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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Effect of Different Drying Methods on Biochemical Composition of *Chlorella vulgaris, Microcystis aeruginosa* and *Haematococcus pluvialis*

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Abstract: As a critical stage in the processing of microalgal biomass, drying has a big impact on the biochemical composition of the biomass. This study assesses how *Chlorella vulgaris, Microcystis aeruginosa*, and *Haematococcus pluvialis'* protein, lipid, and carbohydrate content are affected by drying using oven, lyophilizer and microwave. Because of its low rate of thermal degradation, the findings show that lyophilization maintains the highest protein (30–55%) and lipid (10–35%) content among all species. However, because of the breakdown of other macromolecules, oven-drying and microwave-drying raise the relative carbohydrate content by up to 35%. It was seen that the highest antioxidant activity was determined from *M. aeruginosa.* Similar to biochemical composition exhibit a tendency whereby freeze-dry maintains the highest levels, oven-drying causes moderate losses, and microwave-drying causes considerable deterioration. This is probably because antioxidant chemicals are sensitive to heat and are easily oxidized and degraded in hot environments. These results demonstrate that while oven-drying and microwave-drying may be more suited for applications needing biomass rich in carbohydrates, including the generation of biofuel, freeze-drying is the recommended technique for maintaining high-value biochemical components. Choosing the right drying technique is crucial for maximizing the use of biomass in a range of industrial applications.

Keywords: Antioxidant activity, biochemical composition, drying, microalgae.

Farklı Kurutma Yöntemlerinin *Chlorella vulgaris, Microcystis aeruginosa* ve *Haematococcus pluvialis*'in Biyokimyasal Kompozisyonuna Etkisi

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Öz: Mikroalg biyokütlesinin işlenmesinde kritik bir aşama olan kurutma, mikroalgin biyokimyasal bileşimi üzerinde büyük bir etkiye sahiptir. Bu çalışma, *Chlorella vulgaris, Microcystis aeruginosa* ve *Haematococcus pluvialis*'in protein, lipit ve karbonhidrat içeriğinin etüv, liyofilizatör ve mikrodalga kullanılarak kurutulmasından nasıl etkilendiğini değerlendirmektedir. Bulgular, düşük sıcaklık nedeniyle liyofilizasyonun tüm türler arasında en yüksek protein (%30-55) ve lipit (%10-35) içeriğini koruduğunu göstermektedir. Ancak diğer makromoleküllerin parçalanması nedeniyle etüvde kurutma ve mikrodalga kurutma, göreceli olarak karbonhidrat içeriğini %35'e kadar daha yüksek çıkmasını sağlamıştır. En yüksek antioksidan aktivitenin *M. aeruginosa*'da olduğu belirlenmiştir. Biyokimyasal bileşime benzer şekilde dondurarak kurutma en yüksek seviyeleri korurken, etüvde kurutma ve mikrodalgada kurutma orta ve yüksek düzeyde kayıplara neden olmaktadır. Bunun nedeni muhtemelen antioksidan kimyasalların ısıya duyarlı olması ve sıcak ortamlarda kolayca oksitlenip parçalanmasıdır. Bu sonuçlar, etüvde kurutma ve mikrodalgada kurutmanın biyoyakıt üretimi de dahil olmak üzere karbonhidrat açısından zengin biyokütle gerektiren uygulamalar için daha uygun olabileceğini gösterirken, dondurarak kurutmanın yüksek değerli biyokimyasal bileşenleri korumak için önerilen teknik olduğunu göstermektedir. Doğru kurutma tekniğini seçmek, çeşitli endüstriyel uygulamalarda biyokütlenin kullanımını en üst düzeye çıkarmak için çok önemlidir.

Anahtar kelimeler: Antioksidan aktivite, biyokimyasal bileşim, kurutma, mikroalgler.

