LEAFY BEEFY

SUPPLYING NUTRIENTS
FOR CELLS AND PEOPLE

Table of Contents

1. Introduction

The global demand for meat production is expected to double by 2050[1], but doubling the livestock and farming capacity in a similar time frame is not feasible considering the limited resources and the environmental impact of current agriculture processes. About a quarter of the world's greenhouse gas emissions come from agriculture and other related land use[2]. Furthermore, 14.5 % of all emissions comes from just livestock[3]. Furthermore, the field of alternative proteins includes cultivated meat, an "animal free" meat product that is rapidly developing. Although there is no currently commercially available product in the United States, the cultivated meat industry has seen unprecedented development, from $60 million USD invested in 2019 to $366 million USD invested in 2020[4]. However, to translate this excitement into a product, there are serious production and economic bottlenecks with cultivated meat technology including limited bioreactor sizes, small cell densities, and lack of control over metabolic and differentiation properties. The most concerning bottleneck is the high cost of the media which must be resolved by finding ways to lessen the economic toll without decreasing media quality.

1.1 Current Media Costs

Current mammalian cell culture media are used to produce therapeutics which are low-volume, high-cost products. However, competitiveness in the food industry requires high-volume, low-cost products. To reach scales required in cultured meat production, the adjacent industries that provide the media components will also need to be scaled up as well. The scope of the problem is captured in techno-economic analyses (TEA) on cultivated meat. A TEA by Risner et al. uses the animal-free medium Essential 8™ with prices based on 2017 vendor prices from a Good Food Institute report[5]. The price of TGF-β, a growth factor in media formulations, needed at a concentration of 0.002 mg/L, is priced at $80,900,000 per gram. This high component cost is

reflected in a base case cost of production of $437,000 per kg of cultivated beef to reach a total production volume of 121,000,000 kg cultivated beef (1% of US beef market). On average, a typical beef product is $ 3-5 per kg, and for cultivated beef to reach price parity, this TEA has identified media as a component that contributes >99% of the final consumer costs[5]. Another TEA performed by Humbird uses approximate logarithmic scaling based on historical data of industrial products to predict future prices of media components at the required amounts and captures a more realistic price of cultured beef as supporting industries develop. The baseline cost of production is $37 per kg of beef for a total production volume of 100,000,000 kg of cultivated beef. However, $37 is still not economically competitive with conventional beef, and the media still contributes over 50% of this cost. This study also performed a scenario where macronutrients (glucose + amino acids) in the media are replaced completely with soy plant hydrolysate, bringing the macronutrient component of the media from ~$19.2/kg down to ~$3.4/kg and the cost of production down to a more competitive price of $22 per kg beef[6]. Thus, plant hydrolysates have immense potential to serve as an underutilized nutrient source in cultivated meat.

1.2 Hydrolysates to reduce costs of culture media

Hydrolysates are a good way of providing various amino acids at a low-cost, directly addressing one of the biggest issues facing the cultivated meat industry. In addition to amino acids, there can be other beneficial compounds such as carbohydrates, lipids, and minerals that are present in a final hydrolysate product[7]. Protein hydrolysates are produced using acid, alkaline, or enzymatic treatments. Acid and alkaline treatments are non-specific and typically rely on harsh process conditions that lead to the degradation of amino acids. In acid hydrolysis, amino acids such as tryptophan, methionine, cystine, and cysteine are destroyed and glutamine and asparagine are converted into glutamic acid and aspartic acid. Additionally, safety concerns and large utility

expenditures are associated with maintaining typical process temperatures of 250-280°F and pressures of 32-45psi for 2-8 hours[1]. In alkaline hydrolysis, process conditions are typically milder and reagents such as calcium, sodium, or potassium hydroxide are used, however, amino acids such as serine and threonine are still destroyed during processing[8].

Various plant hydrolysates have been used in plant-based meats, with the most common ones being pea, soy, and wheat. Duckweed hydrolysates are also available as food and nutritional supplements through Parabel USAs Lentein®, which the FDA has designated generally recognized as safe (GRAS), and Hinoman's Mankai® products[9]. However, applying plant hydrolysates to cultured meat media however is a rarer use case. Consequently, this report aims to evaluate the use of duckweed hydrolysates to reduce the cost of cultured meat media.

2. Duckweed Background

Duckweeds are a relatively small family of aquatic floating angiosperms that consists of 37 species divided into 5 genera[10]. Although considered a higher plant, duckweed has a rudimentary morphology that consists only of fronds and a species dependent root. A single individual frond is on the order of a few millimeters in length and roughly oblong in shape[11]. Although sexual reproduction is possible, duckweeds primarily reproduce through the asexual budding of new fronds. This and other genetic factors allow duckweed to have the fastest growth rate of any higher plant[12]. Doubling times have been reported as low as 29.8 hours, allowing for large amounts of biomass to be produced quickly. In one instance 1g of initial biomass grew to 50g in one week[13].

Additionally, duckweed can survive in a wide range of growth conditions. Duckweed's optimum pH and temperature for growth is between 4.5-7.2 and 25 °C respectively, but growth outside of this pH range and temperature tolerances from 2°C - 35°C have been observed[14]. The

robustness of duckweed allows it to proliferate on ponds, inundated fields, and other static water bodies around the world. Because of its rapid proliferation and high tolerance to a wide range of growth conditions duckweed has been used for aquatic phytoremediation in both sewage treatment and wastewater processing[15]. In protein hydrolysate production, these same attributes allow for a robust scale-up and or scale-out process to produce biomass for enzymatic hydrolysis.

