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A Comprehensive Overview Of Protein Extraction From Duckweed: Methods, Challenges, And Applications

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Abstract: Duckweed, a small, fast-growing aquatic plant, is gaining attention as a sustainable and nutrient-rich source of protein. Owing to its high protein content and rapid biomass accumulation, duckweed holds significant potential in addressing global protein demands. This review presents a comprehensive overview of current protein extraction methods from duckweed, including mechanical disruption, enzymatic hydrolysis, solvent extraction, and novel green technologies such as ultrasound- and microwave-assisted extraction. The efficiency, scalability, and impact of each method on protein yield, purity, and functionality are critically analyzed. Furthermore, the review explores the diverse applications of duckweed protein across food, animal feed, nutraceuticals, and biotechnological sectors. Special attention is given to the nutritional profile, digestibility, and functional properties of duckweed-derived proteins. Challenges in large-scale processing, standardization, and regulatory approval are also discussed. Overall, this paper aims to highlight the promising role of duckweed protein in sustainable development and future food systems, while identifying key areas for further research and innovation.

Index Words: Duckweed, Duckweed Protein, Protein Extraction Methods, Application

1. Introduction

Due to its numerous inherent benefits over traditional cereal and grain crops, the aquatic plant duckweed has garnered more and more attention. [1,2] Duckweed, for instance, may be grown without posing a threat to agricultural land and grows quickly. [3,4] Its nutrient absorption efficiency is great, and its protein and dietary fiber concentrations are exceptionally high. [5,6,7,8,9]

Duckweed is a member of the Lemnaceae family, which includes 36 species in five genera: Spirodela, Landoltia, Lemna, Wolffiella (Wa), and Wolffia (Wo) (Fig. 1). [10] With the exception of the polar areas, they can thrive as tiny plants in still, slowly moving water like ponds and lakes wherever in the world. [3] Duckweed species lack genuine leaves and stems, and their morphology is flat and oval, with a maximum length of 5 mm.[11] Under the right circumstances, it can quadruple its biomass in two to three days and expands quickly through clonal growth (mother/daughter fronds). [4] Environmental elements like wind speed, light (exposure duration and intensity), and water quality (nutrient concentration, temperature, and pH) affect duckweed development and biomass accumulation. Furthermore, species like Lemna minor (L. minor) and Lemna aequinoctialis (L. aequinoctialis) have been demonstrated to grow and accumulate more starch when exposed to a medium rich in manure and increased light intensity/photoperiod. [12,13]

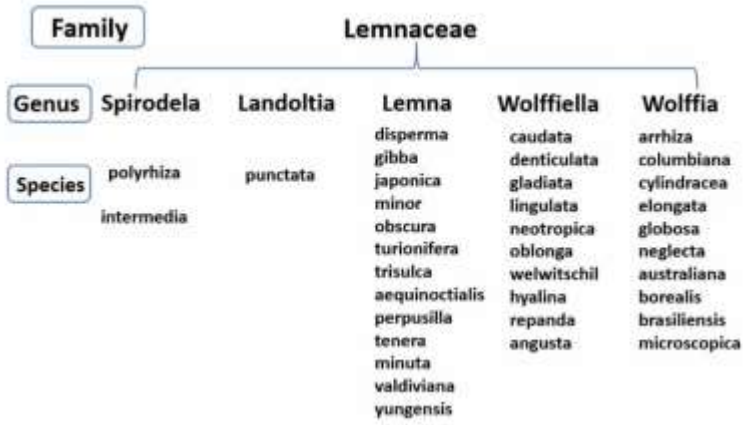

Figure 1. Schematic representation of duckweed family, genus, and species. (Source:[10])

Duckweeds are tiny aquatic monocotyledons. Their leaves, which are a hybrid of stems and leaves, resemble tiny plates or cube-shaped, green ovals known as fronds or thalli. Additionally, they are capable of both asexual (vegetative fronds) and sexual (flowering and seeds) reproduction.[14]

Duckweeds are known to have 35–45% crude protein by dry weight. Compared to other plant proteins, essential amino acids like lysine and methionine are present in higher concentrations. They are similar to animal protein in this sense. Duckweed aquaculture can produce an excellent supplement for poultry and other animal feeds because duckweeds are known to have significant concentrations of trace elements and pigments like carotene and xanthophylls.[15]

