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Duckweed (Lemnaceae) for potentially nutritious human food: A review

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ABSTRACT

With continuous global population growth, the challenges of expanding demand for sufficient and nutritious foods need to be addressed by exploring new and supplemental agricultural and food systems. Duckweed demonstrates a great potential for human food with many advantages. This review covers recent research on duckweed regarding plant cultivation, nutritional composition and quality, product development, and related safety issues and regulations for human food uses. Challenges and future research needs are discussed. Species and cultivation conditions have a significant effect on chemical and nutritional compositions of duckweed. Certain duckweed species contain high protein content (up to 45%) that provides all the essential amino acids meeting FAO references and can support human body growth and development. Duckweed also contains versatile carbohydrates including starch, cellulose, trace hemicellulose, and pectin that deliver functional properties and nutrients. In addition, duckweed is rich in minerals, vitamins, and phytochemicals, especially lutein and β-carotene which are positively associated with reduced risk of many chronic diseases. Duckweed could be used either in conventional cereal-based foods by partial substitution of wheat flour or for extraction of proteins and phytochemicals as functional ingredients. Studies are warranted for developing a duckweed supply chain and establishing duckweed as a novel staple food.

KEYWORDS

Aquatic plant; duckweed; protein; fibers; nutraceuticals; future food

Introduction

Up to now approximately one billion people still suffer from chronic hunger due to food shortages,[1] although global production of crops and animals has been increased by more than 3-fold and 4-fold, respectively, over the last 50 years (https://ourworldindata.org/). The global food shortage will become more critical in the future with the growing world population and the limited and even slowly diminishing agricultural land.[12,3] Recently, the aquatic plant duckweed has attracted increasing interest, attributed to its many intrinsic advantages compared to the conventional cereal and grain crops.[4,5] For example, duckweed grows rapidly and can be produced without competing with agricultural land.[6,7] It has high efficiency of nutrient uptake and extremely high contents of protein and dietary fiber.[8–12] Duckweed could also provide sufficient all essential amino acids (EAA) meeting the World Health Organization (WHO) recommendation, and it is rich in beneficial pigments and antioxidants.[4,13]

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Duckweed belongs to the Lemnaceae family and contains five genera including Spirodela, Landoltia, Lemna, Wolffiella (Wa), and Wolffia (Wo) with 36 different species identified (Fig. 1).[14] They can grow in all geographical regions worldwide except for the polar regions as tiny plants in still and slow-flowing water such as ponds and lakes.[6] Morphologically, duckweed species are flat oval-shape within 5 mm long and without stems or true leaves (Fig. 2).[15] It grows fast through clonal growth (mother/daughter fronds) and can double its biomass in 2–3 days under proper conditions.[7] Duckweed growth and biomass accumulation are influenced by environmental factors such as water condition (nutrient concentration, temperature, and pH), light (exposure time and intensity), and wind speed. In addition, medium rich in manure and increased light intensity/ photoperiod have been shown to increase the growth and starch accumulation of species such as *Lemna minor* (*L. minor*) and *Lemna aequinoctialis* (*L. aequinoctialis*).[16,17]

Duckweed has been characterized for its nutritional compositions, and species and growth conditions have a significant effect on chemical composition and essential nutrients. For example, protein content varied from 16.0 (*Lemna sp.*) to 41.7% (*Landoltia. gibba*) (*L. gibba*), starch from 17.6 (*L. gibba*) to 35.0% (*Lemna sp.*), lipid from 3.4 (*Landoltia. minor*) (*L. minor*) to 9.0% (*Lemna sp.*), crude fiber from 8.8 (*Spirodela. polyrhiza*) (*S. polyrhiza*) to 29.7% (*L. minor*), and ash from 3.5 (*L. gibba*) to 26.0% (*Lemna sp.*) (Fig. 3).[8–12] Duckweed is commonly known as the natural feed for ducks, swans, geese, pig, sheep, cattle, and poultry (GRAS Notice (GRC) No. 742, 2017).[19] Additionally, duckweed has been supplemented to animal feed for complementing nutrition and promoting animal growth. For example, research has been conducted to partially replace fish meal, soybean meal, alfalfa leaf meal, and others with duckweed in animal feed.[20] So far, species of *L. minor* and *S. polyrhiza* have been utilized in animal feed for fish, poultry, pigs, and others.[21,22] In some southeast Asian countries, such as Thailand, Laos, and Myanmar, species of *Wolffia arrhiza* (*Wo. arrhiza*) is localized to be known as *khai nam, kai-pum*, or *kai nhae* and served as human food.[6] However, in the U.S. and western countries, duckweed is generally considered as a pest plant rather than a food source.

Given its high starch and other carbohydrate contents, duckweed biomass can be utilized for heat generation and biofuel production.[23] Xu et al. (2011) reported that the ethanol yield of *S. polyrrhiza* could reach 6,420 L/ha which is about 50% higher than that of corn.[24] Therefore, duckweed-based ethanol production may have potential to supplement corn-based ethanol in the future.