In addition to scalability, the nutritional profile is also of paramount importance. Recent studies have shown that the amino acid distribution profile of duckweed closely matches the World Health Organization's recommendations for protein content in food[16]. This makes using duckweed as an amino acid source highly appealing. Additionally, because of its reduced morphology, duckweed is a plurality protein with one report stating as much as 42% of the plant's dry weight is protein[17]. Duckweed also has been shown to not have any anti-proliferative effects on human cell lines nor produce cytotoxic components[18].

2.1 Duckweed Cultivation Practices

Large scale cultivation of duckweed is currently carried out by companies such as Hinoman, Parabel, and Rubisco Foods, which use greenhouses and large outdoor ponds to cultivate duckweed. The greenhouses used by Hinoman are temperature controlled, utilize automated hydroponic systems, and use no pesticides. In large outdoor ponds, cultivation on manure waste streams to simulate *L. minor* growth on sewage drainage have been utilized[19]. In outdoor growth facilities, the ultimate bottle neck arises from unpredictable weather conditions that could hinder maximum growth schemes. To address the need for a reproducible product and to scale up production in a land efficient way, an indoor vertical farming scheme for duckweed is proposed where cultivation can be moved to any location.

2.2 Proposed Vertical Farming Process

Controlled environment facilities are expensive to operate, and to maximize available space, indoor vertical farming of duckweed is explored for large scale production. The use of indoor facilities allows optimal growth conditions year-round [14]at any location. The proposed duckweed cultivation system will be based off the Nordic Harvest's system, which has implemented a state-of-the-art vertical cultivation system, utilizing robotics to monitor large scale cultivation of plants. In the proposed system, technologies such as flavanol meters and chlorophyll sensors will ensure that the plants are receiving the necessary nutrients, and robotic strainers can be used to harvest the duckweed once the plants are fully grown[20]. Extrapolating from duckweed cultivation yields of 20-180 t/ha/yr in the Southern United States and Israel where optimal growth conditions exist, it is expected that with optimization work the vertical farming system will achieve similar yields[14].

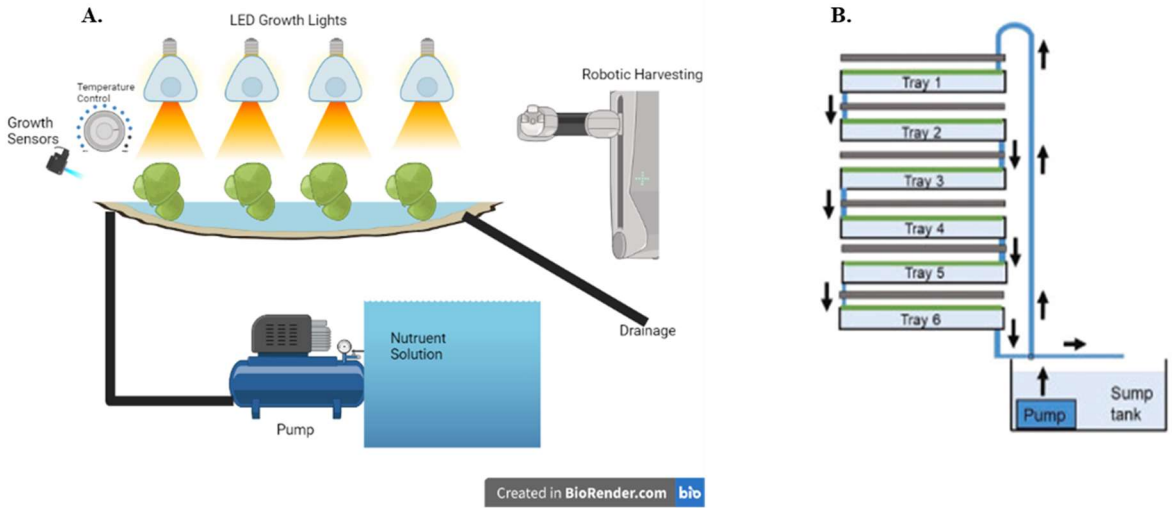


Figure 1 | Use of vertical farming as an alternative to conventional cultivation for production of duckweed on a large scale. A, Enlarged view of a singular tray of duckweed within the larger scale with use of temperature control, LED growth lights, growth sensors such as chlorophyll and flavanol sensors, and robotic harvesting arm. B, Overview of how the vertical farming system will

be structured with multiple growth trays stacked vertically with nutrient supplies being pumped through and drainage going to waste.

3. Duckweed Hydrolysate Production Process

Taking duckweed from the proposed vertical farming system, duckweed hydrolysate will be produced enzymatically utilizing commercially available Flavourzyme, which is a food grade fungal protease produced from *Aspergillus oryzae* with both endoprotease and exopeptidase activities[21]. The optimal pH and temperature of this enzyme are 7.0 and 50°C respectively. The use of this enzyme in the production of *Lemna minor* hydrolysate has been explored at a lab scale with promising results[22].

