Water lentils (duckweed) as new plant protein source

Ingrid van der Meer[1] and Jurriaan Mes[2]

1 Wageningen Plant Research, Wageningen University & Research

2 Wageningen Food & Biobased Research, Wageningen University & Research

'Duckweed, is that really something we can eat? Isn't that the green stuff growing in ditches?'

This is certainly not the image we want people to have of this little plant, which we would like to see as new protein crop. We intend to grow these plants safely and hygienically in sanitised water in a greenhouse or via an indoor farming system. We therefore prefer to call them water lentils, the ancient name for duckweed which is used in many different languages.

As one of the fastest growing plants on earth, water lentils are not only very attractive as a new food crop, they also positively support sustainability goals. Because the plant lacks stems, almost never produces flowers and seeds and hardly has any roots, it can invest a large fraction of its energy in producing leafy biomass. The plant multiplies vegetatively by meristems inside lateral pouches. Daughter plants tie off from the mother plant, almost like the budding of yeast. This enables very rapid, exponential growth of a population. Due to its exponential growth rate, it is often regarded as a pest. We, however, believe water lentils have great potential as a protein source for humans, precisely because of their tremendous capacity for rapid growth and abundant plant material with relatively high protein content.

This little plant is unique: it can double its biomass in two to three days, and continues to do so under optimal environmental conditions. This means there is no specific seasonality for sowing the seeds, growing the plant and harvesting the product or whole crop. For water lentils, cultivation of the plant means a continuous multiplication and harvesting of the biomass, which can be done year-round if the conditions are right. For example, the production of biomass (in dry weight) via water lentils can be 6-7 times higher per hectare per year compared to the production of soybean on that same hectare. As the plant only needs a relative shallow layer of water, some light and nutrients, it can also be grown in multilayer, vertical farming systems, either indoor or in greenhouses. It does not require precious arable land, which means these farming systems can be built on marginal lands in any location around the world where water is available. We therefore see water lentils as one of the most sustainable crops, although a Life Cycle Analysis should be performed when final commercial production systems have been optimised.

The optimal places to cultivate water lentils are geographic regions with warm climates and long daylight periods. However, with sustainable energy sources available, one could also use LED light systems in industrial areas for indoor cultivation. Especially industrial hubs with a surplus of heat and CO_2 could be a perfect location for indoor water lentil cultivation. The heat from surrounding industry can be used to heat up the water or air temperature, and CO_2 can further enhance growth rates and harvestable biomass. Industries could use this cultivation system as part of their strategy to reduce their CO_2 emissions. It is expected that different water lentil species and strains will have differences in optimal growth temperatures and CO_2 concentrations in which they thrive. Therefore, this plant has the potential to enhance global plant biomass and protein production from the subtropical highlands of Africa up to Northern Europe and America.

You might have noticed that not all green floating plants on ponds look the same. Some of these plants indeed may not belong to the Duckweed family. Also, species with the same family can exhibit considerable differences. The Duckweed family includes 37 species divided into five genera. There are species with recognisable leaves, like those within the genera Spirodela (2 species), Landoltia (1 species), and Lemna (13 species, including *Lemna minor* and *Lemna gibba*), as well as species that are very small with barely any recognisable leaves, which float on the water and look like tiny eggs, such as Wolffia (11 species including *Wolffia arrhyza* and *Wolffia globosa*). The species belonging to the genus Wolffiella (10 species) have a more propellor- or spider-like phenotype with thin but long leaf-like structure. Duckweed species are adapted to a wide variety of geographic areas and climatic zones, and many species can survive in extreme temperatures. As the plants easily stick to the feathers of migratory birds, most species have a wide geographic distribution area. Besides differences in plant phenotype, we have seen differences in appearance of senescent leaves and other specific characteristics important for food applications, such as taste.

Officially, duckweeds are the smallest flowering plants in the world, but little is known about how to induce flowering and how do they reproduce sexually. Therefore, breeding for specific traits is not possible at present but will be interesting to study in the future. Also, seed storage is not possible yet. Currently, different species and accessions from all over the world are being stored in biobanks, a type of repository of biological material to store species and lines that can be used to select lines with interesting traits.

