass as 3.45±0.06 g L^{-1}, 1.52±0.04 g L^{-1}, and 1.35±0.02 g L^{-1} in grape juice-based medium, apple juice-based medium, and standard medium, respectively. It was observed that *C. cohnii* cells had a higher growth rate in nutrient medium containing grape juice. This indicates that *C. cohnii* cells provide a higher biomass yield in grape juice-based medium with a higher glucose content, but cannot use fructose efficiently, and fructose is a less metabolized monosaccharide compared to those carried out by *S. limacinum*. Besides, juice-based media enhanced biomass production; standard medium containing glycerol showed a similar result to apple juice-based medium with a high fructose content in terms of biomass yield. In literature, glucose has been mentioned as a commonly used carbon source for *C. cohnii* cells (Mendes et al., 2009). In this case, it was concluded that glucose was metabolized at a higher rate by *C. cohnii* cells compared to fructose and glycerol and showed higher growth.

The results were analysed via two-way ANOVA and Tukey's multiple comparison test. In comparison of all results, the biomass production capacities of *S. limacinum* and *C. cohnii* cultures in different media were found to be statistically meaningful with different *p*-values. The *p-values* for *S. limacinum* cultures in apple juice-based medium and grape juice-based medium, and the cultures in apple juice-based medium and standard medium were <0.0001, while this value was 0.0304 for *S. limacinum* cultures in grape juice-based medium and standard medium. For *C. cohnii* cultures, the *p*-value for the biomass production in apple juice-based medium and standard medium, and the biomass production in grape juice-based medium and standard medium were <0.0001, whereas this value was reported as 0.0034 for *C. cohnii* cultures in apple juice-based medium and standard medium.

The utilization of juice-based media enhanced biomass production in both cultures. Fruit juices, in addition to their high carbon content, are also known to be rich in micronutrients. Those micronutrients, such as iron, magnesium, zinc, and phosphorus, support microalgal growth and lipid accumulation. Moreover, fruit juices contain high amounts of vitamin B and C, and they play a supporting role in growth and lipid biosynthesis. Considering juice-based media and standard media, the contents of these fruit juices provide the opportunity to produce higher biomass compared to those of standard media. The results proved that the selection of carbon sources is an important step in microalgae production and has a great impact on cell growth.

The Lipid Accumulation in Microalgae

As the type of carbon source is important, the ratio between the C and N sources also has an impact on lipid accumulation (Wen & Chen, 2003; Zhu et al., 2007). Therefore, the amounts of fruit juice supplied to carbon-free standard media were determined to maintain the same C/N ratio in each media in order to clearly understand the effect of juice types on lipid production.

One of the most important steps in lipid metabolism in microalgae is acetyl-CoA biosynthesis pathway. The research showed that the addition of organic acids, which existed in the pathway, has an impact on lipid production. Apple juice and grape juice contain high amounts of citric acid and malic acid, which participate in these pathways. Comparing their concentrations, apple juice has more malic acid, while grape juice is richer in citric acid (Li et al., 2020). The reason grape juice-based medium provided highest lipid yield from *S. limacinum* (41.03±0.44%) is that grape juice contains the highest amount of citric acid compared to others, and *S. limacinum* utilizes acetic acid more efficiently compared to malic acid (Figure 5(a)). Besides that, the addition of malic acid has an increasing effect on lipid accumulation, although not as much as the effect of citric acid on *S. limacinum* lipid production (Ren