3.1 Small Scale Duckweed Hydrolysate Production

In brief, on the small scale, duckweed (*L. minor)* powder is defatted using a 1:4 (w/v) ratio of duckweed and hexane at room temperature for 3h. This process is performed twice, and the resulting slurry is air dried in a fume hood overnight. The dried mass was then ground and sieved through a 40-mesh sieve. The defatted powder was then shaken with 50% (v/v) acetone for 2 hours to remove phenolic compounds. The resulting mixture was then centrifuged at 6000 rpm for 20 minutes and the pellet was vacuum evaporated to remove residual solvents at 40°C and 500 mmHg. The dried pellet was then ground into a powder and used for enzymatic hydrolysis. 400 g of *L. minor* powder was mixed with 1.6 kg of water and stirred vigorously in a water bath for 30 min. Once the solution was well mixed, Flavourzyme was added to the reaction vessel up to a 1.5% (w/v) concentration and maintained at pH 7.0 and 55°C for 120 minutes. Once the reaction was carried out to completion, the mixture was filtered through a Whatman paper filter, and the filtrate was analyzed for protein recovery, degree of hydrolysis, and antioxidant activity[22].

3.1 Proposed Large Scale Extraction Scheme

On a large scale, duckweed production will be taken in house and produced through indoor vertical farms as previously described. Duckweed will be wet milled into a powder and added to a mixing tank charged with hexane at a 1:4 (w/v) ratio and allowed to mix at room temperature for 3 hours twice. The defatted duckweed is then dried in a belt dryer and milled to a particle size of 400 μm. The milled powder is then added to a mixing tank with 50% (v/v) acetone and stirred for 2 hours to remove phenolic compounds. The slurry will then be put through a disc stack centrifuge to remove acetone and the solids faction will be vacuum dried at 40°C and 500 mmHg. The dried duckweed will then be fed into another mixing tank and mixed with water at a ratio of 1 kg duckweed powder: 4 kg water. Once the solution is well mixed, Flavourzyme is added to the reaction vessel at a 1.5% (w/v) concentration and maintained at pH 7.0 and 55°C for 120 minutes. The reaction is carried out to completion and terminated by raising the temperature to 75°C for 10 minutes. The mixture is then depth filtered to remove any particulates, and the filtrate is analyzed for protein recovery, degree of hydrolysis, and antioxidant activity to match certain quality control metrics that will be determined experimentally.

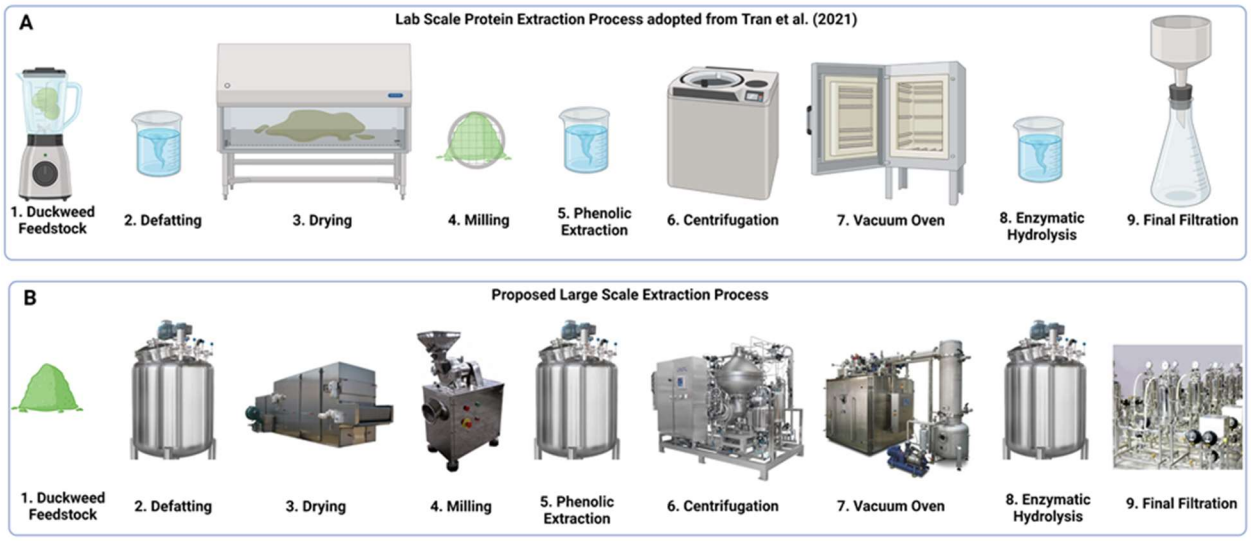


Figure 2 | Production of duckweed hydrolysate through enzymatic hydrolysis at lab and commercial scales. A, schematic of equipment used in the duckweed powder preparation (1-7) and enzymatic hydrolysis and final filtration (8-9) at the lab scale[22]. B, schematic of a proposed scaled up process to produce duckweed hydrolysate as reported by Tran et al. Equipment used in each operation in order or processing (1-9) are, 1. Wet miller, 2. Mixing tank, 3. Belt dryer, 4. Grinder, 5. Mixing tank, 6. Disk stack centrifuge, 7. Vacuum oven chamber, 8. Mixing tank, and 9. Depth filtration skid.

The ultimate sizing of the large-scale equipment will depend on the required production capacity discussed in the next section and how many facilities are going to be utilized to meet the yearly hydrolysate demand.