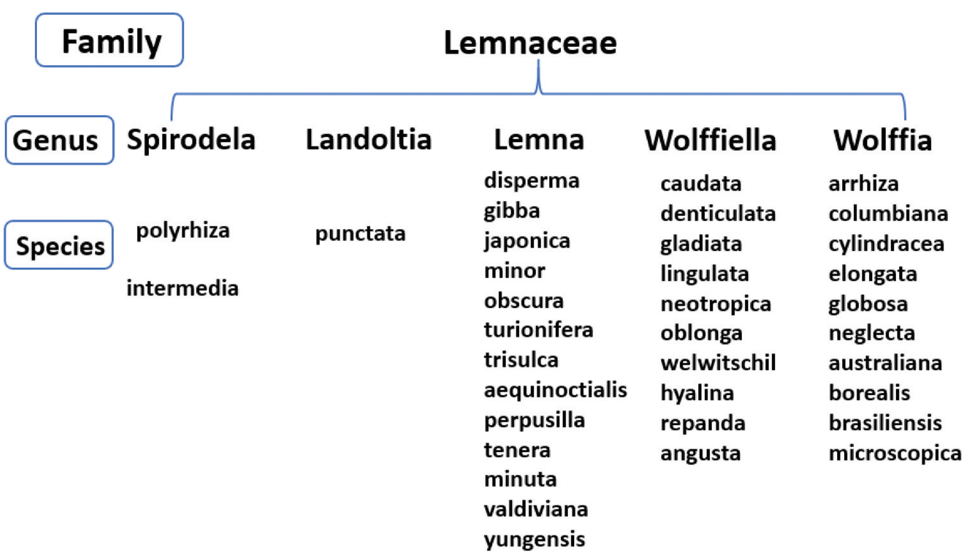

Figure 1. Schematic representation of duckweed family, genus, and species. (Source: [14]).

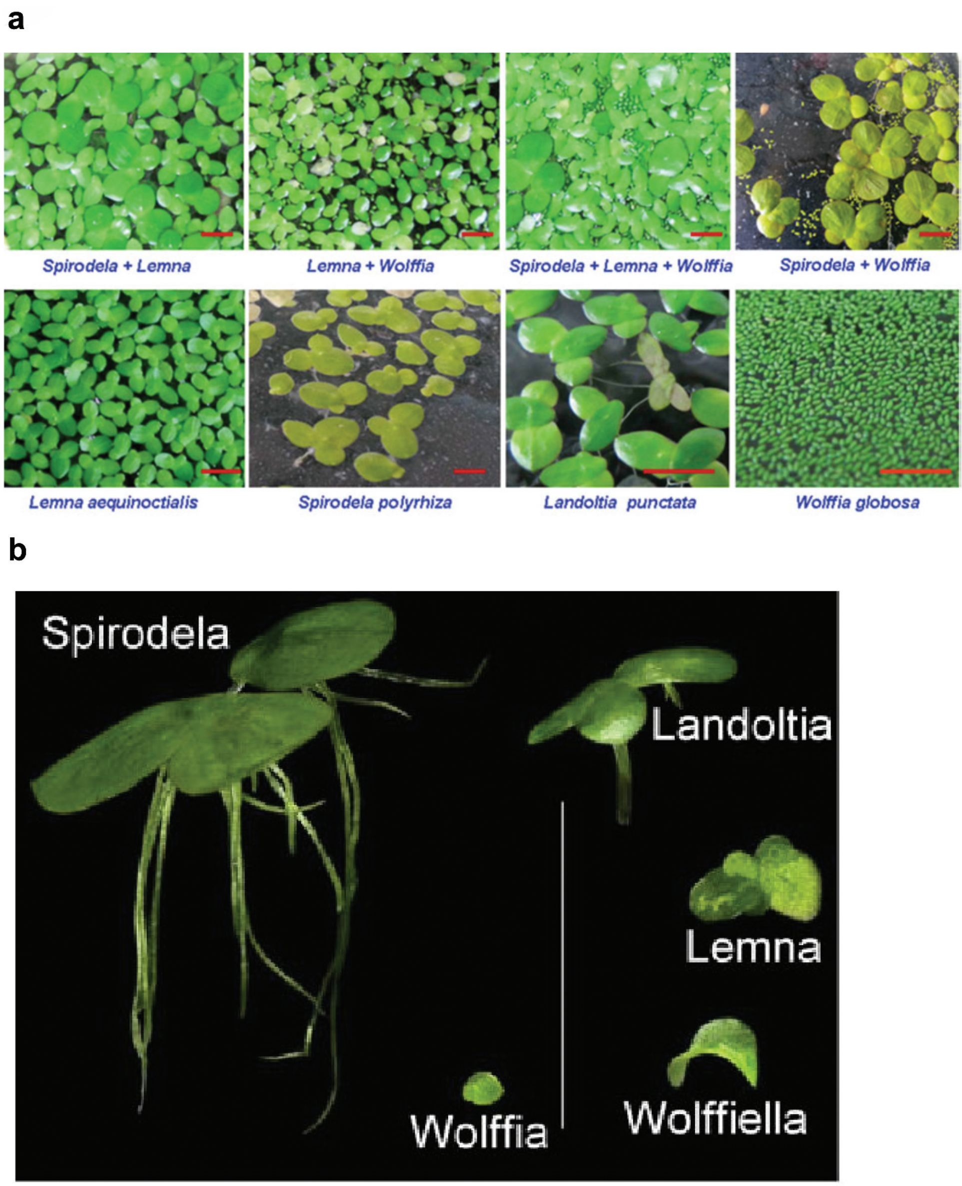

Figure 2. A. Photos of representative species of duckweed (Source: [15]); B. Morphology of representative duckweed species: *S. polyrhiza, L. punctata, L. minor, Wa. lingulate*, and *Wo. arrhiza*. Bar: 1 cm. (Source: [18]).

Existing studies on duckweed have been focused on its cultivation,[17,25] nutritional composition and quality,[4,5,26] animal feed uses,[24,27] and conversion to biofuels.[23] With the growing world population and increasing demand for innovative, nutritious, and healthier foods, duckweed has shown a great potential for human foods. To our knowledge, there is no comprehensive review yet available on the potential production and application of duckweed for human food, especially in terms of nutritional quality, technological limitations and challenges of duckweed processing and utilization, safety issues, regulation, and potential duckweed-containing food development. Hence, this review addresses the aforementioned aspects and identifies future research needs.