2. Chemical Composition

Duckweed has a dry matter content of 3–14%. The quality of the growth media also has a significant impact on the dry matter's protein and other constituent contents. Protein makes up 7–45% (usually 20–45%) of dry matter, followed by fat (2–9%), fiber (12–28%), and carbs (14–44%). Numerous macro- and micronutrients, including Ca, Cl, K, Na, Si, N, H, C, Fe, Mg, Mn, Al, Si, B, P, Cu, and Zn, can be absorbed by duckweed from water. Another benefit of this plant is that it includes carot enoids, amino acids, and vitamins A, B, and E. Dangerous heavy metals are among the many elements from the aquatic environment that duckweed effectively absorbs. Duckweed can be added to feed combinations for livestock and poultry because its elements—such as Cd, N, Cr, Zn, Sr, Co, Fe, Mn, Cu, Pb, Al, and Au—do not endanger human or animal health, according to an FAO report from 1999. [16]

The nutritional composition of duckweed has been described, and species and growth conditions have a major impact on essential nutrients and chemical composition. For instance, the protein content ranged from 16.0 (Lemna sp.) to 41.7% (Landoltia gibba) (L. gibba), 17.6 (L. gibba) to 35.0% (Lemna sp.), 3.4 (Landoltia minor) (L. minor) to 9.0% (Lemna sp.), 8.8 (Spirodela.polyrhiza) to 29.7% (L. minor), and 3.5 (L. gibba) to 26.0% (Lemna sp.) (Fig. 2).[5,-9]

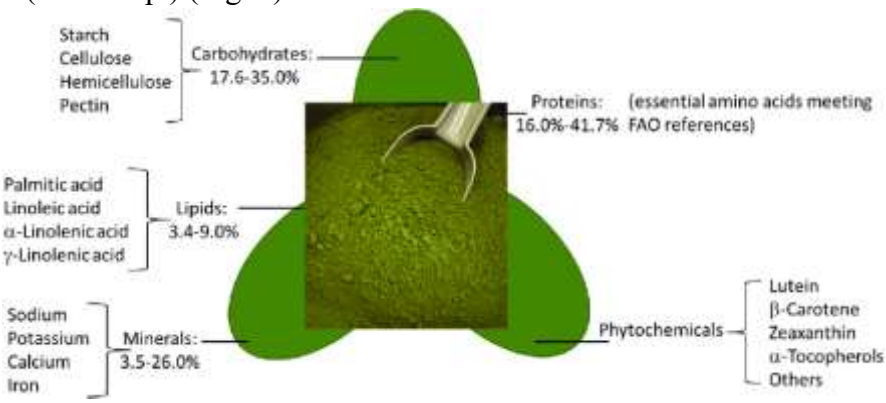

Figure 2 shows the approximate dry weight nutritional contents of duckweed. (Source: [5-9])

2.1 Duckweed Protein

On a dry weight basis, duckweed species of L. minor can have up to 45% protein. [17] Compared to soybean, almonds, rice, and corn, which have average protein outputs of 303, 229, 71, and 179 kg/ha/year, respectively, Wo. arrhiza has been reported to have an average protein production of 2,080 kg/ha/year. [3]

Duckweed species and growth circumstances influence its protein content, and extraction techniques may have an impact on the extract's protein content. [5,18,19] According to Dewanji (1993) and Duan (2013), the protein content of the duckweed species of L. minor and Lemna sp. was 17.5% and 16.3%, respectively [6,9], while the average protein content of the species of L. gibba, L. punctata, S. polyrhiza, and Wo.columbiana was reported to be 25.2, 28.7, 29.1, and 36.5%, respectively [5]. Duckweed, Wo. arrhiza, demonstrates a high potential for protein yield, but it hardly ever contains lectin, which makes up a significant amount of soy grains. Because lectin binds to glycoprotein receptors on intestinal mucosal epithelial cells, it is a well-known anti-nutrient during nutrient absorption. [20] For many plant proteins, thermal processing—such as heating, extrusion, roasting, and steaming—is necessary to reduce anti-nutrients and improve nutrient absorption. [21,22,23] However, duckweed does not require such an expensive and energy-intensive processing as food for humans.

3. Methods for extraction of Duckweed protein

3.1.Mechanical method

3.1.1. Mechanical method for extraction

Step 1: In order to open the cells and enable the solubilization of proteins, mechanical treatment is the initial stage in protein isolation. A slow juicer and a blender were used to compare two different forms of mechanical treatment.

Blender Treatment: A duckweed sample that was kept overnight at 4 °C was employed to optimize the isolation procedure. Fresh duckweed was employed in the final treatment. Demineralized water (861 g) was combined with duckweed (145 g wet weight). Thermomix and Vitamix were the two types of blenders that were used. We utilized two different blender settings and blending times: low for two minutes and high for one minute. To get rid of the solids, the resultant slurry was centrifuged at 16 500×g for 15 minutes (Sorval Lynx). The protein was extracted by coagulation or precipitation after the supernatant was decanted.