3.3 Production Capacity

The required mass of *L. minor* by dry weight is determined using **Equation 1**. The amino acid composition of DMEM/F12 is obtained from ThermoFisher Scientific and the duckweed Flavourzyme hydrolysate composition is taken from experimental results reported in Tran et al.

$$\text{L. minor Production Requirement (kg dry weight)} = \frac{\text{Total media Flow (L)} * \left[\text{DMEM/F12}_{AA_x}\right] \left(\frac{g}{L}\right)}{\text{Duckweed Hydrolysate}_{AA_x} \left(\frac{g}{\text{kg dry weight}}\right)}$$

Equation 1 | Equation used to determine the amount of *L. minor* required to produce the amino acid concentration desired DMEM/F12 at the desired total media flow for 1 year.

Hydrolysate production to satisfy the required yearly demand of each amino acid in DMEM/F12 is summarized in **Table 1**. From this analysis, the concentration of alanine is met first during duckweed supplementation, and if the final concentration of individual amino acids in the DMEM/F12 formulation is used as a constraint, the maximum amount of duckweed that needs to be produced is $4.28 * 10^5$ kg of dry mass. To meet this demand the proposed facility would require about 3 ha of land compared to the nearly 200 ha facility used by Parabel[9]

Table 1 | Calculation of yearly hydrolysate demand scenarios based on matching specific amino acid contents in *L. minor* to concentration of corresponding amino acid in DMEM/F12

Amino Acid	***L. minor* Hydrolysate (g/kg)**	**Amino Acid composition per gram of protein**	**AA Conc. in DMEM/F 12 (mg/L)**	**Total Yearly AA Production Required for DMEM/F12 (g)**	**Required Hydrolysate for yearly AA demand (kg)**
Ala	10.91	6%	4.45	4.67E+06	4.28E+05
Arg	10.99	7%	92.27	9.69E+07	8.81E+06
Asp	14.64	9%	6.65	6.98E+06	4.77E+05
Cys	1.53	1%	12.09	1.27E+07	8.30E+06
Glu	17.93	11%	367.50	3.86E+08	2.15E+07
Gly	10.90	6%	18.75	1.97E+07	1.81E+06
His	7.83	5%	23.24	2.44E+07	3.12E+06
Leu	16.61	10%	59.05	6.20E+07	3.73E+06
lle	8.95	5%	54.47	5.72E+07	6.39E+06
Lys	10.79	6%	72.80	7.64E+07	7.09E+06
Met	3.96	2%	17.24	1.81E+07	4.57E+06
Phe	8.83	5%	35.48	3.73E+07	4.22E+06
Pro	5.16	3%	17.25	1.81E+07	3.51E+06
Ser	9.40	6%	26.25	2.76E+07	2.93E+06
Thr	7.34	4%	53.45	5.61E+07	7.65E+06
Trp	1.51	1%	9.02	9.47E+06	6.28E+06
Tyr	10.99	7%	38.69	4.06E+07	3.70E+06
Val	10.55	6%	52.85	5.55E+07	5.26E+06

4. Economics

The economic analysis in this section will be predicated on the following assumptions:

1. Total yearly meat demand is 100 MT [6])
    a. This is the typically used production volume in the existing TEAs (Humbird, Risner, …) and this is ~0.14% of the annual global beef production.
2. The media needed to meet this demand is $1.05*10^9$ L
    a. Based on internal work performed for cultivated meat TEA with 100 MT production.
3. Media supplementation is based off the amino acid composition of Essential 8™ with a basal media of DMEM/F12
    a. Essential8 is chosen because it is a serum free media, and its amino acid composition is known. The industry is moving towards serum free media formulations and consequently this choice aligns with the future trends of the industry.
4. The *L. minor* sale price of $1.87/kg dry weight is taken as an average of the bulk sale price of pea, soy, and wheat protein hydrolysates from the website Alibaba. These hydrolysates were chosen because they are currently some of the most popular plant proteins used in alternative protein applications.