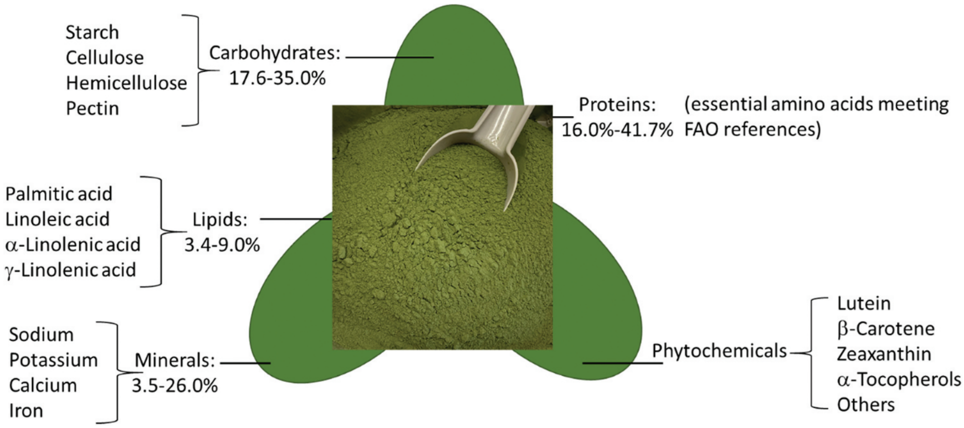

Figure 3. Approximate nutrient compositions of duckweed (on dry weight basis).

Duckweed cultivation

Duckweed is naturally grown in fresh water such as ponds, lakes, and rivers. However, the natural environmental conditions may limit duckweed growth, such as drought, insufficient supply of nutrients, and insect and fungal infestations and contamination. Therefore, research has been conducted to identify more appropriate hygiene culture systems for duckweed to improve the growth and yield while eliminating safety concerns for potential human consumption. Ruekaewma et al. (2015) investigated the effect of various culture systems including static system, vertical aeration (air stone at 400 l/hr flow to circulate water vertically), horizontal surface agitation (a blade paddle wheel driven by a mini-motor at 3500 rpm to circulate water horizontally), system with top water spraying (water moved from the bottom of the culture to the top, sprayed at 900 l/hr), and layer culturing system with top water spraying (water moved from the bottom of the culture to the top, sprayed at 900 l/hr) on *Wolffia globose* (*Wo. globosa*) production.[28] Results showed that the horizontal surface agitation yielded the highest amount of *Wo. globosa* biomass in 28 days, followed by the systems with top water spraying, static, vertical aeration, and layer culturing with top water spraying in that order. The biggest plant size in all the culture systems was achieved at day 7. *Wo. globosa* harvested from the horizontal culture system contained 48.2% protein, 9.6% fat, and 14.5% crude fiber, which are higher than that from a natural pond.[28]

Sharma et al. (2019) attempted to standardize the cultivation technique of *S. polyrhiza* in outdoor tanks. They designed three culture media for *S. polyrhiza* growth, including manure 1 (cattle manure, poultry droppings and mustard oil cake), manure 2 (urea, potash, and triple superphosphate), and manure 3 (cattle manure, urea, potash, and triple superphosphate).[29] The manure 1 (organic manures) resulted in the highest biomass yield of duckweed *S. polyrhiza* than that from manure 2 and 3, and the production of duckweed achieved 2,020 kg ha^{-1} $month^{-1}$ (dry weight basis) from pond culture. This study further demonstrated that temperature, light intensity, ammonia, phosphate, and medium conductivity all significantly influenced duckweed productivity of the water bodies, while temperature and sunlight were more dominant than the nutrient concentrations in influencing the *S. polyrhiza* growth.[29]

To date, very few studies have been conducted on the hygienic cultivation of duckweed for human consumption. Most previous studies have been focused on the utilization of duckweed for wastewater treatment. More research is necessary in developing and optimizing hygienic and mass duckweed cultivation systems to maximize the biomass yield and nutrition potential of this aquatic plant.

Table 1. Chemical compositions of different species of duckweed (dry weight basis).

Duckweed	Crude protein, %	Total carbohydrate, %	Crude fiber, %	Lipid, %	Ash, %	Reference
L. gibba	25.2	-	9.4	4.7	14.1	[8]
L. punctata	28.7	-	9.2	5.5	13.7	[8]
S. polyriza	29.1	-	8.8	4.5	15.2	[8]
Wo. columbiana	36.5	-	11.0	6.0	17.1	[8]
L. minor	17.5	-	29.7	3.4	16.6	[9]
Lemna sp.	16.0	35.0	-	9.0	26.0	[12]
L. punctata	16.3	24.5	-	-	3.5	[11]
L. gibba	41.7	17.6	15.6	4.4	16.2	[10]
L. gibba	21.5	-	-	4.5	20.1	[30]

"-" means not reported.

Chemical and nutritional characteristics of duckweed suitable for human food

Duckweed has great potential as a future food source in terms of its chemical compositions. It can provide energy and macro-/micro-nutrients for human body, maintaining health, and supporting body's structure when consumed as food. Appenroth et al. (2017) characterized the nutritional compositions of duckweed species including *S. polyrhiza, Landoltia punctata* (*L. punctata*)*, Lemna gibba* (*L. gibba*)*, L. minor, Wolffiella hyalina* (*Wa. hyalina*), and *Wolffia microscopica* (*Wo. microscopica*) and reported protein content of 20 (*L. punctata*) – 35% (*Wa. hyalina*), starch content of 4 (*L. minor*) – 10% (*L. punctate*), and fat content of 4 (*L. punctate*) – 6% (*Wa. hyalina*) (dry weight basis).[4] Further investigation on the nutritional compositions of genus of *Wolffia* including eleven species showed that the contents (dry weight basis) of protein, starch, and fat were 20 (*Wo. arrhiza*) – 30% (*Wo. microscopica*), 10 (*Wo. microscopica*) – 20% (*Wo. arrhiza*), 1 (*Wo. columbiana*) – 5% (*Wo. elongata*), respectively, and average content of fiber was around 25%.[5] The chemical compositions of certain duckweed species are summarized in Table 1. The protein content in duckweed is comparable and even higher than that in most cereal grains, and duckweed also contains higher contents of fiber, lipids, and minerals in contrast to cereal grains. This section discusses the nutritional compositions and quality of duckweed including macro-and micro-nutrients such as protein, carbohydrate, fat, minerals, vitamins, and phytochemicals.