INTRODUCTION

Microalgae's tremendous productivity and having no competition with terrestrial food crops have drawn a lot of attention as a possible feedstock for renewable energy. Due to their capacity to fix carbon dioxide, microalgae help lessen the environmental issues brought on by the usage of fossil fuels. In addition to biofuels, microalgae may be used to make high-value goods including food coloring, vitamins, and cosmetics, as well as animal feed (Hosseinizand et al., 2018). In this context, since the algal slurry produced by the upstream harvesting operations might be delicate due to having sensitive components, the drying procedure of microalgae is regarded as an essential phase. Drying algae effectively is crucial for the best possible storage since they can be vulnerable to microbiological degradation, contamination and unfavorable conditions for storage, all of which can reduce the quality of biomass (Aljabri et al., 2023). Also, it is a significant step because of the difficulties in producing biofuel from wet microalgae in in situ transesterification, the most popular process for producing biodiesel (Hosseinizand et al., 2018).

A variety of methods, including, hot air drying, oven drying, traditional sun drying, spray drying, freeze drying and microwave drying are available for drying algae (Aljabri et al., 2023; Hernández et al., 2024). The conventional method of employing solar radiation to dry agricultural products is inexpensive, but it takes a long time, weather-dependent and the biomass is susceptible to contamination from dust, insects and other sources. In contrast to open sun drying, solar drying uses a solar dryer with a chamber that shields the material from direct exposure to dust, insects, rodents, rain, and other environmental factors while performing the drying process. In contrast to open sun drying, solar drying carries out in a vessel that shields the biomass. When compared to open sun drying, solar dryers can also achieve shorter drying times (Agbede et al., 2020; Aljabri et al., 2023). Convective oven drying, also known as hot air drying, is more hygienic and offers more drying uniformity because hot air is fed to the biomass at a specific velocity and air temperature. Unlike convective drying, microwave drying heats the biomass internally using electromagnetic microwaves, which accelerates the evaporation of water due to the rapid absorption of microwave energy by water molecules. Although this results in shorter drying times and faster drying rates, the heat-labile metabolites and bioactive substances may be adversely affected by oven drying (Agbede et al., 2020; Aljabri et al., 2023).

Methods like spray drying and freeze drying have grown in popularity for drying algal biomass. While spray-drying is time efficient and results with high-value goods, freeze-drying can preserve valuable byproducts that could otherwise be lost, making it one of the safest drying methods. These approaches have the drawback of being expensive to operate and maintain (Aljabri et al., 2023; Van De Walle et al., 2024).

In the literature, the studies on microalgal drying are limited and new. Recently, Behera and Balasubramanian reported their studies on convective and microwave drying of microalgae. According to their studies, the lipid content of the microalgal consortium when dried with conventional method at 60 °C, resulted as 24% (w/w). On the contrary, when the mixed culture was dried with microwave method, the lipid content was found as 28% (w/w). Furthermore, it was claimed that the biochemical content of microwave-dried samples relative to oven-dried samples indicated that the former may have had a higher quantity of biochemical content than the latter (Behera & Balasubramanian, 2021). In another, study, it was reported that the freeze-drying method applied to *Tetraselmis subcordiformis* maintained the most proteins, lipids, and chlorophyll where oven drying fared poorly (Aljabri et al., 2023). Similar conclusions were also stated by Madhubalaji et al. who implied that each drying technique has pros and cons. It was highlighted that sun drying reduced the amount of minerals and carbs but increased the amount of soluble material and antioxidant activity. Although there is no control over the drying settings, the sun-drying method may be taken into consideration for financial considerations. Its slower rate of drying and relatively increased bacterial load are its drawbacks. According to Madhubalaji et al., the best approach is freeze drying since it preserves the most nutrients in the biomass, but it uses a lot of energy (Madhubalaji et al., 2021). Guldhe et al. investigated effectiveness of drying methods for recovering microalgal lipids to obtain biodiesel. It was reported that lipid amount after drying using the freeze-drying, oven-drying, and sun-drying methods, yielded 29.65%, 28.63%, and 28.33% lipid (w/w) respectively (Guldhe et al., 2014). Among these studies, it was seen that there are not any studies on a comparative analysis of drying methods on biochemical composition of *Chlorella vulgaris*, *Microcystis aeruginosa*, and *Haematococcus pluvialis.* The purpose of this article is to investigate how different drying methods such as oven drying, lyophilization (freeze-drying), and microwave drying affect the biochemical composition (protein, lipid, and carbohydrate content) and antioxidant activity of three microalgae species (*Chlorella vulgaris*, *Microcystis aeruginosa* and *Haematococcus pluvialis*). By understanding these effects, the study aims to identify the most suitable drying technique that preserves valuable biochemical components and optimizes biomass quality for various industrial applications, such as biofuel

production or health-related products. In order to carry out an effective microalgal processing in a variety of applications, including the production of biofuel, medicines, and functional foods, this study will close this knowledge gap by offering insightful information about how various drying techniques affect important biochemical components.

