4 Protein

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Abstract

Proteins are the building block of the human body. It is recommended to consume 0.8 g of protein per kg of body weight. Quality is equally important: all essential amino acids must be consumed daily. While animal foods (dairy, eggs, fish and meat) offer complete proteins, with high digestibility, their water and carbon footprints present a serious challenge to the planet. Plant foods are more sustainable, yet often incomplete in their amino acidic profile (with cereals low in lysine and legumes low in methionine, for example). Consuming a variety of plant-based protein guarantees access to all essential amino acids. Insects and algae are an area of current interest, although consumer scepticism is present due to unusual looks, taste and challenging logistics (insect farming, algae production). Finally, biotechnology has been implemented to develop mycoprotein and other fermented foods. This could result in high levels of complete protein with low environmental impact.

Keywords

Biotechnology, fish · Footprint · Meat · Plant protein · Protein protein for human nutrition

Protein are essential in human diet. They are the building block of human tissues such as muscles, bones, skin, hair, nails. In addition, they contribute to energy with 3.5 kcal/g, comparably to carbohydrates. Moreover, studies proved the role of specific dietary protein in modulating bone health, cardiovascular disease and diabetes (Qi & Shen, 2020; Shams-White et al., 2017; Tian et al., 2017). Protein are constituted of combinations of up to 20 amino acids, 9 of which are essential, meaning they cannot be synthesised by the human body: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (Wu, 2016).

The recommended daily intake is approximately 0.8 g protein/kg body weight (Bilsborough & Mann, 2006; Wu, 2016). This number varies based on age, gender and level of physical activity, reaching 1.6 g protein/kg body weight in adults who exercise intensely (Wu, 2016). Sources of protein are numerous: meat (red, white), seafood (fish, shellfish), plant (seeds,

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nuts, grains and even fruits and vegetables) and insects. Quantity is just as important as quality. Bioavailability refers to the body's ability to digest, absorb and metabolize a certain nutrient or supplement. In terms of protein, bioavailability is measured using a Protein Digestibility Corrected Amino Acid Score (PDCAAS) as a tool to show protein quality. The PDCAAS is a value scored from 0.0 to 1.0 that calculates limiting amino acid score multiplied by protein digestibility (FAO, 2011). For example, cereals like rice and wheat are typically limited in the amount of lysine, whereas legumes like beans and chickpeas are usually low in methionine, thus resulting in low PDCAAS (0.4–0.6). High PDCAAS scores (close to 1.0) are typical of animal protein, with a few exceptions in the plant kingdom (soy and buckwheat for example) (Joye, 2019).

It is important to mention that different sources of protein also delivers a "package" of other nutrients: for example, saturated fats and cholesterol are found in red meat, hormones and vitamins in dairy and eggs, unsaturated fats in fish, phytochemical compounds and antinutrients in grains and mushrooms. It is the matrix that determines protein quality. Finally, the environmental impact must be considered. It is known that the production of animal protein requires more land, water and emits more carbon dioxide (CO_2) than plant protein (Moughan, 2021).

Therefore, this chapter will compare different sources of protein, traditional and innovative, for their protein content and quality, as well as for their environmental impact and consumer acceptability. An example of the modern trajectory of protein-rich food products is depicted in Fig. 4.1.

4.1 Traditional Food Sources of Protein

Protein are found in numerous sources of animal and plant origin. Table 4.1 offers a representative summary of the highest sources of dietary protein. As it can be seen, and perhaps contrary to popular belief, several options are available for those looking to obtain protein. Dairy and meat are the top sources, in terms of quantity: from 25.2 to 33.3 g/100 g of protein in chicken and skim milk powder, respectively (Food Data Central, 2021; NZ Food Composition Data, 2021). Other sources include seeds, nuts and eggs: 7.5–22.4 g/100 g of protein (Food Data Central, 2021; NZ Food Composition Data, 2021). What separates these foods is their environmental impact and consumer acceptability.

Skim milk powder is produced from cow's milk where fat and water have been removed, this increases storage time and allows for lower shipping requirements. Skim milk powder requires the highest resource use of all the proteins in this case study, this is because water is needed to grow the pasture, process the milk, and clean the equipment, dairy production is also heavy in land use and degradation if not carefully managed.. Skim milk powder contains all the essential amino acids and the absorption and bioavailability of the amino acids is very high as there are no anti-nutritive factors in milk (van Lieshout et al., 2020). The cooked flavour of skim milk powder may come from the drying process. The high level of protein and some sugars causes the Maillard reaction, the other flavours are typical of that of milk. However, astringency may be related to a textural defect (Lemieux & Simard, 1994). The price is moderate (1.20 NZD/100 g (Countdown, 2021) thus making it accessible to most consumers.