Slow Juicer: A duckweed sample that was kept overnight at 4°C was used to optimize the isolation procedure. Fresh duckweed was employed in the final treatment. A slow juicer (Angel Juicer 7500) was used to press the duckweed, producing a solids fraction and a juice fraction. A protein concentrate product was created using the juice fraction. Protein was isolated using two methods: coagulation and precipitation.

Step 2: Juice Protein Isolation. Either heat treatment, isoelectric point precipitation, or a mild heat treatment followed by filtration were used to further treat the juice fraction in the second step of protein isolation in order to produce a protein isolate.

Heat Treatment- To denature and precipitate the protein, the juice fraction was heated to 70 °C and maintained there for one hour. The sample was then centrifuged for 15 minutes at 16500×g (Sorval Lynx). The particle was gathered after the supernatant was decanted.

Isoelectric Point Precipitation- Using HCl, the pH of the juice fraction was adjusted to 4.2. The sample was centrifuged at 16 500×g for 15 minutes after 60 minutes (Sorval Lynx). The particle was gathered after the supernatant was decanted.

Mild Heat Treatment Followed by Filtration- 12.5–15 kg of fresh duckweed were needed for a protein synthesis study that processed 10 L of duckweed juice. The fresh duckweed juice was combined with a 20 weight percent sodium metabisulphite solution (pH 5.9) until the juice contained 1 weight percent sodium metabisulphite. After using HCl to get the juice's pH down to 6.0, it was heated to 52 °C and maintained there for 33 minutes in a jacketed, agitated metal container. The juice was then rapidly chilled in the jacketed vessel using tap water to less than 21.5 °C. In 1-liter buckets (Sorval Lynx 6000), the juice was

centrifuged for 45 minutes at 17000×g. After being decanted, the supernatant was gathered for filtration. The process's pH, duration, and temperature were changed to maximize decolorization.

To get rid of the remaining green material and the majority of the microorganisms, the first filtration stage was cross-flow microfiltration using a Sartorius Sartojet cross-flow system with two Sartocon slice cassettes (Hydrosart regenerated cellulose, 0.45 µm pore size), which added up to 0.2 m2 membrane area. Water with salts and phenols were eliminated in the second step, which involved ultrafiltration using a Sartorius Sartojet cross-flow system with three Sartocon slice cassettes (Hydrosart regenerated cellulose 100 kDa cutoff, adding up to 0.3 m2 membrane area). The final step, diafiltration, involved washing out salts and phenols until the final conductivity in the permeate was less than 0.2 mS using demineralized water (Sartorius Sartojet cross-flow system with three Sartocon slice cassettes (Hydrosart regenerated cellulose 100 kDa cutoff, adding up to 0.3 m2 membrane area)). The concentrated final product underwent freeze-drying.[24]

3.1.2. Ultrasound-Assisted Protein Extraction

A 400 W, 24 kHz, high-intensity probe ultrasonic generating system was employed. The device was the Hielscher Ultrasonics GmbH (Teltow, Germany) model UP400St, for which time and amplitude settings (10–100%) were employed. Prior to identifying the optimal traditional protein extraction method, the ultrasonic parameters were optimized ("Optimization of Protein Extraction Using Response Surface Methodology"). In order to prevent protein denaturation, the treatment was conducted in an ice bath with a maximum temperature of 48 °C and a restart temperature difference of 5 °C. Starting with a natural pH of 6.3, a suspension of duckweed powder at a concentration of 2.5 weight percent and deionized water was combined in a 100 ml plastic beaker and set on a magnetic stirrer for 15 minutes. To enable total immersion, the beaker was set in an ice bath. A thermometer and a sonotrode (22 mm in diameter) were submerged in the middle of the sample, about 2 cm down in the beaker. Given the sonotrode's 22 mm diameter, an amplitude of 40 µm peak-to-peak is produced by setting the amplitude to, say, 40% power. Following centrifugation, the Kjeldahl method (N × 5.8, see "Specific Nitrogen Conversion Factor" section) was used to test the protein content of the supernatant (10 ml) in triplicate.[25]

3.2.Chemical method

For extraction of proteins from duckweed plants were extracted using four different chemical techniques. Below is an outline of the main processes in these methods (Fig. 1). About 0.4 g of fresh duckweed plants were used as the starting material for protein extraction in each method. They were ground to a fine powder in a mortar with liquid N2. The acetone used below and the 10% TCA/acetone were both precooled to -20°C and contained 5 mM dithiothreitol (DTT). Every step listed below was carried out at room temperature unless otherwise noted. Five separate biological replicates were used to assess the four approaches.