MATERIAL AND METHOD

Microalgae Cultivation: In this study, *Chlorella vulgaris 211-11b, Haematococcus pluvialis 34-1a* and *Microcystis aeruginosa 1450-1* cultures were selected to evaluate the effect of various drying techniques on biochemical content and radical scavenging characteristic of these species. In this context, at room temperature, cultures were cultivated with 10% (v/v) inoculum in 500 mL Erlenmeyer flasks in BG-11 media at pH 7.8 in a shaking incubator with constant light (180 µmol/m^2/s). Microalgae were collected by centrifugation at 5000 rpm for 15 minutes after reaching the stationary phase, and they were subsequently dried with different methods (Sert et al. 2018).

Drying Process of the Microalgae: Microalgae were dried by lyophilization using a freeze-dryer (Christ Alpha, 1–2 LD plus, Germany), in an oven (Binder, Germany), microwave (Beko, MD1505, Türkiye). As for the lyophilization, cultures were freeze-dried at the temperature of -48 °C for 24 h. In the drying process in oven, cultures were exposed to 60°C overnight. For the cultures that were dried with microwave was carried out at 600 W for a minute. All drying steps were carried out until approximately 1 gr dry microalgae were obtained. Once the samples were dried, they were ground into small pieces with a mortar and pestle and stored in a desiccator for characterization (Hosseinizand et al. 2018, Stramarkou et al. 2017).

Also, the samples with almost 70±2% moisture were evaluated for drying kinetics. Their weight was continuously recorded throughout the drying process of lyophilization and oven in order to determine the drying kinetics. Examination of a first-order kinetic model was carried out in order to describe the moisture transfer during the drying operations.

$$-dX/dt = k\,(X - X_e) \qquad (1)$$

According to Kyriakopoulou et al., X represents material moisture content on dry basis during drying (g water g^{-1} dry solids), X_e represents equilibrium moisture content of dehydrated material (g water g^{-1} dry solids), k represents drying rate (min^{-1}), and t represents processing time (min) (Kyriakopoulou et al., 2013). In this step, only freeze-dry and oven have been considered since measurements from microwave drying could not be possible.

Determination of Biochemical Content: The protein, lipid and carbohydrate content of dried microalgae were specified by the phenol Lowry method (Lowry et al., 1951), Bligh and Dyer method (Bligh & Dyer, 1959) and sulfuric acid method (Dubois et al., 1956), respectively. Every characterization analysis was performed three times and given as percentage of dry weight.

Determination of Radical Scavenging Characteristic: The total antioxidant activities of *Chlorella vulgaris, Microcystis aeruginosa*, and *Haematococcus pluvialis* were determined using the 1,1-diphenyl-2-picryl hydrazil (DPPH) free radical scavenging method (Brand-Williams et al., 1995). In this method, firstly, lipid extracts of the samples were dissolved in methanol at the concentration of 1% w/v. After that, lipid extracts in methanol (0.1 ml) was added to 3.9 mL of a 6×10^{-5} mol/L methanol DPPH solution and absorbance at 515 nm was determined after 30 min.

The DPPH radical scavenging activity was calculated using the following formula:

$$\text{DPPH Radical Removal Activity (\%)} = [(A0 - A1)/A0] \times 100 \qquad (2)$$

A0: Absorbance value of the control

A1: Absorbance value of sample or standard BHT was used as control.

Statistical Analysis: The data expressed as mean values ± standard deviation and one-way analysis of variance was carried out and a p value < 0.05 was marked as significant. All experiments were performed in triplicate (n = 3). For each triplicate, at least three measurements were performed.