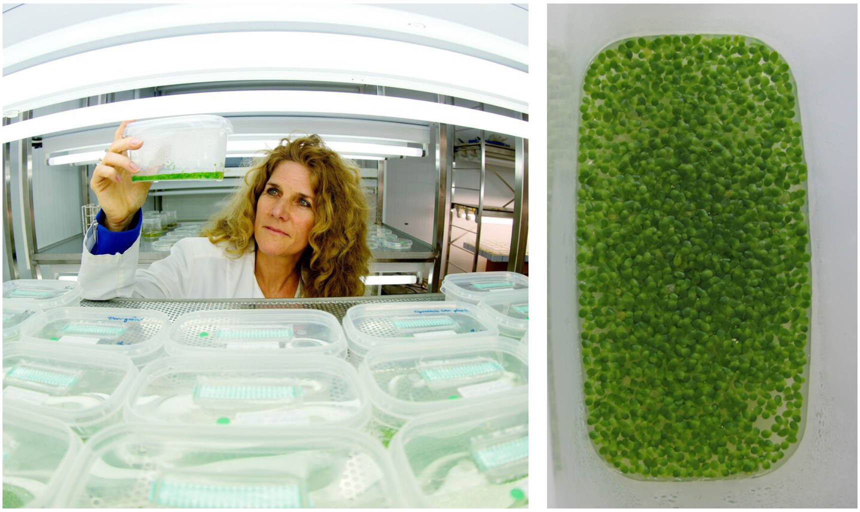

Figure 1: Repository of the species and accessions via sterile storage of the living plant material (biobanking)

Duckweed plants are perfect for use as feed for a wide variety of animals, including cows, goats, pigs, chicken and fish. Without having an effect on growth performance, they can replace up to 20% of the animals' usual diets. From the point of view of the protein transition, duckweed could replace good vegetable protein sources like soybean which is now being fed to animals, so that these protein sources can be used for direct human consumption. Several companies have set up small, local production systems, even trying to close the nitrogen and phosphate cycle by cultivating using locally produced manure. These efforts have not yet led to large volumes of sales due to legislation restrictions, but this might change in the future as research is ongoing and more companies are showing interest in producing duckweed as feed either in Europe, Africa or South-East Asia.

But water lentils also have potential as a food source for humans. Plants from the duckweed family have long been consumed in Asian countries. The specific species used are not well documented but are often classified as *Wolffia arrhiza* and *Wolffia globosa* (also known and marketed under the name Mankai). The plants that are consumed in Asia are mostly collected from open ponds. The European Food Safety Authority (EFSA) has stated that the use of these species is safe for consumers in Europe, provided they are clean and cultivated in a hygienic way. In December 2021, this resulted in EU legislation, authorising the placing on the market of fresh plants of *Wolffia arrhiza* and/or *Wolffia globosa* as a 'traditional food from a third country' under Regulation (EU) 2015/2283 of the European Parliament. Production in good hygienic cultivation systems (as performed by Hinoman and GreenOnyx Ltd., both in Israel) is currently very limited but will soon increase. All other species of the duckweed family, and derived products, are seen as 'novel food' for which approval via EFSA/EC applications is required before they can enter the European market. Over the past six years, all scientific and technological evidence needed for such an approval has been generated at Wageningen University & Research to show that water lentils can be produced and consumed as vegetable in a safe and controlled way and can be used for human consumption. In May 2020, Wageningen Plant Research submitted the Novel Food dossier for the use of *Lemna minor* and *Lemna gibba* as vegetable for human consumption to the EFSA. However, in November 2022 the EFSA concluded that the concentration of manganese (5-21 mg/kg FW in the five batches provided for each species) was higher compared to spinach and that safety could therefore not be guaranteed. This is remarkable as, firstly, no Upper Limits for manganese intake are set by EU legislation, and secondly, since many plant-based food products that we consume, such as potato, oats, wheat, nuts and chickpeas contain similar or much higher concentrations of manganese as found in Lemna. Furthermore, the two accepted duckweed species, *Wolffia globosa* and *Wolffia arrhiza* had manganese concentration between 20-32 mg/kg FW but still were approved by the EFSA panel as long as they would try to get lower level than 6 mg/kg FW. As mineral content of the plants is highly influenced by the fertilisers given during cultivation, we already demonstrated that we can produce Lemna with manganese levels <6mg/kg FW. Discussions with the EFSA are continuing hoping to get Lemna approved and allowed as new sustainable, protein-rich vegetable for human consumption in Europe.