3.2.1. Phenol extraction (PE)

Duckweed plant tissue powder was frozen, cooled, and then homogenized in a mortar using SDS buffer (1% SDS, 0.1 M Tris HCl, pH 6.8, 50 mM DTT, 5 mM EDTA, and 2 mM PMSF; 5 ml/g fresh weight) after a brief chilling period. To encourage protein extraction, the crude extracts were put into Eppendorf tubes and incubated for ten minutes at 70 °C. The combined extracts were centrifuged for five minutes at 4 °C at 10,000 g. With minor adjustments, the resultant supernatants were moved to fresh tubes (600 µl per 1.5 ml tube; 800 µl per 2.0 ml tube) and put through the phenol extraction process as previously mentioned.

In a nutshell, the protein extract was mixed with equal parts Tris-buffered phenol (pH 7.8) and completely inverted for five minutes. The two-phase mixture was separated into two immiscible liquid phases—the phenol-rich organic phase and the aqueous phase—after being centrifuged at 10,000 g for five minutes. Using five volumes (1,500 µl per tube) of 0.1 M ammonium acetate in methanol at -20°C overnight, the lower organic phase (containing proteins) was transferred into 2.0-ml tubes (300 µl per tube) without disturbing any pellets in the bottom. Centrifugation at 10,000 g for 10 min at 4 °C was used to recover the protein pellets, and each tube was twice washed with 1500 µl of acetone.

As previously mentioned, centrifugation and complete pellet resuspension were required for each wash phase. The resulting protein pellets were utilized for protein determination and 2DE after being air-dried

and dissolved in 200 µl of 2DE lysis solution (7 M urea, 2 M thiourea, 2% CHAPS, and 20 mMDTT with or without 0.5%pH4–7IPGbuffer).

3.2.2. TCA/acetone precipitation (TA)

The procedure was carried out as previously explained. In short, the frozen tissue powder of duckweed plants was kept at -20°C for the entire night after being re-suspended in a 10% TCA/acetone solution (10 milliliters per gram of fresh weight). Ten percent TCA/acetone solution means that 10 grams of TCA are dissolved in 100 milliliters of acetone, or 10% TCA (w/v) in acetone. The samples were then centrifuged at 5000 g for 30 minutes at 4 °C. The resulting pellets were centrifuged at 10,000 g for 10 min at 4°C after being washed twice with cold acetone. Following complete air drying, the tissue pellets were resuspended in 200 µl of 2DE lysis solution (or homogenized in a tiny mortar). After 30 minutes of shaking incubation, the mixture was centrifuged as previously described to achieve clarity. For protein determination and 2DE, the resulting supernatant (protein extract) was utilized straight away.

3.2.3. TCA/acetone precipitation and phenol extraction (TAP)

Combining the TA and PE described above. In short, 10% TCA/acetone was used to purify the frozen tissue powder of duckweed plants, and then acetone washed. SDS buffer (3 ml/0.1 g dry weight) was used in a mortar to homogenize the dried tissue pellets. After 10 minutes of incubation at 70 °C, the crude extract was centrifuged at 10,000 g for 5 minutes at 4 °C. If required, the extraction process might be carried out once more. To prepare protein samples for 2DE, the protein extracts were extracted using phenol as previously mentioned.

3.2.4. TCA/acetone/TCA precipitation (TAT)

Two clean-up processes (TCA/acetone precipitation and aqueous TCA precipitation) were added to this TA technique modification, which was connected by SDS extraction. Prior to being placed on ice for 30 minutes with periodic vigorous vortexing, the frozen tissue powder of duckweed plants was purified using TCA/acetone suspended in 10% TCA/acetone 10 ml/g fresh weight. For 10 minutes at 4°C, the mixture was centrifuged at 5000 g. To get rid of any remaining TCA, the tissue pellets were thoroughly cleaned with acetone three times after discarding the supernatant. The pellets were thoroughly resuspended for each wash stage, and they were centrifuged at 10,000 g for 10 minutes at 4 °C.