RESULTS AND DISCUSSION

Figure 1 represented the results of weight change in microalgal samples before and after drying process. It was seen that, the highest moisture removal during the drying process time was observed with the microwave, and the oven and lyophilization resulted as close values, respectively. The calculation of drying kinetics was carried out using the experimental data that was presented in Figure 2. The estimation of the drying constants k was as follows: $k = 2.22 \times 10^{-6}$ 2.42×10^{-6}, and 2.18×10^{-6} min^{-1} and $k = 1.22 \times 10^{-6}$, 1.32×10^{-6} min^{-1} and 1.25×10^{-6} for freeze-dry and oven drying, respectively.

It was seen that the ultimate moisture content of the oven-dried microalgae was similar that of the freeze-dried microalgae samples. Although the material's surface dehydrated more quickly in the oven-treated samples, some moisture remained beneath the surface despite the sample's thin layer. The intense drying process produces a hard, dry

shell on the surface that prevents the remaining moisture from diffusing to the upper surface and, eventually, the air (Aljabri et al., 2023; Shekarabi et al., 2019; Stramarkou et al., 2017).

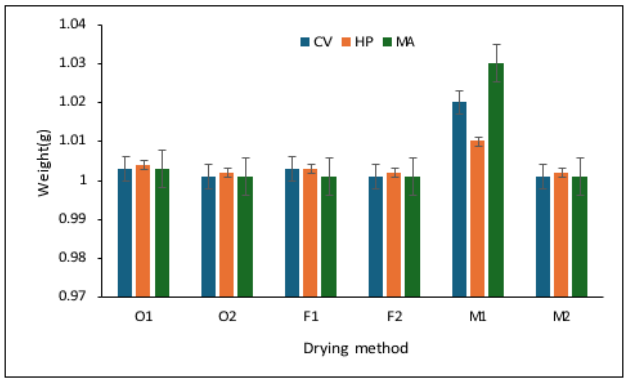

Figure 1. Results of weight change in microalgal samples before and after drying process. CV, HP and MA mean *Chlorella vulgaris*, *Haematococcus pluvialis* and *Microcystis aeruginosa*, respectively. O1 and O2, F1 and F2, and M1 and M2 mean the weights of the samples drying in oven, freeze-dry and microwave at the beginning and at the end of the process. Values are expressed as mean ± standard deviation, n=3. Values in the same column with different letters are significantly different ($p<0.05$).

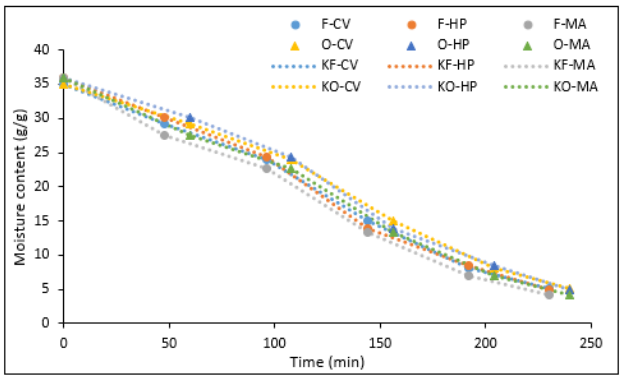

Figure 2. Drying kinetics of freeze-dried (F) and oven-dried (O) microalgae cultures. CV, HP and MA mean *Chlorella vulgaris*, *Haematococcus pluvialis* and *Microcystis aeruginosa*, respectively. KF and KO mean kinetics of the microalgae cultures.

Table 1. Biochemical composition of *H. pluvialis*, *M. aeruginosa*, and *C. vulgaris* determined after different drying methods.

Component (%)	Drying Method	*C. vulgaris*	*M. aeruginosa*	*H. pluvialis*
Protein	Freeze-Dry	50±4	45±2	30±2
	Oven-Dry	40±2	35±3	25±2
	Microwave-Dry	42±1	38±2	28±1
Lipids	Freeze-Dry	15±2	10±3	25±2
	Oven-Dry	10±3	7±2	18±2
	Microwave-Dry	12±1	8±1	20±2
Carbohydrates	Freeze-Dry	10±2	15±3	20±3
	Oven-Dry	15±2	20±2	25±3
	Microwave-Dry	12±1	18±1	22±2

As for the biochemical content of microalgae samples, it was seen that type of the drying process affected the biochemical content determination clearly, especially for the lipid and content (Table 1).