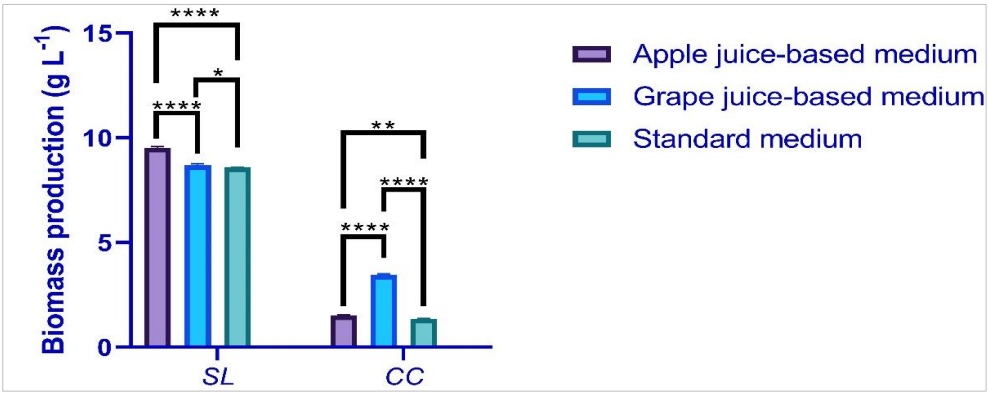

Figure 4. The biomass production from *Schizochytrium limacinum* and *Crypthecodinium cohnii* during the cultivations in juice-based and standard media (SL: *Schizochytrium limacinum*, CC: *Crypthecodinium cohnii*, **** means P value≤0.0001, *** means P value≤0.001, ** means P value≤0.01, * means P value≤0.05).

et al., 2009). On the other hand, *C. cohnii* accumulated 15.13±0.45% and 14.49±0.50% lipids in its biomass during the cultivation in apple juice-based medium and grape juice based medium. The result showed that *C. cohnii* has the capacity to consume both citric acid and malic acid at the same time. As each juice-based medium contains malic acid in different concentrations, the lowest lipid production was obtained from the culture grew in each standard medium as there is no additional acid in those media. There was a statistically meaningful difference between the lipid yields of the cultures in juice-based media and standard media, except between the samples obtained from *C. cohnii* produced in apple juice-based medium and grape juice-based medium, as the *P*-value was higher than 0.05.

It was observed that the lipid production from *S. limacinum* increased by 17.6% in the medium with apple juice (2.54±0.02 g L^{-1}), while this increase was 65.3% in grape juice-based medium (3.57±0.02 g L^{-1}) (Figure 5(b)). The highest amount of lipids was obtained from production using grape juice-based medium, and this value was followed by the cultures in apple juice-based medium and standard medium. Apple juice has a higher fructose/glucose ratio compared to grape juice, while there is no fructose in standard medium of *S. limacinum* (Can, 2021; Patil & Gogate, 2015). The reason the culture produced more lipids in juice-based media compared to standard medium can be the fructose content in fruit juices. *C. cohnii* culture produced lipids at 0.08±0.01 g L^{-1} concentration in standard medium. The addition of apple juice caused an increase in the lipid amount 1.9 times higher (0.23±0.02 g L^{-1}), while grape juice induced 5.3 times more lipid production in *C. cohnii* culture (0.50±0.03 g L^{-1}). There was a slight difference in the lipid amounts between the productions carried out in the standard medium and the medium containing apple juice due to glycerol content of the standard medium and low glucose density of the apple juice-based medium.

The results were statistically analysed via two-way ANOVA and Tukey's multiple comparison test. The difference between the capacity of lipid production from *S. limacinum* and *C. cohnii* in each of the media was found to be statistically meaningful. The *p-values* for *S. limacinum* and *C. cohnii* cultures for lipid production in juice-based media and standard media were <0.0001.