The tissue pellets were then allowed to air dry completely, mixed in a small mortar with 3 ml of SDS buffer (0.1 g dry weight), and incubated for 10 minutes at 70 °C while being vigorously vortexed periodically. The extraction was done once more. The combined extracts were centrifuged for 10 minutes at 4 °C at 10,000 g. The resulting supernatants were put into fresh tubes (600 µl in a 1.5 ml tube, 800 µl in a 2.0 ml tube, etc.) and precipitated for 30 minutes on ice using an equivalent volume of aqueous 20% TCA. The proteins were extracted using centrifugation as described above and then washed three times with acetone. The pellets were thoroughly resuspended for each wash stage, and centrifugation was performed as previously mentioned. The resulting protein pellets were used for 2DE and protein determination after being air dried and treated in an appropriate amount of 2DE lysis solution. [26]

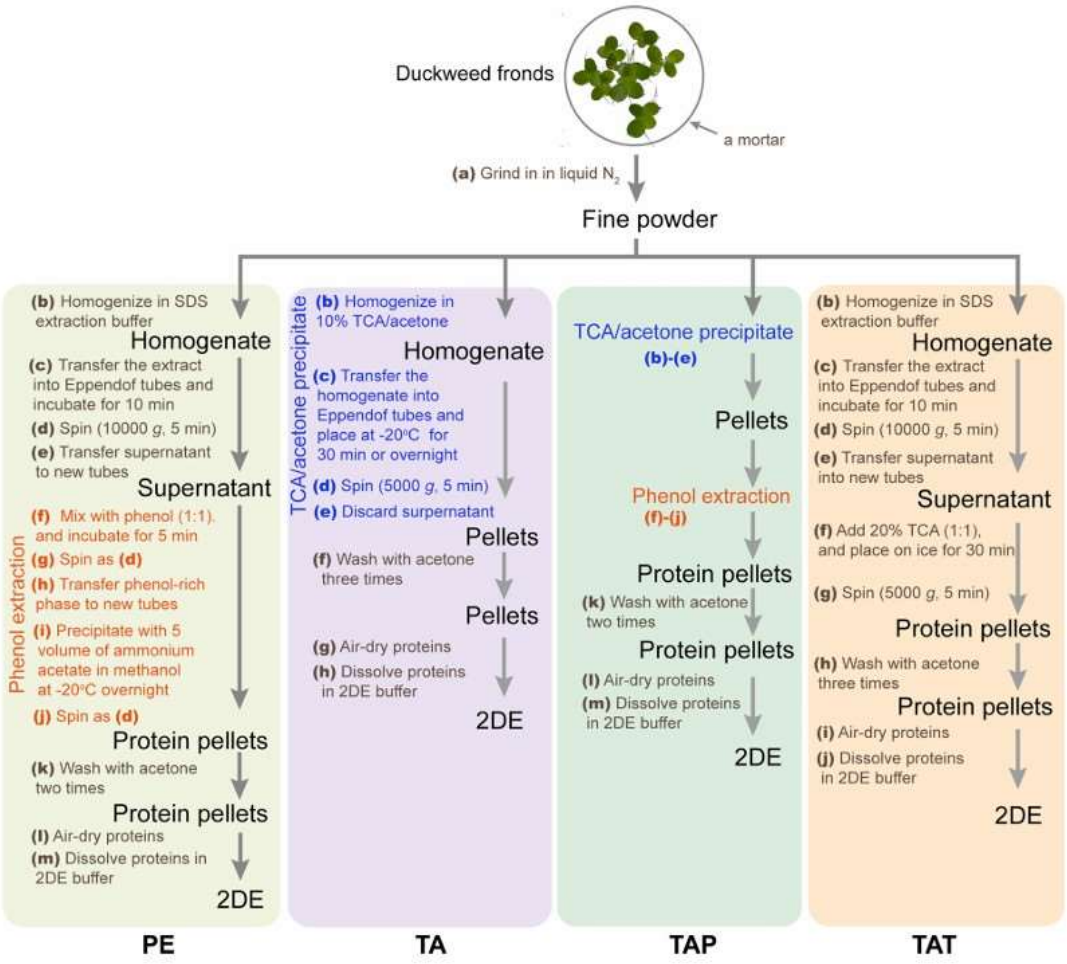

Figure 4 Outline of Chemical methods used for extracting proteins in duckweed plants. Source: [26]

4. Challenges for extraction

Scalability of culture systems is one of the main technological issues in the production of aquatic plant proteins. Despite the development of photobioreactors and other controlled cultivation systems to maximize growth conditions for duckweed and microalgae, a major obstacle still stands in the way of scaling these systems to an industrial scale while keeping costs low. When compared to more well-established agricultural protein sources like soy and wheat, these systems' high capital and operating costs—particularly in terms of energy consumption and nutrient supply—can limit their economic sustainability.

The effectiveness of protein extraction and purification procedures is another significant obstacle. Due to the high water content of duckweed and the intricate cell walls of microalgae, advanced extraction methods are necessary, which can be expensive and energy-intensive. Furthermore, it is challenging to guarantee the quality and uniformity of protein isolates across several production batches, despite the fact that this is essential for their acceptability in pharmaceutical and food applications. Therefore, it is essential to create extraction and purification methods that are more effective, economical, and scalable.