Protein

Duckweed species of *L. minor* can contain up to 45% protein on a dry weight basis.[31] The average protein yield of *Wo. arrhiza* has been reported to be 2,080 kg/ha/year which is much higher than that of soybean, nuts, rice, and corn of which the average protein yields are 303, 229, 71, and 179 kg/ha/year, respectively.[6] The protein content of duckweed is affected by duckweed species and growth conditions, and the protein content of the extract could be affected by extraction methods.[8,32,33] For example, the average protein content of species of *L. gibba, L. punctata, S. polyrhiza*, and *Wo. columbiana* were reported to be 25.2, 28.7, 29.1, and 36.5%, respectively,[8] while Dewanji (1993) and Duan (2013) reported that the duckweed species of *L. minor* and *Lemna sp.* contained 17.5% and 16.3% protein, respectively.[9,12] Duckweed *Wo. arrhiza* shows high yield potential of protein; however, duckweed rarely contains lectin which accounts for large proportion in soy grains. Lectin is a well-known anti-nutrient during nutrient absorption due to its binding to glycoprotein receptors on the epithelial cells in the intestinal mucosa.[34] Thermal processing is required for reducing anti-nutrients for many plant proteins, such as cooking, extrusion, roasting, and steaming for improving the nutrient absorption.[35–37] Whereas such an energy- and cost-consuming processing is not necessary for duckweed as human food uses.

Fasakin (1999) studied protein extraction from species of *S. polyrhiza* and reported that crude protein content of the resulted protein concentrate and residual pulp fiber was 64.6% and 19.9%, respectively.[38] In addition, processing method of duckweed, such as fresh preparation, frozen, and drying, also influences the protein extractability. Yu et al. (2011) reported that protein contents in the extracts of species of *S. polyrhiza* that was processed differently (i.e., fresh, frozen, and ambient temperature dried) were 46.1, 45.2, and 67.8%, respectively.[33] The molecular weight of the extracted *S. polyrhiza* protein ranged from 14 to 160 kDa, and the denaturation endothermic peak of *S. polyrhiza* protein was at 103.6 C.[33] These researchers also investigated the thermal stability of *S. polyrhiza* protein and found 10% weight loss at 250 C, 74% thermal degradation between 250 and 500 C, and 25% residue remained beyond 750 C. Comparing the effect of drying methods including blanching with sun drying (blanched with water at 100 C for 3 min, direct sunlight at 27–30 C), sun-drying (direct sunlight at 27–30 C), and shade-drying (under roofs) on the *L. minor* nutrients, Ifie et al. (2020) demonstrated that blanching with sun drying yielded the highest contents (dry weight basis) of protein (30.0%) and crude fiber (27.1%).[39]

Duckweed protein can provide a full spectrum of amino acids required by human body. It contains all the nine essential amino acids (EAA), as well as various non-essential amino acids. The content of individual amino acid in duckweed can meet the recommendations of WHO.[4,13] The average values of amino acids in species of *L. gibba, S. polyrhiza, L. punctata*, and *Wo. columbiana* were reported to be (g/100 g of protein): 4.0 Lys, 3.6 Ile, 6.7 Leu, 0.9 Met, 4.2 Phe, 3.1 Thr, and 4.4 Val.[8] The amino acid profiles of species including *S. polyrhiza, L. punctata*, and *L. aequinoctialis* were reported to be similar.[40] Table 2 summarizes the EAA compositions of certain duckweed species compared to cereal grains including wheat, corn, rice, soy, chickpea, and lentil. The individual amino acid content in duckweed varies among species. Generally, EAA contents in duckweed are either comparable or superior to the FAO reference. For example, the contents of leucine, phenylalanine, and valine in the species of *L. minor, L. gibba, S. polyrhiza, L. punctata*, and *Wo. columbiana* are far above the FAO reference. The EAA contents especially tryptophan and methionine in duckweed seem to be superior to those in chickpea, corn, lentil, rice, soy, and wheat.

Protein digestibility is one of the important criteria evaluating protein quality. Dewanji (1993) showed that the *in vitro* digestibility of *L. minor* protein was greater than 77.9% based on the pepsin-pancreatin method, indicating that *L. minor* protein was relatively easy to be broken down to amino acids for absorption and metabolism.[9]

The bioavailability of EAA in duckweed has also been studied. Kaplan et al. (2019) investigated the bioavailability of amino acids of *Wo. globosa* in healthy men (n = 36) for continuous 3 days with 12 h overnight fast through the blood samples test at 0, 30, 90, and 180 min.[42] It was found that EAA concentrations of blood fluctuated more with the increase of the sampling time, and the overall bioavailability of EAA was firstly found to increase at 90 min while the EAA concentrations including His, Phy, Thr, Lys, and Trp were higher than the baseline.[42] In addition, the bioavailability of EAA of *Wo. globosa* was similar to that of soft cheese (n = 12) and pea (n = 11) EAA.[42] However, a few limitations of this study may affect the data reliability, such as the effect of carbohydrate and fat on protein digestibility, no female subjects, and large variation of the data. In addition, the short blood

Table 2. Comparison of essential amino acids in different species of duckweed compared to cereal grains, legumes, and FAO reference (g/100 g protein).