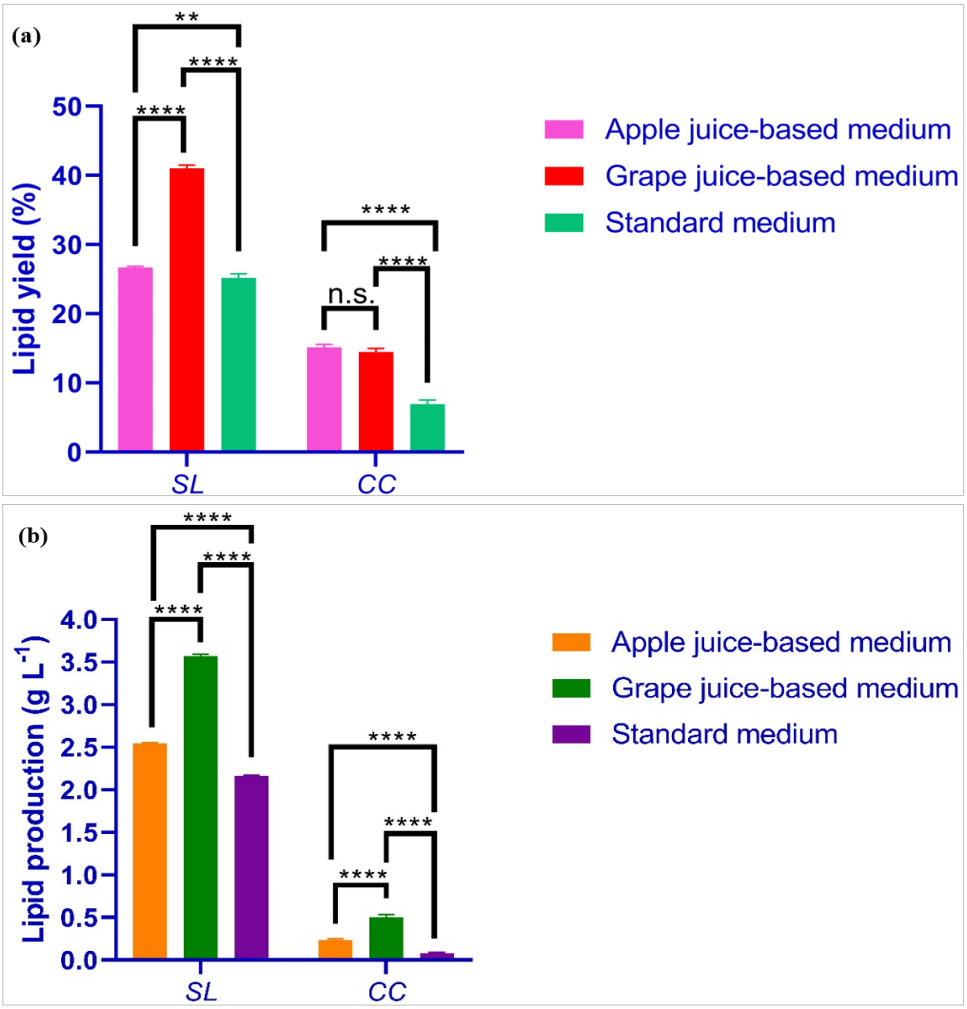

Figure 5. (a) The lipid yield of *Schizochytrium limacinum* and *Crypthecodinium cohnii* biomass and (b) the lipid production of the cultures produced in juice-based and standard media (SL: *Schizochytrium limacinum*, CC: *Crypthecodinium cohnii*, n.s.: non- significant, **** means P value≤0.0001, ** means P value≤0.01).

Conclusions

Many experiments have been conducted to predict the potential of various carbon sources in microalgae cultivation to reduce costs in the commercial production of biomass and lipids from microalgae. These include beer production waste, sweet sorghum juice, crude glycerol, molasses, orange peel extract, date syrup, pineapple extract, waste food syrup, hydrolyzate, and coconut water. In this study, *S. limacinum* and *C. cohnii* cultures were heterotrophically produced in apple and grape juice-based media in order to underline their biomass and lipid production capacities. These cultures stand out with their high lipid content, which has important potential for industrial production. The production efficiency was compared with those cultures grown in standard media, including glucose or glycerol as carbon sources. For both cultures, the fruit juice-based media provided microalgae cultures with more efficient biomass production and lipid accumulation than the use of pure glucose and glycerol due to the glucose, fructose, and micronutrient contents of the fruit juices. It was concluded that waste and pulp produced in fruit juice production, which are rich in valuable compounds such as carbons, vitamins, and organic acids, are promising candidates as nutrients that offer alternative solutions for establishing more sustainable, economic, and eco-friendly microalgae productions. For further studies (i) in order to maximize both growth and fatty acid biosynthesis in industrial scale production with heterotrophic microalgae species, it is recommended to combine different fruit wastes generated in the fruit juice industry, (ii) it is thought that optimization studies in which fruit juice industry wastes are applied in different concentrations in heterotrophic microalgae production, as well as trials of different operating modes, will be useful in scale-up studies, and (iii) genetic modification efforts in commercially produced microalgae are at a promising point, it is recommended to modify the genes involved in carbon metabolism and lipid biosynthesis pathways, which affect microorganism growth of *S. limacinum* and *C. cohnii* species in terms of increasing productivity.