The market's acceptance of aquatic plant proteins is still in its infancy from an economic standpoint. Traditional plant proteins are more well-known to consumers and businesses, despite the increased interest in plant-based proteins. It will take a large investment in marketing, product development, and education to overcome consumer reluctance and create a substantial market presence for aquatic plant proteins. Adoption of these new protein sources may also be slowed by regulatory obstacles pertaining to their approval and standardization in various jurisdictions.[27]

5. Applications of duckweed protein

5.1.As a alternative of protein source

Duckweed is a desirable plant substitute for protein production due to its high protein content and sufficient amounts of key amino acids and vitamins. Leucine, threonine, valine, and isoleucine are abundant in duckweed protein, which also has 2.7% methionine and cysteine, 7.7% phenylalanine and threonine, and 4.8% lysine. Duckweed has been acknowledged as a high-quality source of protein for aquaculture and farm animals, and it may one day be used by people as well (Ziegler, Adelmann, Zimmer, Schmidt, & Appenroth, 2015).

The following are some benefits of using duckweed as a substitute protein source:

(a) Its rapid growth and broad stress tolerance make it appropriate for biological production.

(b) Duckweed cultivation water can be recycled with a recovery rate greater than 95%. Because it floats on the water's surface, less water is lost through evaporation, requiring less water to grow than other crops.

(c) duckweed can be grown in water without the addition of fertilizer or pesticides, and it can even be grown in wastewater, so it doesn't compete with arable land.

(d) duckweed production emits less CO2. About 0.4 kg of CO2 equivalent is created for every kilogram of duckweed, whereas 37–85 kg of CO2 equivalent are produced for every kilogram of beef.

Depending on the species and development conditions, duckweed's protein content can range from 15% to 45% of dry weight. As duckweed absorbs more nitrogen, its protein concentration rises. Ammonium ($NH+4$) is preferred over nitrate ($NO-3$) for the synthesis of amino acids.

5.2.Using duckweed as a feed/supplement

Duckweed's composition is determined by the water's nutrient concentration and the current weather. Harvested duckweed plants can be utilized as a complete fish feed without additional processing because they contain up to 43% protein on a dry weight.

Duckweed protein is more similar to animal protein and has a greater variety of necessary amino acids than most vegetable proteins (Hillman and Culley 1978). Therefore, it is a high-quality protein source that can be used to produce domestic animals. In addition to being a rich source of vitamins A and B for humans, duckweed growing on nutrient-rich water contains a high concentration of pigments, especially carotene and xanthophyll, and trace minerals, K and P, which make duckweed meal a very excellent supplement for chickens and other animals.

5.3.Use of duckweed in fish nutrition

Meals with high biological value and protein content are costly and frequently unavailable locally, which is a significant barrier to fish farming. Duckweeds with a high protein content (about 40%) and high biological value are cultivated on water with 10–30 mg NH3-N/liter (Hillman and Cully 1978; see Table 2). Certain fish, such as carp and tilapia, effectively convert fresh duckweed to liveweight, making it ideal for intensive fish farming systems with relatively quick water exchange for waste removal (Hepher and Pruginin 1979; Robinette et al 1980; Van Dyke and Sutton 1977; Hassan and Edwards 1992).

5.4.Poultry nutrition studies

Duckweed has long been recognized for its possible nutritional significance in poultry diets (Lautner and Mueller 1954; Musaffarov 1968; Abdullaev 1969). In traditional poultry diets, dehydrated duckweed has been utilized as a protein source in place of alfalfa (lucerne)meal. Compared to chickens fed traditional protein sources, those fed 10 dehydrated duckweed showed better weight increases. However, depending on the ingredients in the growing medium, different reactions are frequently recorded based on the source of the duckweed, which can be either low protein/high fiber or high protein/low fiber.[29]

5.5.Duckweed as A Dietary Staple for Humans

As of right now, animals, dairy products, and plants provide the majority of the proteins in human diets 46 percent, 16 percent, and 30 percent, respectively [30]. Animal-derived protein production has a number of established negative effects on the economy, human health, and the environment. Thus, plant protein appears to be the clear substitute for animal-derived protein. Plant protein is preferred over animal protein,

which reduces ecological disturbance and a number of health problems. Therefore, more study to integrate duckweed into society must be done to make this possible.