Amino acid	Trp	Thr	Ile	Leu	Lys	Met	Phy	Val	His	Reference
L. minor	4.24	5.13	5.67	9.6	5.93	1.38	6.01	6.43	2.65	[9]
L. gibba	N/A	3.2	3.87	7.15	4.13	0.83	4.45	4.96	1.89	[8]
S. polyrhiza	N/A	3.45	3.75	6.85	4.3	0.83	4.2	4.4	2.15	[8]
L. punctata	N/A	3.31	3.76	6.88	4.26	1.07	4.38	4.71	1.9	[8]
Wo. columbiana	N/A	2.55	3.06	5.83	3.37	0.87	3.6	3.49	1.18	[8]
FAO	1.4	2.8	4.2	4.8	4.2	2.2	2.8	0.9	1.9	[8]
Chickpea	1.1	3.15	3.51	6.35	6	1.39	5.06	3.71	2.03	[26]
Corn	0.1	3.8	3.6	12.3	2.8	2.1	4.9	5.1	3.1	[8]
Lentil	1	3.9	4.7	7.9	7.6	0.9	5.4	5.4	3.1	[26]
Rice	1.2	3.6	4.3	8.3	3.6	2.3	5.3	6.1	2.3	[8]
Soy	1.4	4.1	4.6	7.7	6.3	1.3	4.9	4.7	2.6	[26]
Wheat	1.3	2.7	3.9	6.8	2.2	1.6	5	4.4	2.4	[41]

"NA" means not available

collection time before test cannot reflect the bioavailability of EAA in a long-term study. Therefore, further studies are recommended to expand the number of subject and examine the effect of a long-term intake of protein isolate/concentrate from duckweed on amino acid bioavailability.

The effect of *Wo. globosa* on blood glucose concentration was also studied through a randomized controlled crossover trial with 20 abdominally obese subjects (51 years old and 91 kg).[42] Participants took either green shake containing *Wo. globosa* or an iso-carbohydrate/protein/calorie yogurt shake for a 2-week flash glucose evaluation. Green shake ingestion of *Wo. globosa* resulted in a lower postprandial glucose concentration in contrast to the yogurt shake at 15, 30, 45, 60, and 150 min after the ingestion. Compared to the yogurt ingestion, the ingestion of *Wo. globosa* retarded the increase of glucose concentration to the peak level but accelerated the decrease of glucose concentration to the baseline.[43] Meanwhile, ingestion of *Wo. globosa* reduced the next-morning fasting glucose levels.[43] Thus, the ingestion of *Wo. globosa* is beneficial for glycemic response, i.e., species of *Wo. globosa* may be utilized to develop functional foods for controlling blood glucose level.

Carbohydrate

Duckweed contains versatile carbohydrates such as starch, cellulose, trace hemicellulose, pectin, and others. The specific carbohydrate contents depend on the species and growth conditions. For example, duckweed starch could vary greatly from 3 (*L. aequinoctialis*) to 75% (*L. punctata*) on the dry basis, dependent on the species and varieties, locations, nutrient availability, and other growth conditions.[17,44] Starch granules are accumulated quickly during *L. punctata* growth and distributed in fronds (Fig. 4).[45] de Souza Moretti et al. (2019) studied the starch content in species of *L. punctata and L. minor* from different locations (Australia and Brazil), and found that the starch content ranged from 75.4 (*L. minor* from The University of Queensland, Australia) to 85.7% (*L. punctata* from Watergarden Paradise, Australia), wherein, amylose content of the starch ranged from 29.4 (*L. minor* from Brazil) to 39.2% (*L. punctate* from Brazil).[46] In addition to starch, *L. punctata* has been reported to consist of 20.3% pectin (i.e., galacturonan, xylogalacturonan, and rhamnogalacturonan) and 3.5% hemicellulose.[47] Ge et al. (2012) reported that cellulose in *L. minor* was as low as 10% (dry basis), whereas lignin was undetectable.[48]

Starch content of species *S. polyrhiza* has been further characterized and compared with corn starch by Lee et al. (2016), and they reported that *S. polyrhiza* contained 23.3% starch (dry basis), which was less than B73 corn (66.5%).[49] The starch granules of *S. polyrhiza* displayed disk/dome shapes with one side of the granule flat and diameters of 4 ~ 9 μm (Fig. 5). The *S. polyrhiza* starch contained 35.7% amylose and displayed a B-type polymorph, wherein, the average amylopectin branch-chain length was 26.5 of degree of polymerization.[49] The *S. polyrhiza* starch had a higher gelatinization temperature and greater percentage of retrogradation compared to corn starch.[49] Resistant starch (RS) was the predominant starch in the uncooked species of *S. polyrhiza* which accounted for 80.3%, followed by slowly digestible starch (SDS) (14.9%) and rapidly digestible starch (RDS) (4.7%).[49] Resistant starch is referred to a carbohydrate that resists digestion in the small intestine but ferments in the large intestine, therefore being beneficial for gut health. However, up to

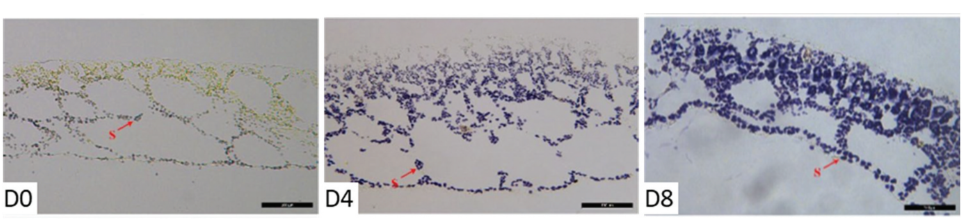

Figure 4. Starch distribution of *L. punctata* in fronds at day 0, 4, and 8. “Red arrow” and “S” point to starch granules. Scale bar, 100 μm. (Source: [45]).