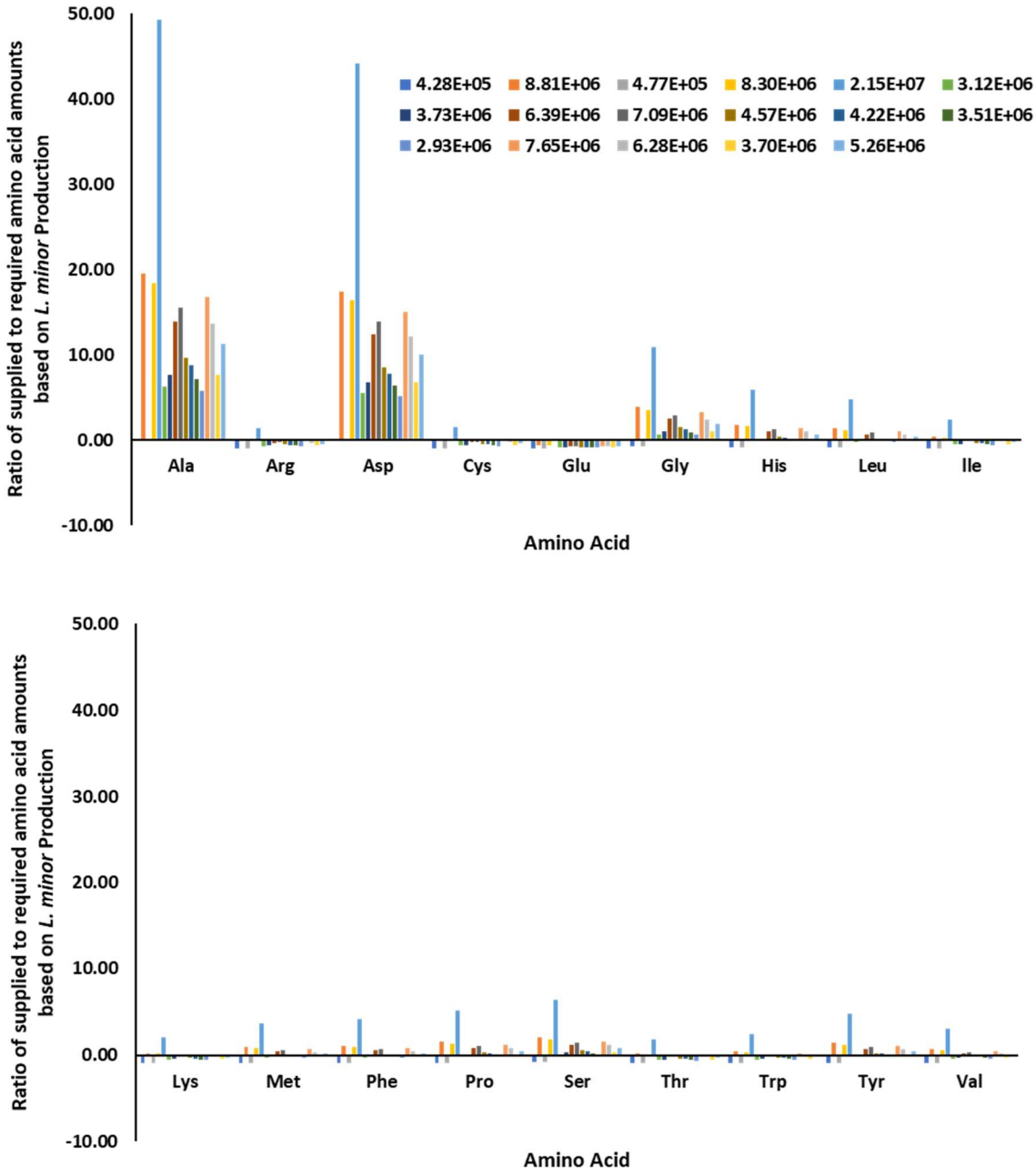
The base media cost of DMEM/F12 is determined by taking the total yearly required amino acid amount and multiplying it by the cost of commercial production taken from Humbird's TEA analysis. The breakdown of costs per amino acid stream is summarized in **Table 2**. Using the assumptions listed above, the cost to produce $1.05*10^9$ L of DMEM/F12 is $65,404,428.77.

Table 2 | A comparison of relative amino acid content present in *L. minor* and the chemically defined basal medium, DMEM/F12. The most obvious mismatch occurs with alanine, asparagine, and glutamine. Using the hydrolysate to match alanine or asparagine requirement creates a severe oversupply of the other amino acids, and doing this for glutamine creates a severe undersupply of all other amino acids.

Amino Acid	Total required for Yearly Production (g)	AA Cost ($) / kg for commerical production	Yearly Cost for Trad. Commercial Prod. ($)
Ala	4.67E+06	$71.00	$331,747.50
Arg	9.69E+07	$70.00	$6,782,203.79
Asp	6.98E+06	$85.00	$593,512.50
Cys	1.27E+07	$137.00	$1,738,925.49
Glu	3.86E+08	$40.00	$15,435,000.00
Gly	1.97E+07	$87.00	$1,712,812.50
His	2.44E+07	$118.00	$2,878,846.00
Leu	6.20E+07	$63.00	$3,906,157.50
Ile	5.72E+07	$83.00	$4,747,060.50
Lys	7.64E+07	$62.00	$4,739,315.57
Met	1.81E+07	$156.00	$2,823,912.00
Phe	3.73E+07	$85.00	$3,166,590.00
Pro	1.81E+07	$90.00	$1,630,125.00
Ser	2.76E+07	$74.00	$2,039,625.00
Thr	5.61E+07	$76.00	$4,265,310.00
Trp	9.47E+06	$146.00	$1,382,766.00
Tyr	4.06E+07	$81.00	$3,290,551.91
Val	5.55E+07	$71.00	$3,939,967.50

By utilizing *L. minor* hydrolysate, the cost of DMEM/F12 can be reduced by $4,763,726.26 which is a cost reduction of 7.28%. The supplementation amount is restricted off the basis of maintaining the final amino acid composition of the DMEM/F12 and not over supplementing any other amino acids, however if this limitation is relaxed, the amount of *L. minor* that can be added to the formulation can be increased. With increased supplementation amounts, the cost savings can be increased up to 61.38%. In the scenarios explored and summarized in **Table 3** and **Figure 3**, there are cases where Ala is over supplemented by almost a factor of 50. This specific scenario is likely unfeasible; however, it indicates that with additional processing of the hydrolysate to remove over supplemented amino acids, there is potential to realize additional cost savings to using duckweed hydrolysates that have not been explored in this report.

Figure 3 | Various production volumes in kg of duckweed (indicated in the legend) to satisfy single amino acid requirements with duckweed hydrolysate. Given the differences in the amino acid composition of duckweed hydrolysate and DEME/F12, majority of scenarios tested over supplement alanine and asparagine by almost 10x. The bottom image is a continuation of the top image to display all the amino acids.