The biochemical contents of *H. pluvialis*, *M. aeruginosa*, and *C. vulgaris* is strongly influenced by the drying process. Each of the three drying methods examined-freeze-drying, oven-drying, and microwave-drying have a unique effect on the retention of protein, lipid, and carbohydrates. The main causes of these fluctuations, which affect macromolecular stability, include oxidation, water loss, and thermal degradation (Aljabri et al., 2023; Chaijan et al., 2017; Stramarkou et al., 2017).

The high protein content in freeze-dry can be due to the absence of thermal degradation, as water is removed by sublimation at low temperatures. On the other hand, oven-drying and microwave-drying can reduce the protein content of microalgae due to thermal denaturation and Maillard reactions, which can alter amino acid composition (Van De Walle et al., 2024; Zhang et al., 2022). As for the lipid content, the low processing temperature and lack of oxidation, which stop lipid peroxidation, are responsible for comparatively good lipid preservation in freeze-drying. On the contrary, lipid breakdown results with oven-drying and microwave-drying, with reductions of 5–10% in all species. Lipid oxidation, which happens when polyunsaturated fatty acids (PUFAs) react with oxygen at high temperatures, is the main cause of these losses (Schmid et al., 2022). The overall biomass quality for uses like the manufacture of biofuel is impacted by lipid breakdown under oven-drying and microwave-drying in *Haematococcus pluvialis*, making this effect especially noteworthy. Although lipid degradation was observed in *Haematococcus pluvialis* under oven and microwave-drying, particularly in polyunsaturated fatty acids (PUFAs), this effect may not negatively impact biodiesel production. In fact, PUFAs are associated with poor oxidative stability in biodiesel, as reported by Stansell et al. (2012). Therefore, a reduction in PUFA content could potentially enhance the fuel properties of the resulting biodiesel.

Carbohydrate concentration often rises during oven-drying and microwave-drying, in contrast to proteins and lipids. This pattern is seen in *Microcystis aeruginosa* (20–30% under oven drying) and *Haematococcus pluvialis* (rising to 25–35% under oven-drying). The breakdown of proteins and lipids is responsible for the apparent increase in the proportion of carbohydrates, which results in a relative enrichment of carbohydrates in the residual dry biomass (Megawati et al., 2020; Schmid et al., 2022).

The drying characteristics of marine *Chlorella sp.* biomass have been investigated by Amin et al. (2021) using three distinct techniques: freeze drying, oven drying, and solar drying. According to the study, oven drying was the quickest method for achieving a final moisture content below 10%, whereas freeze drying took the longest. Also, they discovered that the biomass that was freeze-dried had the maximum lipid content. Similarly, Kröger et al. (2019) (Sun et al., 2020) have examined how freeze-drying affects *Scenedesmus rubescens* microalgae extraction behavior. They discovered that the cell wall of microalgae is damaged by freeze-drying, which affects the amount of lipids that are extracted. The isolated lipids' quality is

unaffected by freeze-drying, though. The findings suggest that drying may change the composition or structure of microalgae, which could affect how well industrial-scale operations work. According to Ruiz-Domínguez et al. (2020), the strain *Muriellopsis* sp. (MCH35) grown in an open-raceways reactor in the north of Chile with seawater culture media and dry outdoor conditions is a viable option for economical lutein production, particularly in desert regions and for various biotechnological uses. Additionally, they discovered that compared to other methods like spray-drying, the freeze-drying process increased the lutein concentration and recovery by 0.3 to 2.5 times.

Behera and Balasubramanian, (2021) evaluated the performance of microwave-based drying using a power range of 360-900 W, and contrasted it with traditional oven drying (OD) at temperatures between 40 and 100 °C. With a larger effective diffusivity than OD, the microwave's efficiency is referred to its capacity to heat both evenly and volumetrically through dipolar contact. Because the microwave method ensured uniform heating throughout the interior subsurface, it reduced cell distress. As a result, the lipid production increased by 14.4%, and the biochemical components that can be used to produce valuable products and bioenergy in microalgal biorefineries were successfully preserved.