In certain regions of Southeast Asia, such as Laos, Thailand, and Myanmar, where Wolffia arrhiza and Wolffia globosa are the most common species utilized for human consumption, duckweed has already been embraced as a source of protein in the form of a vegetable known as "Khai-Nam" [31]. as these areas, the plant is consumed as soups, stir-fries, chips, and bread. Nonetheless, the reduced attractiveness of duckweed as a meal in other regions of the world might be because some species contain oxalic acids, which give the plant a bad taste and make it harder to separate the bacteria, worms, snails, and protozoa from the plants [32]. Nonetheless, duckweed has promise for human consumption on a worldwide scale.

According to one study on the acceptability of duckweed for human consumption in the Netherlands, the acceptance of duckweed as human food (as a suitable meal) in the country increased when information about its nutritional and environmental advantages was made available [33]. Proteins, polyunsaturated fats, fibers, micronutrients, and a variety of other bioactive substances are among the essential macronutrients found in abundance in certain duckweed species, including L. minor, L. gibba, S. polyrhiza, L. punctata, Wo. globosa, and Lemna sp. These plants are also excellent candidates for creating human food because of their well-balanced amino acid profile.

Reference

[1] Appenroth, K. J., Sree, K. S., Böhm, V., Hammann, S., Vetter, W., Leiterer, M., & Jahreis, G. (2017). Nutritional value of duckweeds (Lemnaceae) as human food. *Food chemistry*, *217*, 266-273.

[2] Appenroth, K. J., Sree, K. S., Bog, M., Ecker, J., Seeliger, C., Böhm, V., ... & Jahreis, G. (2018). Nutritional value of the duckweed species of the genus Wolffia (Lemnaceae) as human food. *Frontiers in chemistry*, *6*, 483.

[3] Bhanthumnavin, K., & MCGARRY, M. G. (1971). Wolffia arrhiza as a possible source of inexpensive protein. *Nature*, *232*(5311), 495-495.

[4] Said, M. Z. M., Culley, D. D., Standifer, L. C., Epps, E. A., Myers, R. W., & Boney, S. A. (1979, March). Effect of harvest rate, waste loading, and stocking density on the yield of duckweeds. In *Proceedings of the World Mariculture Society* (Vol. 10, No. 1-4, pp. 769-780). Oxford, UK: Blackwell Publishing Ltd.

[5] LL, R. (1980). Duckweeds (Lemnaceae family): A potential source of protein and amino acids. *Journal of Agriculture and Food Chemistry*, *28*, 848-850.

[6] Dewanji, A. (1993). Amino acid composition of leaf proteins extracted from some aquatic weeds. *Journal of agricultural and food chemistry*, *41*(8), 1232-1236.

[7] Skillicorn, P., Spira, W., & Journey, W. (1993). *Duckweed aquaculture: a new aquatic farming system for developing countries* (pp. x+-76pp).

[8] Chen, Q., Jin, Y., Zhang, G., Fang, Y., Xiao, Y., & Zhao, H. (2012). Improving production of bioethanol from duckweed (Landoltia punctata) by pectinase pretreatment. *Energies*, *5*(8), 3019-3032.

[9] Duan, P., Chang, Z., Xu, Y., Bai, X., Wang, F., & Zhang, L. (2013). Hydrothermal processing of duckweed: effect of reaction conditions on product distribution and composition. *Bioresource technology*, *135*, 710-719.

[10] Sree, K. S., & Appenroth, K. J. (2020). Worldwide genetic resources of duckweed: stock collections. *The duckweed genomes*, 39-46.

[11] Zhang, J., & Azizullah, A. (2020). Genetic diversity and DNA barcoding in the duckweed family. *The Duckweed Genomes*, 59-65.

[12] Chakrabarti, R., Clark, W. D., Sharma, J. G., Goswami, R. K., Shrivastav, A. K., & Tocher, D. R. (2018). Mass production of Lemna minor and its amino acid and fatty acid profiles. *Frontiers in chemistry*, *6*, 479.

[13] Yin, Y.; Yu, C.; Yu, L.; Zhao, J.; Sun, C.; Ma, Y.; Zhou, G. The Influence of Light Intensity and Photoperiod on Duckweed Biomass and Starch Accumulation for Bioethanol Production. Bio. Technol. 2015, 187, 84–90.

[14] Jaiprasert, A. O. R. N. P. I. L. I. N. (2018). *Development of duckweed transformation technique for biological application* (Doctoral dissertation, Thesis. Burapha University).