The three amino acids with the largest relative differences based on each respective concentrations are alanine, asparagine, and glutamine (**Figure 4**). Thus, in processing, it would be helpful to remove alanine and asparagine from the hydrolysate to get a profile that is much closer to what is seen in DMEM/F12. Additionally, the cost of production for glutamine using traditional commercial methods is the cheapest of all amino acid production streams. Thus, although there is a large discrepancy in the glutamine content between the hydrolysate and DMEM/F12, the supplementation cost would likely not outweigh the added cost savings of using duckweed hydrolysates.

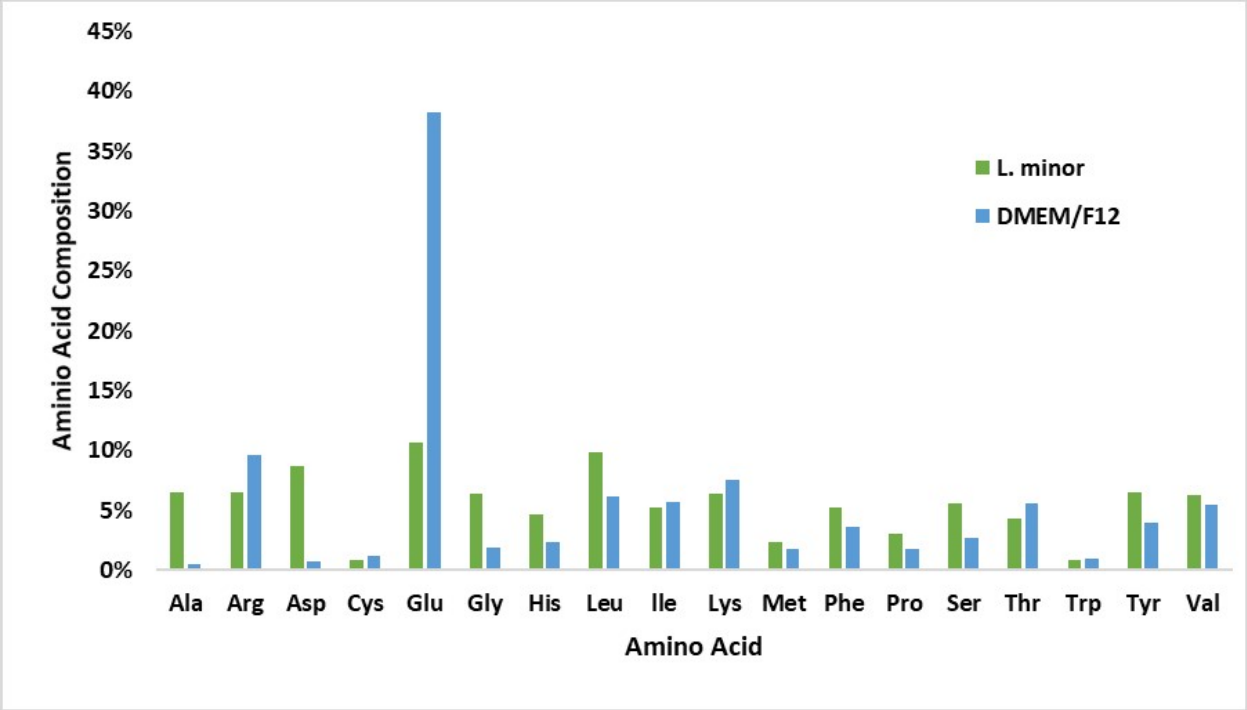


Figure 4 | Relative amino acid content present in *L. minor* hydrolysate and DMEM/F12. Disparities between alanine, asparagine, and glutamine lead to restrictions in the amount of hydrolysate that can eventually be supplemented into culture media without modifications.

Table 3 | Comparison of the cost savings using *L. minor* hydrolysate that meets 100% supplementation requirements of a single amino acid. All scenarios except for alanine (Ala) results in the over supplementation of an amino acids with the largest over supplementation approaching 50x of what is required in DMEM/F12.

Amino Acid	*L. minor* Production Requirement to Meet Demand (kg)	Cost of Production ($)	Additional Supplementation Cost ($)	Cost savings	Percent Savings
Ala	4.28E+05	$ 799,461.04	$59,841,241.47	$ 4,763,726.26	7.28%
Arg	8.81E+06	$ 16,450,978.53	$9,114,722.06	$ 39,838,728.18	60.91%
Asp	4.77E+05	$ 890,069.52	$59,248,326.01	$ 5,266,033.24	8.05%
Cys	8.30E+06	$ 15,492,456.75	$9,878,142.55	$ 40,033,829.47	61.21%
Glu	2.15E+07	$ 40,175,583.42	$0.00	$ 25,228,845.35	38.57%
Gly	1.81E+06	$ 3,373,029.53	$44,656,237.24	$ 17,375,162.00	26.57%
His	3.12E+06	$ 5,816,785.35	$31,662,306.51	$ 27,925,336.91	42.70%
Leu	3.73E+06	$ 6,967,488.70	$26,615,682.50	$ 31,821,257.57	48.65%
Ile	6.39E+06	$ 11,933,759.21	$14,276,774.81	$ 39,193,894.75	59.93%
Lys	7.09E+06	$ 13,229,406.49	$12,248,256.61	$ 39,926,765.68	61.05%
Met	4.57E+06	$ 8,532,390.63	$21,847,288.15	$ 35,024,749.99	53.55%
Phe	4.22E+06	$ 7,876,095.07	$23,693,875.07	$ 33,834,458.64	51.73%
Pro	3.51E+06	$ 6,555,565.25	$28,336,521.18	$ 30,512,342.34	46.65%
Ser	2.93E+06	$ 5,476,258.90	$33,363,797.05	$ 26,564,372.82	40.62%
Thr	7.65E+06	$ 14,279,561.10	$10,980,300.15	$ 40,144,567.53	61.38%
Trp	6.28E+06	$ 11,727,884.84	$14,680,993.70	$ 38,995,550.24	59.62%