Ethical Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding Information

The authors did not receive support from any organization for the submitted work.

Author Contribution

Funda Can: Conceptualization, Data curation, Formal analysis, Methodology, Writing-original draft, Çağla Yarkent: Conceptualization, Data curation, Formal analysis, Methodology, Writing-original draft, Deniz Ş. Öncel: Conceptualization, Data curation, Formal analysis, Methodology, Writing-original draft, Suphi S. Oncel: Supervision, Conceptualization, Data curation, Formal analysis, Methodology, Writing- review & editing. All the authors have read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

References

Abdel-Wahab, M. A., El-Samawaty, A. E. R. M. A., Elgorban, A. M., & Bahkali, A. H. (2021). Fatty acid production of thraustochytrids from Saudi Arabian mangroves. *Saudi Journal of Biological Sciences*, *28*(1), 855–864. https://doi.org/10.1016/j.sjbs.2020.11.024

Adarme-Vega, T. C., Lim, D. K. Y., Timmins, M., Vernen, F., Li, Y., & Schenk, P. M. (2012). Microalgal biofactories: a promising approach towards sustainable omega-3 fatty acid production. In *Microbial Cell Factories* (Vol. 11). https://doi.org/10.1186/1475-2859-11-96

Béligon, V., Christophe, G., Fontanille, P., & Larroche, C. (2016). Microbial lipids as potential source to food supplements. In *Current Opinion in Food Science* (Vol. 7, pp. 35–42). Elsevier Ltd. https://doi.org/10.1016/j.cofs.2015.10.002

Brennan, L., & Owende, P. (2010). Biofuels from microalgae-A review of technologies for production, processing, and extractions of biofuels and co-products. In *Renewable and Sustainable Energy Reviews* (Vol. 14, Issue 2, pp. 557–577). https://doi.org/10.1016/j.rser.2009.10.009

Calder, P. C., & Yaqoob, P. (2009). Omega-3 polyunsaturated fatty acids and human health outcomes. In *BioFactors* (Vol. 35, Issue 3, pp. 266–272). https://doi.org/10.1002/biof.42

Can, F. (2021). *Investigarion of the oil production potential of heterotrophic microalgae by using fruit juice based culture medium*.

Cheirsilp, B., & Torpee, S. (2012). Enhanced growth and lipid production of microalgae under mixotrophic culture condition: Effect of light intensity, glucose concentration and fed-batch cultivation. *Bioresource Technology*, *110*, 510–516. https://doi.org/10.1016/j.biortech.2012.01.125

Chew, K. W., Chia, S. R., Show, P. L., Yap, Y. J., Ling, T. C., & Chang, J. S. (2018). Effects of water culture medium, cultivation systems and growth modes for microalgae cultivation: A review. In *Journal of the Taiwan Institute of Chemical Engineers* (Vol. 91, pp. 332–344). Taiwan Institute of Chemical Engineers. https://doi.org/10.1016/j.jtice.2018.05.039

Chi, Z., Pyle, D., Wen, Z., Frear, C., & Chen, S. (2007). A laboratory study of producing docosahexaenoic acid

from biodiesel-waste glycerol by microalgal fermentation. *Process Biochemistry*, *42*(11), 1537–1545. https://doi.org/10.1016/j.procbio.2007.08.008

De Morais, M. G., Vaz, B. D. S., De Morais, E. G., & Costa, J. A. V. (2015). Biologically Active Metabolites Synthesized by Microalgae. *BioMed Research International*, *2015*, 1–15. https://doi.org/10.1155/2015/835761