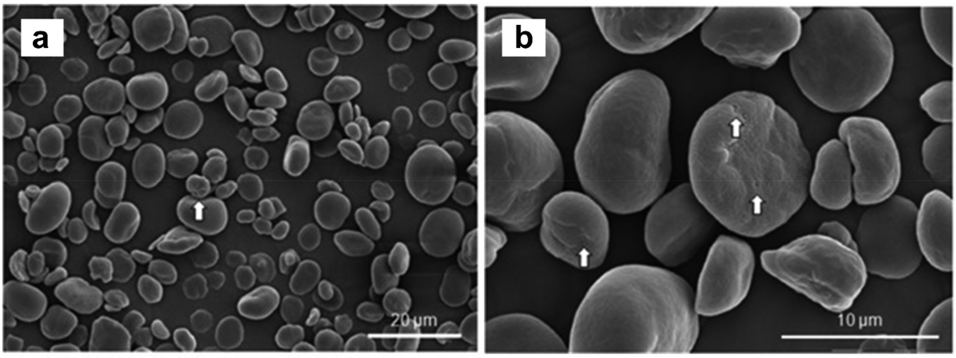

Figure 5. Scanning electronic micrographs of starch granules of *S. polyrhiza* (Source: [49]).

now, little is known about the RS in duckweed in terms of its specific types and function. In addition, few *in vitro, in vivo*, or on-human studies have been done about the digestibility and absorption of the RS from duckweed after ingestion.

Duckweed also contains significant amount of dietary fiber. The fiber content was approximately 25% of dry weight based on the analysis of 11 species of the genus Wolffia.[5] The high content of dietary fiber in duckweed could improve the Western diet by providing low-energy food components and preventing cardiovascular diseases. So far, studies on duckweed carbohydrates are still very limited. Future studies can be focused on the RS and other types of dietary fibers in duckweed to further elucidate their unique functions and properties.

Fat

Fat is an important nutrient and provides energy to fuel life activities. Fatty acid (FA) profile plays a critical role in human health because FAs could either protect against or induce coronary heart disease depending on the type of FAs. Yan et al. (2013) characterized the FA distribution of 30 species of duckweed and demonstrated that the total fatty acid (TFA) contents (dry weight basis) ranged from 4.6 (*Wolffiella welwitschii*) (*Wa. welwitschii*) to 14.2% (*Wolffia borealis*) (*Wo. borealis*), wherein triacylglycerol (TAG) ranged from 0.02 (*S. polyrhiza*) to 0.15% (*Wa. lingulata*).[50] Palmitic acid (PA), linoleic acid (LA), and α-linolenic acid (ALA) accounted for more than 80% of the TFA in duckweed.[50] Within the tested thirty species of duckweed, seven species of Lemna and one species of Wolffiella contained linolenic acid (GLA) and ALA.[50] Another study conducted by Tang et al. (2015) showed that the TFA contents varied from 1.05 (*L. punctate* and *S. polyrhiza*) to 1.62% (*Wo. globosa*) (dry weight basis), wherein, PA, LA, and linolenic acid also accounted for more than 80% of the TFA.[51] In addition, there was no significant difference of TAG among species of *L. punctata, L. aequinotialis, S. polyrhiza*, and *Wo. globosa*.

Appenroth et al. (2017) analyzed FA distribution of species of *S. polyrhiza, L. punctata, L. minor, Wa. hyalina*, and *Wo. microscopica* and found that *Wo. microscopica* contained the lowest contents of saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA), which were 25.1 and 3.8%, respectively, and the highest amount of polyunsaturated fatty acid (PUFA) of 71.1%.[4] *L. punctata* contained the highest SFA and MUFA and lowest PUFA of 46.2, 5.7, and 48.1%, respectively. The total omega-3 FA contents varied from 32.7 (*L. punctata*) to 53.1% (*L. gibba*) and total omega-6 FA contents ranged from 10.9 (*S. polyrhiza*) to 26.8% (*Wo. microscopica*).[4] The ratio of omega-6 FAs/omega-3 FAs of tested duckweed species was less than 1.0.[4] A ratio of approximately 6:1 was suggested for adequate intake for healthy populations.[52] However, evidence showed that dietary intake of less than 50% omega-6 FAs in high unsaturated fatty acids (UFAs) contents could effectively prevent cardiovascular disease.[53] In another study, the fat in eleven species of Wolffia contained 34% SFAs with particularly high levels of PA.[4] In species of *Wo. globosa* and *Wo. arrhiza*, PUFA dominated the FA content, wherein, ALA, LA, and GLA were the three predominant PUFAs.[4]

The FA distribution in duckweed varies among duckweed genera and species. Duckweed contains a greater proportion of PUFA and MUFA, and omega-3 and omega-6 FAs are the predominant UFAs in duckweed. So far, little *in vitro* or *in vivo* research has been conducted about the UFA in duckweed associated with the risk of cardiovascular diseases. The oxidation of UFAs in duckweed and their shelf stability are rarely known. Hence, more studies are needed to better understand the functions, properties, and health benefits of UFAs in duckweed.

Minerals, vitamins, and phytochemicals

In addition to the aforementioned macro-nutrients, duckweed is also rich in minerals, vitamins, and phytochemicals.[30] The average content of ash content, which is mainly mineral, in genus of Wolffia was reported to be around 18% (dry weight basis).[4] Ifie et al. (2020) revealed that K, Ca, Fe, and Na were the predominant minerals in *L. minor* with their respective contents of 6,780, 1,154, 230, and 201 mg/kg fresh *L. minor*, followed by Mn (29.35 mg/kg) and Zn (6.35 mg/kg).[39] The contents of macro- and micro-minerals in duckweed are summarized and compared to those in the common cereal grains (Table 3).

Vitamin B12 has also been identified in species of *Wo. globose*,[42] which shows great potential to be promising alternative food source for vegan, since most plant foods lack vitamin B12 due to the absence of cobalamin-dependent enzymes. Kaplan et al. (2019) prepared tested meals for providing 30 g protein from the following food sources including 333 soft cheese, 600 green peas and 410 g *Wo. globosa*.[42] Vitamin B12 content was reported to be 0.00, 1.16, and 2.81 μg in soft cheese, green peas and *Wo. globosa* respectively. Though the detection method of vitamin B12 in the study conducted by Kaplan et al. (2019) is not available, it should be noted that vitamin B12 occurred in active and inactive forms. Wherein, the vitamin B12 in soft cheese is known to be active. Therefore, the vitamin B12 in the study of Kaplan et al. is considered in the pseudo form[5857]. Although *Wo. globosa* shows great potential of vitamin B12 for vegan, more studies are warranted for discovering the bioavailability of vitamin B12 for human nutrition.