[15] Sil, S. K., Gupta, S., & Neela, F. A. (2023). Anatomical features and antimicrobial activity of duckweed. *Bangladesh Journal of Botany*, *52*(1), 105-110.
[16] Sońta, M., Rekiel, A., & Batorska, M. (2019). Use of duckweed (Lemna L.) in sustainable livestock production and aquaculture–a review. *Annals of Animal Science*, *19*(2), 257-271.
[17] Escobar, C.; Escobar, A. Duckweed: A Tiny Aquatic Plant with Enormous Potential for Bioregenerative Life Support Systems. In 47th International Conference on Environmental Systems, Charleston, U.S., July 16-20, 2017.
[18] Casal, J. A.; Vermaat, J. E.; Wiegman, F. A Test of Two Methods for Plant Protein Determination Using Duckweed. Aqua. Bot. 2000, 67, 61–67.
[19] Yu, G.; Liu, H.; Venkateshan, K.; Yan, S.; Cheng, J.; Sun, X.; Functional, W. D. Physiochemical, and Rheological Properties of Duckweed (Spirodela Polyrhiza) Protein. Trans. ASABE. 2011, 54, 555–556.
[20] Liener, I. E.;. Implications of Antinutritional Components in Soybean Foods. Crit. Rev. Food Sci. Nutr. 1994, 34, 31–67.
[21] Hamad, S. A. A.; Mustafa, A. I.; Magboul, B. I.; Qasem, A. A. A.; Ahmed, I. A. M. Nutritional Quality of Raw and Cooked Flours of a High β-glucan Sorghum Inbred Line. J. Cereal Sci. 2019, 90, 102857.
[22] Wu, G.; Ashton, J.; Simic, A.; Fang, Z.; Johnson, S. K. Mineral Availability Is Modified by Tannin and Phytate Content in Sorghum Flaked Breakfast Cereals. Food Res. Int. 2018, 103, 509–514.
[23] Xiong, Y.; Zhang, P.; Luo, J.; Johnson, S. K.; Fang, Z. Effect of Processing on the Phenolic Contents, Antioxidant Activity and Volatile Compounds of Sorghum Grain Tea. J. Cereal Sci. 2019, 85, 6–14.
[24] Nieuwland, M., Geerdink, P., Engelen-Smit, N. P., Van Der Meer, I. M., America, A. H., Mes, J. J., ... & Mulder, W. J. (2021). Isolation and gelling properties of duckweed protein concentrate. *ACS Food Science & Technology*, *1*(5), 908-916.
[25] Maag, P., Cutroneo, S., Tedeschi, T., Grüner-Lempart, S., Rauh, C., & Karslioglu, Ö. Ö. (2025). Optimization of Protein Extraction from Duckweed Using Different Extraction Processes. *Food and Bioprocess Technology*, 1-22.
[26] Wang, Y., Wang, X., Yang, R., Niu, L., & Wang, W. (2020). Comparison of protein extraction methods for 2DE-based proteomic analysis of duckweed Spirodela polyrhiza, a small aquatic model plant. *Aquatic Botany*, *163*, 103216.
[27] Song, Y., Hu, Z., Liu, S., Luo, S., He, R., Yang, X., ... & Lu, Y. (2025). Utilization of Microalgae and Duckweed as Sustainable Protein Sources for Food and Feed: Nutritional Potential and Functional Applications. *Journal of Agricultural and Food Chemistry*.
[28] Liu, Y., Xu, H., Yu, C., & Zhou, G. (2021). Multifaceted roles of duckweed in aquatic phytoremediation and bioproducts synthesis. *Gcb Bioenergy*, *13*(1), 70-82.
[29] Leng, R. A., Stambolie, J. H., & Bell, R. (1995). Duckweed-a potential high-protein feed resource for domestic animals and fish. *Livestock Research for Rural Development*, *7*(1), 36.
[30] S. M. Pasiakos, S. Agarwal, H. R. Lieberman, and V. L. Fulgoni, “Sources and Amounts of Animal, Dairy, and Plant Protein Intake of US Adults in 2007–2010,” Nutrients, vol. 7, no. 8, Art. no. 8, Aug. 2015, doi: 10.3390/nu7085322.
[31] K. Bhanthumnavin and M. G. Mcgarry, “Wolffia arrhiza as a Possible Source of Inexpensive Protein,” Nature, vol. 232, no. 5311, Art. no. 5311, Aug. 1971, doi: 10.1038/232495a0.
[32] S. Iqbal, “Potentials, Possibilities and Limitations for Combined Wastewater Treatment and Animal Feed Production in Developing Countries,” 2017.
[33] Diwan, F. (2023). Duckweed and its broad-spectrum applications. *Climate Survival Solutions, Inc., and Climate Survival Solutions Pvt. Ltd*, 1-18.