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric Method for Determination of Sugars and Related Substances. *Analytical Chemistry*, *28*(3), 350–356. https://doi.org/10.1021/ac60111a017

FAO. (2019). Food and Agriculture Organization of the United Nations. http://www.fao.org

Fernández, F. G. A., Reis, A., Wijffels, R. H., Barbosa, M., Verdelho, V., & Llamas, B. (2021). The role of microalgae in the bioeconomy. *New Biotechnology*, *61*, 99–107. https://doi.org/10.1016/j.nbt.2020.11.011

Gantar, M., & Svirčev, Z. (2008). Microalgae and cyanobacteria: Food for thought. In *Journal of Phycology* (Vol. 44, Issue 2, pp. 260–268). https://doi.org/10.1111/j.1529-8817.2008.00469.x

Gouveia, L., Batista, A. P., Sousa, I., Raymundo, A., & Bandarra, N. M. (2008). Microalgae in Novel Food Products. In *Food Chemistry Research Developments* (pp. 1–37). Nova Science Publishers.

Gouveia, L., Oliveira, A. C., Congestri, R., Bruno, L., Soares, A. T., Menezes, R. S., Filho, N. R. A., & Tzovenis, I. (2017). Biodiesel from microalgae. In *Microalgae-Based Biofuels and Bioproducts: From Feedstock Cultivation to End-Products*. Elsevier Ltd. https://doi.org/10.1016/B978-0-08-101023-5.00010-8

Gupta, A., Barrow, C. J., & Puri, M. (2022). Multiproduct biorefinery from marine thraustochytrids towards a circular bioeconomy. *Trends in Biotechnology*, *40*(4), 448–462. https://doi.org/10.1016/j.tibtech.2021.09.003

Gürlek, C., Yarkent, Ç., Köse, A., Tuğcu, B., Gebeloğlu, I. K., Öncel, S., & Elibol, M. (2020a). Screening of antioxidant and cytotoxic activities of several microalgal extracts with pharmaceutical potential. *Health and Technology*, *10*(1), 111–117. https://doi.org/10.1007/s12553-019-00388-3

Gürlek, C., Yarkent, Ç., Köse, A., Oral, İ., Öncel, S., & Elibol, M. (2020b). Evaluation of several microalgal extracts as bioactive metabolites as potential pharmaceutical compounds. *IFMBE Proceedings*, *73*, 267–272. https://doi.org/10.1007/978-3-030-17971-7_41

Gurlek, C., Yarkent, C., Oral, I., Kose, A., & Oncel, S. S. (2019). Nutraceutical Aspects of Microalgae Will Our Future Space Foods Be Microalgae Based? *Handbook of Algal Technologies and Phytochemicals*, 177–184. https://doi.org/10.1201/9780429054242-18

Guschina, I. A., & Harwood, J. L. (2013). Algal lipids and their metabolism. In *Algae for Biofuels and Energy* (pp. 17–36). Springer Netherlands. https://doi.org/10.1007/978-94-007-5479-9

Jacob-Lopes, E., Mérida, L. G. R., Queiroz, M. I., & Zepka, L. Q. (2015). Microalgal Biorefineries. In *Biomass Production and Uses*. InTech. https://doi.org/10.5772/59969

Kaya, K., Nakazawa, A., Matsuura, H., Honda, D., Inouye, I., & Watanabe, M. M. (2011). Thraustochytrid aurantiochytrium sp. 18w-13a accummulates high amounts of squalene. *Bioscience, Biotechnology and Biochemistry*, *75*(11), 2246–2248. https://doi.org/10.1271/bbb.110430

Li, J., Zhang, C., Liu, H., Liu, J., & Jiao, Z. (2020). Profiles of Sugar and Organic Acid of Fruit Juices: A Comparative Study and Implication for Authentication. *Journal of Food Quality*, *2020*. https://doi.org/10.1155/2020/7236534