Tyr	3.70E+06	$ 6,902,257.23	$26,862,017.98	$ 31,640,153.56	48.38%
Val	5.26E+06	$ 9,816,577.66	$18,659,052.88	$ 36,928,798.22	56.46%

5. Conclusion

This body of work has identified the feasibility of using a Flavourzyme enzymatic process to produce a duckweed hydrolysate to supplement the amino acids used in the formulation of DMEM/F12 which is the basal media in Essential 8^{TM}. A cost savings of 7.28% is achieved while maintaining the final concentrations of amino acids in DMEM/F12 media. Because of the discrepancies in the ratio of alanine and asparagine as shown in **Figure 4**, work to further remove these excess components and refine duckweed hydrolysates can lead to higher cost savings. In a promising scenario investigated in **Table 3** a theoretical cost savings of 61.38% is achieved, however this would require the removal of excess amino acids from the duckweed hydrolysate to be feasible. The amount of excess alanine in this scenario is 16x more than what is required. Next steps in this work would be to culture bovine cells at the shake flask scale on media supplemented with duckweed hydrolysate and observe to see if there are any abnormalities in growth rate, morphology, and viability of these cultures to provide a proof of concept for further large-scale studies.**Table 3 |** Comparison of the cost savings using *L. minor* hydrolysate that meets 100% supplementation requirements of a single amino acid. All scenarios except for alanine (Ala) results in the over supplementation of an amino acids with the largest over supplementation approaching 50x of what is required in DMEM/F12.

5.1 Circular economy applications of duckweed

Natural Resources Institute Finland and a Finnish company called Kalankasvatus Vääräniemi utilized duckweed in in its aquaculture operations. Duckweed sequesters waste ammonia from fish metabolism and lowers energy demands compared to conventional industrial filtration systems[23]. A similar project that aims to integrate duckweed into a broader agrarian circular economy is “BRAINWAVES”[24]. The project aims to design a stacked phytoponic flow

system that integrates compact bioreactor processes with larger outdoor cultivation systems. The intent is to have a working system easily deployable in farming regions to reduce the instances of water pollution due to excess fertilizers that can cause contamination and eutrophication in lakes, rivers and other water bodies. Another EU funded program for usage of duckweed in circular economy applications is the "Lemna Project" which focuses on the usage of 48 strains from across the Iberian Peninsula. The project showed promise in feeding agricultural digestate as a main source of nutrients for duckweed, resulting in the removal of 2.7 tons of nitrogen per hectare and 1.2 tons of phosphorus per hectare per year with 17 tons of dry weight duckweed[25]. Additionally, extracting the starch and fat content from duckweed prior to extracting amino acids could allow a co-existing biofuel production to exist, furthering the commercial viability of the operation. A study published in 2015 compared the possible ethanol yield from duckweed to corn kernel derived starch (B73), and the experiment showed that the ethanol yield from duckweed was a comparable 12 grams per 100 grams of dry weight compared to the 33.7 grams of ethanol per 100 grams of dry weight in B73. The project aims at designing a stacked phytoponic flow system that integrates compact bioreactor processes with larger outdoor cultivation systems. The intent is to have a working system easily deployable in farming regions to reduce the instances of water pollution due to excess fertilizers that can cause contamination and eutrophication in lakes, rivers and other water bodies. Another EU funded program for usage of duckweed in circular economy applications is the "Lemna Project" which focused on the usage of 48 strains from across the Iberian Peninsula. The project showed promise in feeding agricultural digestate as a main source of nutrients for duckweed, resulting in the removal of 2.7 tons of nitrogen per hectare and 1.2 tons of phosphorus per hectare per year with 17 tons of dry weight duckweed [27]. The integration of aquaculture-based duckweed with existing food production systems may bring other viable resources that may further

progress the growing cultivated meat industry. Specifically, other waste items from already existing aquaculture systems could be integrated, including Chitosan from crustacean exoskeletons that could be used as a scaffolding biopolymer laboratory cultivation of meat cells. Additionally, extracting the starch and fat content from duckweed prior to extracting amino acids could allow a co-existing biofuel production to exist, furthering the commercial viability of the operation. A study published in 2015 compared the possible ethanol yield from duckweed to corn kernel derived starch (B73), and the experiment showed that the ethanol yield from duckweed was a comparable 12 grams per 100 grams of dry weight compared to the 33.7 grams of ethanol per 100 grams of dry weight in B73 [5]..

5.2 Growth Experiment

A more direct way to integrate duckweed into the circular economy would be to utilize a waste stream directly from the cultured meat facility. An option would be to utilize spent animal cell media i.e. media that has been depleted of nutrients and exchanged and use it as a base medium to grow duckweed, which could then be converted into plant hydrolysate and fed back into the protein production system. A small-scale experiment measuring the growth rate of *L. minor* in spent animal cell media comparing the growth of plants in standard Schneck and Hildebrandt (SH) media would help to determine the viability of this potential integration. Assuming the cell media will act as a growth inhibitor, the protocol outlined in Test No. 221[26] by the Organisation for Economic Co-operation and Development outlines a good experimental setup. Following this one could also determine what percentage of spent cell media to fresh SH media produces an appreciable amount of duckweed. The additional space required to cultivate duckweed on site or transit costs of moving spent cell medium to cultivation sites could be high. However, we believe

it is still something worth looking into as the highest long term costs of a cultured meat production facility are related to supplying nutritious medium to the cells.