Carotenoids are the major health-promoting phytochemicals in genus of Wolffia with lutein and β-carotene being the predominant carotenoids, which average content were reported to be 40–80 mg/100 g and 10–30 mg/g (dry weight basis), respectively.[4] In addition, zeaxanthin was also found in genus of Wolffia, though at a lower level. Appenroth et al. (2018) showed that α-tocopherol of Wolffia genus varied from 0.5 to 13 mg/100 g (dry weight basis) from eleven species.[5] Total sterol content in genus of Wolffia accounted for 2.4–5.3%, mainly as β-sitosterol, campesterol, and stigmasterol. Saponin, flavonoids, alkaloids in *L. minor* were found to be 23.25 mg/g, 0.83 mg/g, and 6.40 mg/g (dry weight basis).[39] Further studies should be conducted to understand the health benefits of duckweed phytochemicals.

Table 3. Mineral content of duckweed compared to cereals and grains (dry weight basis).

Plant	Ca (g/100 g)	P (g/100 g)	Na (g/100 g)	K (g/100 g)	Mg (mg/100 g)	Fe (mg/100 g)	Mn (mg/100 g)	Cu (mg/100 g)	Zn (mg/100 g)	Reference
Duckweed (genus of *Wolffia*)	1.22–3.25	1.21–2.13	0.01–0.07	3.97–9.37	190–460	<0.1	8–34	0.2–0.4	2.6–9.2	[5]
Chickpea (Desi)	0.17	0.45	-	0.994	169.00	4	3	-	4	[54]
Corn	-	-	0.06–0.20	-	-	-	3.00–7.00	2–4	10–30	[55]
Rice	-	-	<0.05	0.15–0.28	80–150	10–20	20–50	1–7	10–50	[56]
Soybean	-	-	0.04–0.33	-	-	-	30–60	13–18	40–60	[55]
Wheat	0.03–0.06	0.09–0.20	0.37–0.40	0.09–0.22	22–68	1.60–2.60	0.62–1.96	0.37–0.55	0.90–1.90	[]

"-" means not reported.

Duckweed utilization for human food

Certain duckweed species (i.e., *L. minor, L. gibba, S. polyrhiza, L. punctata, Wo. globosa, Lemna sp.*) are rich in crucial macro-nutrients (i.e., protein, polyunsaturated lipids, and fibers), valuable micro-nutrients and other bio-active compounds. Its protein has a well-balanced amino acid profile; thus, it has great potential for human food products. Cereal-based foods, particularly wheat products, such as bread, cookie, pasta, and snacks are staple food and a major energy source worldwide. These foods are abundant in starch carbohydrate with high glycemic index. Partial substitution of wheat flour with duckweed flour could greatly improve the nutritional aspects of conventional cereal foods and also provide additional health benefits to prevent chronic diseases, due to the better amino acid and FA profile and higher amount of vitamin, mineral, and antioxidant in duckweed compared to wheat. Duckweed contains up to three times more protein than wheat; thus, it can be used for protein supplementation in snacks or nutrition bars.

There has been a strong demand for plant-based proteins. Protein ingredients, such as protein concentrate or isolate, could be developed from duckweed whole meal and used as functional ingredients in various foods to enhance food texture and functional attributes. Soy proteins, wheat gluten, and pea proteins have been dominating the current plant-based protein market; however, food industries and consumers are seeking additional or even alternative protein sources. Duckweed could become an innovative source for plant proteins that can be developed into alternative and animal-free meat, dairy, and egg products. However, it must be mentioned that protein extraction from duckweed can be more challenging than from cereals or pulses, as expected for any leaf proteins. The protein bodies are located inside the cell walls and may be tightly bonded to polysaccharides. Economically feasible technologies need to be developed for duckweed protein extraction with desirable protein yield and purity.

Bioactive compounds such as carotenoids, phytosterols, and other pigments are naturally synthesized in duckweed. Genus of Wolffia contains high content of lutein, β-carotene, α-tocopherol, and zeaxanthin,[4] which are great antioxidants and have anti-inflammatory properties that have been widely used in many foods and nutraceuticals to protect against cancer, coronary heart disease, cataract, and free radicals. In addition, Stewart et al. (2020) also reported species of *L. gibba* contained carotenoid.[59] They could also be used as food colorants in dressing, ice-cream, puddings, chewing gums, and dairy products, given their unique and attractive appearance and taste as well as their nutritional benefits.[59] Genus of Wolffia is also rich in phytosterols (i.e., β-sitosterol, campesterol, and stigmasterol)[4] which possess many anti-cancer and anti-inflammatory properties and could be valuable additives in conventional food products to promote human health.

Space foods are designed to supply astronauts with recommended calories and a balance of vitamins and minerals for performing well in the space environment.[60] The recent technology roadmap of National Aeronautics and Space Administration (NASA) identifies that the importance of self-sufficiency of life support system for life sustainability on a long-term mission. The life support system requires to meet the demands for resource recovery, system closure, and minimal use of expendables. Therefore, the food production in the limited space habitat needs to provide the essential nutrients for humans and close the carbon loop including carbon dioxide removal, oxygen production, water recovery, and waste recycling. Therefore, certain duckweed species (i.e., *L. minor, L. gibba, S. polyrhiza, L. punctata, Wo. globosa, Lemna sp.*) may be a good plant-based food candidate for seeding and cultivating in the space.

Up to now, duckweed is rarely known in the Western diet. de Beukelaar et al. (2019) conducted a study to investigate the acceptance of duckweed (species not mentioned) as human food to Dutch people who have never eaten duckweed and reported that participants (n = 10) tasted duckweed like vegetables with positive feedbacks.[61] An online survey involving 669 participants indicated that consumers would accept the duckweed-containing food if served in a fitting meal properly such as salad, cake, sandwich, and drink.[61] Thus, duckweed has a great potential to be accepted in diet by Western consumers if served properly.