Liang, Y., Sarkany, N., Cui, Y., Yesuf, J., Trushenski, J., & Blackburn, J. W. (2010). Use of sweet sorghum juice for lipid production by Schizochytrium limacinum SR21. *Bioresource Technology*, *101*(10), 3623–3627. https://doi.org/10.1016/j.biortech.2009.12.087

Mendes, A., Reis, A., Vasconcelos, R., Guerra, P., & Lopes Da Silva, T. (2009). Crypthecodinium cohnii with emphasis on DHA production: A review. *Journal of Applied Phycology*, *21*(2), 199–214. https://doi.org/10.1007/s10811-008-9351-3

Mittal, V., & Ghosh, U. K. (2023). Potential of microalgae for phytoremediation of various wastewaters for nutrient removal and biodiesel production through nanocatalytic transesterification. *Asia-Pacific Journal of Chemical Engineering*, *18*(1). https://doi.org/10.1002/apj.2847

Molino, A., Mehariya, S., Di Sanzo, G., Larocca, V., Martino, M., Leone, G. P., Marino, T., Chianese, S., Balducchi, R., & Musmarra, D. (2020). Recent developments in supercritical fluid extraction of bioactive compounds from microalgae: Role of key parameters, technological achievements and challenges. *Journal of CO2 Utilization*, *36*, 196–209. https://doi.org/10.1016/j.jcou.2019.11.014

Nazir, Y., Halim, H., Al-Shorgani, N. K. N., Manikan, V., Hamid, A. A., & Song, Y. (2020). Efficient conversion of extracts from low-cost, rejected fruits for high-valued Docosahexaenoic acid production by Aurantiochytrium sp. SW1. *Algal Research*, *50*. https://doi.org/10.1016/j.algal.2020.101977

Oncel, S., & Sukan, F. V. (2008). Comparison of two different pneumatically mixed column photobioreactors for the cultivation of Artrospira platensis (Spirulina platensis). *Bioresource Technology*, *99*(11), 4755–4760. https://doi.org/10.1016/j.biortech.2007.09.068

Panchal, B. M., Padul, M. V., & Kachole, M. S. (2016). Optimization of biodiesel from dried biomass of Schizochytrium limacinum using methanesulfonic acid-DMC. *Renewable Energy*, *86*, 1069–1074. https://doi.org/10.1016/j.renene.2015.09.027

Patil, K. P., & Gogate, P. R. (2015). Improved synthesis of docosahexaenoic acid (DHA) using Schizochytrium limacinum SR21 and sustainable media. *Chemical Engineering Journal*, *268*, 187–196. https://doi.org/10.1016/j.cej.2015.01.050

Perez-Garcia, O., Escalante, F. M. E., de-Bashan, L. E., & Bashan, Y. (2011). Heterotrophic cultures of microalgae: Metabolism and potential products. In *Water Research* (Vol. 45, Issue 1, pp. 11–36). Elsevier Ltd. https://doi.org/10.1016/j.watres.2010.08.037

Pulz, O., & Gross, W. (2004). Valuable products from biotechnology of microalgae. In *Applied Microbiology and Biotechnology* (Vol. 65, Issue 6, pp. 635–648). https://doi.org/10.1007/s00253-004-1647-x

Quero-Jiménez, P. C., Montenegro, O. N., Sosa, R., De La Torre, J. B., Valero Acosta, J., López Pérez, D., Santana Rodríguez, A., Ramos Méndez, R., Alonso, A. C., Corrales, A. J., & Broche Hernández, N. (2019). *Total carbohydrates concentration evaluation in products of microbial origin*.