References

1. FAO. FAO's Director-General on How to Feed the World in 2050. *Popul. Dev. Rev.* **35**, 837–839 (2009).
2. Intergovernmental Panel on Climate Change. *Climate change 2014 mitigation of climate change*. (Cambridge University Press, 2014). doi:10.1017/CBO9781107415416.
3. Tackling climate change through livestock // FAO's Animal Production and Health Division Tackling climate change through livestock // FAO's Animal Production and Health Division. http://www.fao.org/ag/againfo/resources/en/publications/tackling_climate_change/index.htm.
4. Byrne, B. & Murray, S. *2020 State of the Industry Report Cultivated Meat*. https://gfi.org/wp-content/uploads/2021/04/COR-SOTIR-Cultivated-Meat-2021-0429.pdf (2021).
5. Risner, D. *et al.* Preliminary Techno-Economic Assessment of Animal Cell-Based Meat. *Foods* **10**, (2020).
6. Humbird, D. Scale-up economics for cultured meat. *Biotechnol. Bioeng.* **118**, 3239–3250 (2021).
7. Ho, Y. Y. *et al.* Applications and analysis of hydrolysates in animal cell culture. *Bioresour. Bioprocess.* **8**, 93 (2021).
8. *Protein hydrolysates in biotechnology*. (Springer Netherlands, 2010). doi:10.1007/978-1-4020-6674-0.
9. Watson, E. Parabel USA unveils neutral-tasting, beige-colored protein hydrolysate from water lentils. *Food Navigator-usa* (2019).

10. Bog, M. *et al.* Genetic characterization and barcoding of taxa in the genus Wolffia Horkel ex Schleid. (Lemnaceae) as revealed by two plastidic markers and amplified fragment length polymorphism (AFLP). *Planta* **237**, 1–13 (2013).

11. Klaus, J. A., Nikolai, B. & Eric, L. Telling duckweed apart: genotyping technologies for the lemnaceae. *Chinese Journal of Appplied Environmental Biology* **19**, 1–10 (2013).

12. Ziegler, P., Adelmann, K., Zimmer, S., Schmidt, C. & Appenroth, K. J. Relative in vitro growth rates of duckweeds (Lemnaceae) - the most rapidly growing higher plants. *Plant Biol (Stuttg)* **17 Suppl 1**, 33–41 (2015).

13. Sree, K. S., Sudakaran, S. & Appenroth, K.-J. How fast can angiosperms grow? Species and clonal diversity of growth rates in the genus Wolffia (Lemnaceae). *Acta Physiol. Plant.* **37**, 204 (2015).

14. Yang, G.-L. *et al.* Research progress of a potential bioreactor: duckweed. *Biomolecules* **11**, (2021).

15. Liu, Y., Xu, H., Yu, C. & Zhou, G. Multifaceted roles of duckweed in aquatic phytoremediation and bioproducts synthesis. *Glob. Change Biol. Bioenergy* **13**, 70–82 (2021).

16. Appenroth, K.-J. *et al.* Nutritional value of duckweeds (Lemnaceae) as human food. *Food Chem.* **217**, 266–273 (2017).

17. Baek, G., Saeed, M. & Choi, H.-K. Duckweeds: their utilization, metabolites and cultivation. *Appl. Biol. Chem.* **64**, 73 (2021).

18. Sree, K. S. *et al.* Duckweed for Human Nutrition: No Cytotoxic and No Anti-Proliferative Effects on Human Cell Lines. *Plant Foods Hum. Nutr.* **74**, 223–224 (2019).

19. Chakrabarti, R. *et al.* Mass Production of Lemna minor and Its Amino Acid and Fatty Acid Profiles. *Front. Chem.* **6**, 479 (2018).

20. Coughlan, N. E. *et al.* Duckweed bioreactors: Challenges and opportunities for large-scale indoor cultivation of Lemnaceae. *J. Clean. Prod.* **336**, 130285 (2022).

21. Novozyme. Flavourzyme | Novozymes. https://biosolutions.novozymes.com/en/animal-protein/products/flavourzyme.

22. Tran, H. C., Le, H. A. T., Le, T. T. & Phan, V. M. Effects of Enzyme Types and Extraction Conditions on Protein Recovery and Antioxidant Properties of Hydrolysed Proteins Derived from Defatted Lemna minor. *j.asep* (2021) doi:10.14416/j.asep.2021.05.003.

23. Laajala, P. Circular economy at its finest. *Luke Natural Resources Institute Finland* (2018).

24. University College Cork. A plant that can help clean farm waste? Unbe-leaf-able. *News | /iniversity College Cork* https://www.ucc.ie/en/bees/news/a-plant-that-can-help-clean-farm-waste-unbe-leaf-able.html (2021).

25. Vidal, A. P. *Layman's Report 2020*. (2020).

26. Development Organisation for Economic Co-operation and. Test No. 221: Lemna Sp. Growth Inhibition Test. *Test No. 221: Lemna Sp. Growth Inhibition Test* (2006).