The first thing most people ask when discussing water lentils as new food crop is: 'How does it taste?'. This depends on how you eat it and what processing steps were involved in the preparation of the ingredient or final food product. The first application is to use the fresh or fresh-frozen plants as vegetable. Water lentils have a very neutral taste when harvested fresh and eaten as part of a green salad or added to a stir fry, vegetable-based sauce or mashed potato base. The cooked vegetable can also be added to meals, where it will take on the flavour of the dish. As a vegetable, water lentils have a mild, leafy or nutty flavour and a firm bite. Their neutral flavour makes them versatile, so they can be incorporated into a wide range of meals, dishes and food products. In a human trial to study the safety of the product, people had to consume water lentil-based products for 11 days in a row (Mes et al., 2022b). The respondents' scores on flavour were very similar to those of the control group, who consumed the same meals containing spinach. In our research, we also collaborated with chefs that developed very tasty meals with substantial amounts of water lentils plant material. These dishes were evaluated by a consumer taste panel and were very well appreciated. On a fresh weight basis, the plant leaves contain 2-3% protein. We also analysed how efficiently the proteins present in water lentils are released by our intestinal tract (Zeinstra et al., 2019).

Figure 2: Various meals containing water lentils that were evaluated by a consumer taste panel

On a dry weight basis these plants can contain 35-43% of protein which is very high for plant leaf material. A second application of the water lentils is therefore just using it as dry powder by drying the plants, potentially followed by milling (likely not needed for the smaller Wolffia species). The dried material tends to have a more 'grassy' smell and taste, but this might differ between species or cultivation methods and most likely can be modified by specific treatments. Taste can often be masked by other ingredients or by the food matrix. That also means the dried powder as a product can be used in many applications after optimisation of product formulation. Companies like Parabel Ltd. in the US already sell such dried products (a mixture of different types of Lemnaceae species) under the names LENTEIN™ Complete and De-greened LENTEIN™ Complete as ingredient in bars and drinks. This dried water lentils powder is allowed in the US and some other countries, but it has not been accepted in Europe as can be concluded from EFSA's scientific opinion (EFSA NDA panel, 2021).

A third use of the plant as new protein crop is based on the extracted protein. Methods have been developed to isolate total protein or specifically RuBisCO protein which is the major protein in plant leaves (Nieuwland et al., 2021). The extraction of RuBisCO protein from water lentils resulted in an off-white protein concentrate with appealing functionalities for a diverse set of food applications. The amino acid composition matches very well with the optimum amino acid requirements for human consumption. The digestibility of water lentil protein is 60.4%, comparable to that of casein, a very widely consumed animal-based protein (Mes et al., 2022a). However, water lentil protein is also not yet allowed to enter the European food market, and its EFSA dossier is still pending. To make the product even more sustainable, food applications should also be developed for the co-stream of this extraction method that will contain dietary fibres and other nutrients. But such co-stream will again require EFSA approval.

But why have we not consumed this product before? Are there still any challenges that need to be overcome? We are not sure why water lentils were not yet consumed on a large scale before 1997. In Asian countries it is actually considered a poor man's diet. Probably because it is often collected from open ponds and eaten by people who cannot afford other products. These ponds do not form a good hygienic culture system and might not produce a very healthy and tasty product. This can all be changed by using proper agronomic cultivation systems as our knowledge on hydroponic cultivation has grown enormously in the past decade. Also, the introduction of fully controlled indoor farming systems, and recycling of water and nutrients has generated the need to have a very sustainable cultivation leading to high plant quality and control of pest.

What do we see as challenges? The plants are known for their efficient uptake of contaminants and minerals from the water, which is one of the reasons they are used for water remediation (i.e., purifying the water from contaminants such as heavy metals and pesticides). Attention should therefore be given to the water used in the production systems as low traces of copper or cadmium can result in levels that exceed the allowance for some food products. A second challenge might be the number of bacteria that are allowed to be present on food products. For products like lettuce this is already challenging. But with floating plants that are continuously in contact with fertilised water containing nutrients, we have to develop good sanitary treatments to keep bacterial counts low. The same holds for a putative co-cultivation of algae that might also grow in the same cultivation water. We are aiming for a cultivation of water lentils without the use pesticides or other decontaminating agents. But with large and widescale production also other pests, like water insects or pathogenic fungi, may pop up. At this moment we foresee that EFSA/EC approval to enter the European market is still the biggest hurdle.