Quilodrán, B., Hinzpeter, I., Quiroz, A., & Shene, C. (2009). Evaluation of liquid residues from beer and potato processing for the production of docosahexaenoic acid (C22:6n-3, DHA) by native thraustochytrid strains. *World*

Journal of Microbiology and Biotechnology, *25*(12), 2121–2128. https://doi.org/10.1007/s11274-009-0115-2

Rashid, N., Ur Rehman, M. S., Sadiq, M., Mahmood, T., & Han, J. I. (2014). Current status, issues and developments in microalgae derived biodiesel production. *Renewable and Sustainable Energy Reviews*, *40*, 760–778. https://doi.org/10.1016/j.rser.2014.07.104

Ratledge, C. (2004). Fatty acid biosynthesis in microorganisms being used for Single Cell Oil production. In *Biochimie* (Vol. 86, Issue 11, pp. 807–815). Elsevier B.V. https://doi.org/10.1016/j.biochi.2004.09.017

Ratledge, C. (2008). Microbial Lipids. In *Bitechnology* (pp. 133–197).

Razzak, S. A., Ali, S. A. M., Hossain, M. M., & deLasa, H. (2017). Biological CO2 fixation with production of microalgae in wastewater – A review. *Renewable and Sustainable Energy Reviews*, *76*(September 2015), 379–390. https://doi.org/10.1016/j.rser.2017.02.038

Ren, L. J., Huang, H., Xiao, A. H., Lian, M., Jin, L. J., & Ji, X. J. (2009). Enhanced docosahexaenoic acid production by reinforcing acetyl-CoA and NADPH supply in Schizochytrium sp. HX-308. *Bioprocess and Biosystems Engineering*, *32*(6), 837–843. https://doi.org/10.1007/s00449-009-0310-4

Senturk, T. (2024). Phycoremediation of Phenolphytalein, Indigo Carmine, Methyl Orange and Eriochrome Black T By Using Chlamydomonas and Chlorella sp. (Chlorophyta). *Turkish Journal of Fisheries and Aquatic Sciences*, *24*(6). https://doi.org/10.4194/TRJFAS25765

Singh, S. P., & Singh, P. (2014). Effect of CO2 concentration on algal growth: A review. *Renewable and Sustainable Energy Reviews*, *38*, 172–179. https://doi.org/10.1016/j.rser.2014.05.043

Suparmaniam, U., Lam, M. K., Uemura, Y., Lim, J. W., Lee, K. T., & Shuit, S. H. (2019). Insights into the microalgae cultivation technology and harvesting process for biofuel production: A review. In *Renewable and Sustainable Energy Reviews* (Vol. 115). Elsevier Ltd. https://doi.org/10.1016/j.rser.2019.109361

Sülük, K., Tosun, Ġ., & Ekinci, K. (2018). Determination of Properties of Apple Processing Waste and Investigation of Appropriate Disposal Methods. *Bilge International Journal of Science and Technology Research*, *2*, 98–108. http://doi.org/10.30516/bilgesci.497147

Statista. (2022). Global Fruit Production Volume, by selected region. https://www.statista.com/outlook/cmo/food-/fruitsnuts/turkey

Tandon, P., & Jin, Q. (2017). Microalgae culture enhancement through key microbial approaches. *Renewable and Sustainable Energy Reviews*, *80*(May), 1089–1099. https://doi.org/10.1016/j.rser.2017.05.260

Tokgöz, M., Yarkent, Ç., Köse, A., & Oncel, S. S. (2023). The potential of microalgal sources as coating materials: A case study for the development of biocompatible surgical sutures. *Letters in Applied Microbiology*, *76*(8). https://doi.org/10.1093/lambio/ovad086

Torres-Tiji, Y., Fields, F. J., & Mayfield, S. P. (2020). Microalgae as a future food source. In *Biotechnology Advances* (Vol. 41). Elsevier Inc. https://doi.org/10.1016/j.biotechadv.2020.107536

Unagul, P., Assantachai, C., Phadungruengluij, S., Suphantharika, M., Tanticharoen, M., & Verduyn, C. (2007). Coconut water as a medium additive for the production of docosahexaenoic acid (C22:6 n3) by Schizochytrium mangrovei Sk-02. *Bioresource Technology*, *98*(2), 281–287. https://doi.org/10.1016/j.biortech.2006.01.013