Agbede et al., 2020 have investigated the process of drying a thin layer of green microalgae (*Chlorella* sp.) paste biomass using a variety of drying methods, including microwave drying, open sun, solar, hot air. In contrast to hot air drying, the results showed that microwave drying had a higher moisture diffusivity and a lower specific energy consumption. Additionally, microwave drying had a lower activation energy than hot air drying. In comparison to conventional hot air-drying techniques, the authors propose that microwave drying may be a more effective and efficient way to dry microalgae biomass.

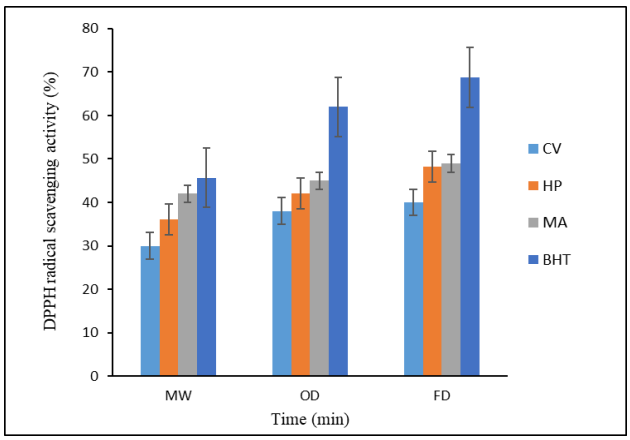

Figure 3. DPPH radical scavenging activity of *H. pluvialis*, *M. aeruginosa*, and *C. vulgaris* determined after different drying methods (MW:microwave-drying, OD:oven-drying, FD: freeze-drying) CV, HP and MA mean *Chlorella vulgaris*, *Haematococcus pluvialis* and *Microcystis aeruginosa*, respectively.

In Figure 3, DPPH radical scavenging activity of *H. pluvialis*, *M. aeruginosa*, and *C. vulgaris* determined after different drying methods were presented. It was seen that the highest antioxidant activity was determined from *M. aeruginosa*. Similar to biochemical composition exhibit a tendency whereby freeze-dry maintains the highest levels, oven-drying causes moderate losses, and microwave-drying causes considerable deterioration. This is probably because antioxidant chemicals are sensitive to heat and are easily oxidized and degraded in hot environments. The highest concentrations of phenolic compounds and antioxidants in microalgae and other algal types are reliably preserved during freeze-drying. Because high temperatures are not used in this process, sensitive bioactive chemicals are less likely to degrade (Shekarabi et al., 2019). Moderate amounts of phenolic chemicals and antioxidants are lost during oven-drying. These delicate chemicals are degraded by the heat used in this process, though not as severely as with microwave-drying (Le Lann et al., 2008).

CONCLUSION

Protein, lipid, and carbohydrate levels are all impacted by the drying process, which is essential for maintaining the microalgae's biochemical composition. Because of its low temperature, which reduces oxidation and thermal deterioration, freeze-drying is the best technique for preserving proteins and lipids. On the other hand, although more energy-efficient, oven-drying and microwave-drying cause substantial lipid oxidation and protein denaturation as a result of high temperature exposure. However, the percentage carbohydrate content may rise as a result of these thermal drying techniques, which might be advantageous for the manufacture of biofuel.

In the end, the planned use of the biomass should guide the drying technique chosen. Freeze-drying is better if maintaining the protein and lipid content is important, as it is for pharmaceuticals or nutraceuticals. On the other hand, heat drying techniques might be more appropriate for uses like the generation of biofuel where biomass rich in carbohydrates is preferred. Additional research aimed at improving drying conditions for distinct microalgal species will improve the use of biomass in a range of industrial applications.

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Ethical Approval: There is no need for ethical approval.

Data Availability: The original contributions presented in the study are included in the article; further inquiries can be also directed to the corresponding author.

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