And when it is accepted, how long before we can eat this new plant protein crop and what changes will it bring for the protein supply and the sustainability of our food production system? This will heavily depend on investments and pioneers that start to work on further developing the cultivation systems, and optimise closed and efficient fertilisation systems. Control of water quality and fertilisers is key as well as putative contaminations by algae and bacteria for which strict hygiene and sterilisation processes must be implemented.

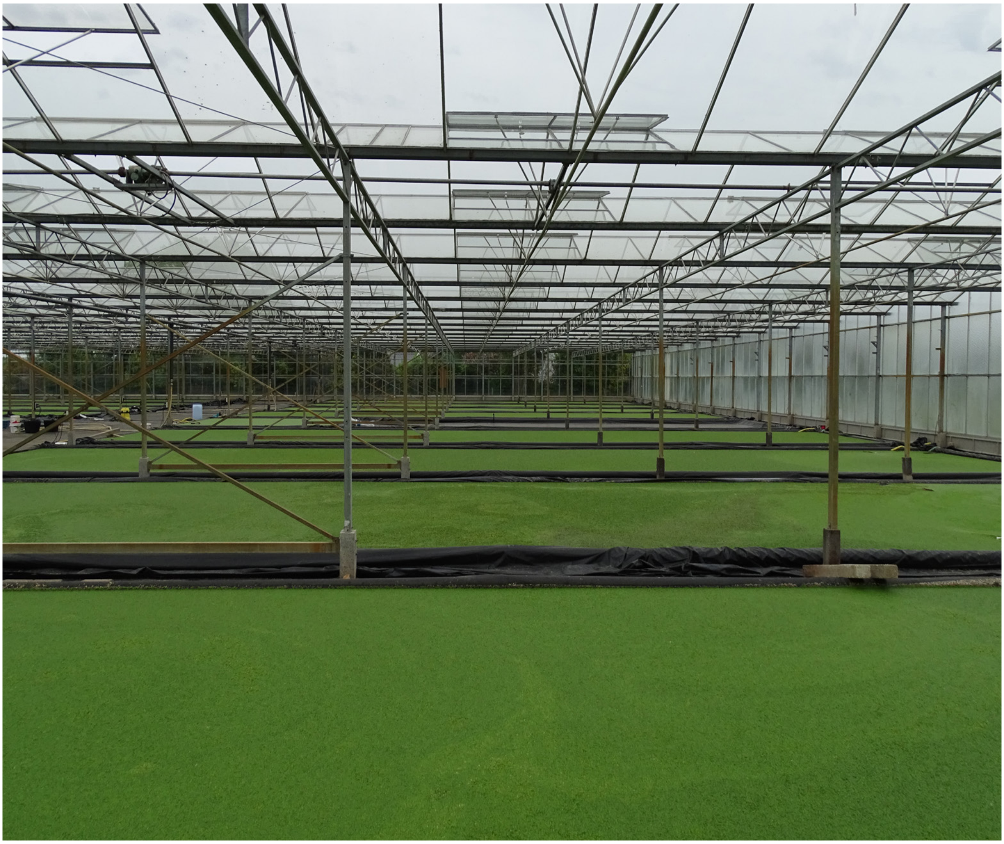

Figure 3: Large scale production of water lentils in a greenhouse

Another crucial player in this process are food producers that will have to include water lentils (protein) in their product portfolio. Profitable market concepts on protein concentrates or isolates will be challenging and might only grow in volume when these proteins have unique food functionalities.

Concluding, water lentils are very easy to grow and easy to harvest, which makes them suitable for use as new protein crop for smallholder farmers in developing countries, as well as in developed countries with an overdependence on soy protein. The very high biomass production rate results in a high putative protein yield (per ha) which is six to seven times higher than soybean on a yearly basis. And if we can grow this plant in ten levels on top of each other via vertical farming, the production per hectare will really make a difference. This little plant can therefore contribute to the protein transition we are aiming for and to the increased sustainability of our food production system.

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