Verma, M. L., Kishor, K., Sharma, D., Kumar, S., & Sharma, K. D. (2019). Microbial production of omega-3 polyunsaturated fatty acids. In *Biotechnological Production of Bioactive Compounds* (pp. 293–326). Elsevier. https://doi.org/10.1016/B978-0-444-64323-0.00010-2

Wen, Z. Y., & Chen, F. (2003). Heterotrophic production of eicosapentaenoic acid by microalgae. In *Biotechnology Advances* (Vol. 21, Issue 4, pp. 273–294). Elsevier Inc. https://doi.org/10.1016/S0734-9750(03)00051-X

Yadavalli, R., Rao, C. S., Rao, R. S., & Potumarthi, R. (2014). Dairy effluent treatment and lipids production by Chlorella pyrenoidosa and Euglena gracilis: Study on open and closed systems. *Asia-Pacific Journal of Chemical Engineering*, *9*(3), 368–373. https://doi.org/10.1002/apj.1805

Yarkent, Ç., Aslanbay Güler, B., Imamoglu, E., & Oncel, S. S. (2024). Microalgae-factories as potential antimicrobial agents: a comprehensive review. *Biologia*, 1–14. https://doi.org/10.1007/s11756-024-01616-6

Yarkent, C., Guler, A., Gurlek, C., Sahin, Y., Kose, A., Oncel, S. S., & Imamoglu, E. (2021). Algal Alginate in Biotechnology: Biosynthesis and Applications. In *Properties and Applications of Alginates* (pp. 1–26). https://doi.org/10.5772/intechopen.101407

Yarkent, Ç., Gürlek, C., & Oncel, S. S. (2020). Potential of microalgal compounds in trending natural cosmetics: A review. In *Sustainable Chemistry and Pharmacy* (Vol. 17). Elsevier B.V. https://doi.org/10.1016/j.scp.2020.100304

Yarkent, Ç., & Oncel, S. S. (2022). Recent Progress in Microalgal Squalene Production and Its Cosmetic Application. *Biotechnology and Bioprocess Engineering*, *11*, 1–11. https://doi.org/10.1007/s12257-021-0355-z

Yarkent, Ç., & Öncel, S. Ş. (2023). Biotechnological Applications of Haematococcus: Future Perspectives. In *Haematococcus* (pp. 293–320). Springer Nature Singapore. https://doi.org/10.1007/978-981-99-2901-6_17

Yen, H. W., Hu, I. C., Chen, C. Y., Ho, S. H., Lee, D. J., & Chang, J. S. (2013). Microalgae-based biorefinery - From biofuels to natural products. *Bioresource Technology*, *135*, 166–174. https://doi.org/10.1016/j.biortech.2012.10.099

Yilmaz, H. A., Kumlu, M., Evliyaoğlu, E., Praiboon, J., Duman, M. B., Çimen, B. A., Işik, O., & Eroldoğan, O. T. (2023). Effects of the Alga Aurantiochytrium mangrovei FIKU008-Enriched Artemia on Early Stages of the Green Tiger Shrimp, Penaeus semisulcatus. *Turkish Journal of Fisheries and Aquatic Sciences*, *23*(11). https://doi.org/10.4194/TRJFAS23912

Yin, Z., Zhu, L., Li, S., Hu, T., Chu, R., Mo, F., Hu, D., Liu, C., & Li, B. (2020). A comprehensive review on cultivation and harvesting of microalgae for biodiesel production: Environmental pollution control and future directions. In *Bioresource Technology* (Vol. 301). Elsevier Ltd. https://doi.org/10.1016/j.biortech.2020.122804

Yokochi, T., Honda, D., Higashihara, T., & Nakahara, T. (1998). Optimization of docosahexaenoic acid production by Schizochytrium limacinum SR21. *Applied Microbiology and Biotechnology*, *49*, 72–76.

Zhu, L., Zhang, X., Ren, X., & Zhu, Q. (2007). Effects of culture conditions on growth and docosahexaenoic acid production from Schizochytrium limacinum. *Journal of Ocean University of China*, *7*(1), 83–88. https://doi.org/10.1007/s11802-008-0083-4