With so many potential opportunities in developing duckweed into nutritious and healthy human foods and food ingredients, there may still be safety concerns associated with duckweed cultivated and harvested from natural fresh water for human diets, such as high-level residues of heavy metals and pesticide, toxicants, dioxins, and pathogens. The accumulation of heavy metals by duckweed generates a potential safety hazard for humans because the heavy metals may enter the food chain, although previous study has been reported that the heavy metals and pesticides in fish grown in sewage stabilization ponds fed on duckweed were at acceptable levels.[62] A previous FAO report (1999) stated that levels of the heavy metals (i.e., Cd, Cr, and others) in duckweed do not threat human or animal health, but it must be mentioned that this would heavily depend on the water quality.[63] Recently, Appenroth et al. (2018) analyzed the heavy metal contents of duckweed genus of Wolffia including eleven species collected from Zurich, Switzerland and then cultivated in a medium containing various nutrients and minerals, and showed that the average contents of Cd, Pb, Hg, and As were 76 ± 145, 244 ± 301, 18 ± 4, and 53 ± 18 g/kg, respectively.[5] Thus, safety concerns regarding the heavy metals in duckweed require close monitoring, risk assessment, and further evaluation. Fujisawa et al. (2006) investigated the uptake of pesticides by *L. gibba* in Hoagland's medium and reported that *L. gibba* absorbed 3-methy-4-nitrophenol and 3,5-dichloroaniline to certain extent, whereas, the pesticides (R,S)-2-(4-chlorophenyl)-3-methylbutanoic acid and (1RS)-*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid were scarcely transported into *L. gibba* from the medium.[64] More studies are necessary to further understand the adsorption and transformation mechanisms of pesticides and the toxicity of pesticide metabolites in duckweed and associated food chains. Hygienic cultivation of duckweed for human food uses should also be addressed. Many previous studies were focused on wastewater treatment using duckweed, and the resultant products may be potentially contaminated by heavy metals, pesticide, toxicants, dioxins, and pathogens. Therefore, hygienic culture in natural clean and fresh water or designed culture media with the needed nutrients for duckweed growth should be explored.

Anti-nutritious factors such as oxalate, phytate, trypsin inhibitor, protease inhibitor, and tannin are known to be present in the plant protein that affected the digestion and absorption of nutrients.[65,66] Oxalate is a known chelating agent which affects the calcium absorption in human body and phytate is known to be associated with impairment of mineral bioavailability, though little is known about the toxic dose of oxalate and phytate to human health. Ifie et al. (2020) reported the average content of oxalate and phytate contents was 1.81 and 10.30 mg/g, respectively, in fresh species of *L. minor*.[39] To reduce the anti-nutritional contents, processing techniques such as drying, soaking, cooking, or using phytic acid-degrading enzymes may be used, which have been effective in reducing anti-nutritional contents in other vegetables and plants. Sree et al. (2019) reported the anti-proliferative and cytotoxic effects of *S. polyrhiza, L. punctata, L. gibba, L. minor, Wa. Hyaline, Wo. globosa*, and *Wo. microscropica* on human cell lines *in vitro*.[67] Results showed that duckweed did not affect the proliferation or cytotoxicity of human cell lines including HUVEC, K-562, and Hela cells, indicating that the extracts from the tested duckweed species is non-toxic for cells.[67]

Species of *Wo. arrhiza* and *Wo. globosa* have long been accepted as a food source in Thailand and Laos.[3,22] Recently, the U.S. FDA has issued a "GRAS No Objection Letter" for Parabel USA Inc.'s trademarked Lentein plant protein, which is a commercially available duckweed product (https://www.fda.gov/media/113614/download). Parabel Ltd. concluded that the LENTEIN Complete (protein powder from duckweed) and Degreened LENTEIN Complete (protein powder from duckweed) meet the requirement of Good Manufacturing Practice (GRAS Notice (GRC) No. 742, 2017).[19] The appropriate food-grade product specifications set for Section II.E.2. of the provided safety evaluation and required by FDA regulation, 21 CFR 182.1 (b)(1), can be considered to be Generally Recognized As Safe (GRAS) when consumed as a commercial food product at 210 g per serving size per person per day (GRAS Notice (GRC) No. 742, 2017).[19] Overall, it can be concluded that duckweed should be generally safe for human consumption if its production and handling follow food safety principles and guidance.

Conclusions and future research

New food sources should be continuously explored and developed to feed the increasing population worldwide in the future. Duckweed is a potential food source to relieve food shortage stress globally in future due to many advantageous features such as aquatic production and rapid growth. In addition, certain duckweed species contain many critical nutrients such as high content of protein and dietary fiber, sufficient essential amino acids, health-promoting micronutrients and antioxidants. Previous research demonstrated promising acceptance of duckweed as human food if served in appropriate forms such as substitution to cereal foods and snacks, duckweed protein concentrates and isolates. Duckweed whole meal has been available from a few commercial suppliers; however, techno-functional properties of the whole meals and protein extracts need to be thoroughly investigated, and innovative and palatable food products need to be developed and made available to consumers. Food safety regarding duckweed protein allergy and hygienic cultivation for eliminating toxins such as heavy metals, pesticide, dioxins, and pathogens is warranted for more focus. To develop a duckweed supply chain and establish duckweed as a new staple food, substantial research and development efforts will be required, such as developing continuous and economic duckweed farming systems, efficiently harvesting and processing fresh duckweed into staple dry food materials, deriving ingredients and food products from duckweed, consumer and marketing study, economic assessment, among many others. Although duckweed constituents such as proteins, carbohydrates, fats, and phytochemicals have demonstrated some great functional and nutritional benefits, further research is necessary to better understand and elucidate their chemistry, structure, function, and health beneficial effects.

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Disclosure statement

The authors declare that they have no known conflict of interests.

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