A more common dairy product is cheese. Let's look, for example, at Parmesan cheese. Approximately 14 litres of milk are needed to produce a kilogram of parmesan; and milk is a resource that takes over six-hundred litres of water to produce one single litre; approximately 5,000 L of water would be needed to produce 1 kilogram of parmesan (Mekonnen & Hoekstra, 2011). Depending on what milk is used, and the ageing process, the total amount of protein per hundred grams varies, with an average value of 32.6 grams of protein per 100 grams (NZ Food Composition Data, 2021). Milk is the main source of protein in cheese, so it is high in all essential amino acids like tyrosine, valine, and especially lysine. Furthermore, these amino acids can be assimilated efficiently as they are hydrolyzed by proteolytic enzymes into peptones, pep-

Fig. 4.1 Representative sources of protein: traditional (beef steak) and innovative (peas)

tides and free amino acids during the ageing process (Summer et al., 2017). Parmesan cheese is known for its piquant flavour, dry, crumbly texture, and strong aroma, and salty aftertaste. This cheese is often used to complete Italian inspired dishes or on a charcuterie board (Loffi et al., 2021). The price bracket varies for Parmesan cheese. For instance, within the Countdown line of supermarkets across New Zealand, the price ranges from $5/100 g to $6.20/100 g (Countdown, 2021). Big factors that contribute to the cost is ageing of cheese and bacteria used. The longer it takes to age, the higher the cost of production. Only certain strains of bacteria can be used to make Parmesan.

When thinking of protein, most people think of meat, particularly read meat such as beef. Its protein content is high, up to 30 g/100 g (NZ Food Composition Data, 2021), and of excellent quality (PDCAAS 1.0) (Ertl et al., 2016). Most consumers appreciate the umami flavour and the tender, juicy texture (Legako et al., 2016). The price is high, anywhere from 2 to 6 NZD/100 g based on the quality (Countdon, 2021). The problem is the environmental weight of such food. The amount of water required is massive: 15,712 L/kg beef (Gerbens-Leenes et al., 2013). This is due to the high demand of cows, which results in large use of plants and water to sustain their growth. In addition, cow farming for meat production causes large production of CO_2: as large as 24 kg CO_2 per kg beef (Vitali et al., 2018). To put it in perspective, dry beans are responsible for production of only 2.0 kg CO_2/kg product (Rahmadi et al., 2021), that is 12 times lower, while delivering comparable protein content of moderate quality (PDCAAS 0.75) (Hoffman & Falvo, 2004). The reason is that plants are digested by cows, their nutrient partially accumulated in the meat and partially excreted via feces and urine. The process is slow, due to cows being ruminants, thus processing foods through four stomachs. In addition, because of forage digestion, cows' metabolism releases methane at variable quantities based on their body weight (Van Lingen et al., 2019). Dietary strategies, such as the introduction of higher quantities of digestible grass and replacement of traditional forage with corn silage, have been trialed to mitigate methane emissions (Van Gastelen et al., 2019). Nonetheless, results were not sufficient.

Tofu is produced from coagulated soybean beverage that is subsequently pressed, this

Table 4.1 Representative food sources of protein: products, nutritional value (quantity, PDCAAS), sustainability (water and carbon footprint) and acceptability (price, taste)

	Nutrition		Sustainability		Taste	
Food products	Protein quantity (g/100 g)	PDCAAS[a]	Water Footprint (L water/kg product)	Carbon Footprint (kg CO_2/kg product)	Price (NZD/100 g)	Sensory profile
Skim Milk powder	33.3 Food Data Central (2021)	1.00 Chalupa-Krebzdak et al. (2018)	4,745 Mekonnen and Hoekstra (2011)	9.0 Flysjö et al. (2014)	1.20 Countdown (2021)	Milky, sweet, cooked Cheng et al. (2020)
Pumpkin seeds, roasted	32.9 NZ Food Composition Data (2021)	0.97 ESHA Docs (2021)	336 Mekonnen and Hoekstra (2011)	0.14 Schäfer and Blanke (2012)	*2.49* Countdown (2021)	Dark green, hard, nutty taste and aroma Uddin et al. (2016)
Parmesan cheese	32.6 NZ Food Composition Data (2021)	1.00 Summer et al. (2017)	5,000 Mekonnen and Hoekstra (2011)	10.3–16.9 (Grana Padano) Canellada et al. (2018)	*9.00* Countdown (2021)	Light yellow colour, butter aroma, nut smell, salty, pungent, friable Loffi et al. (2021)
Beef steak	29.9 NZ Food Composition Data (2021)	1.00 Ertl et al. (2016)	15,712 Gerbens-Leenes et al. (2013)	24 Vitali et al. (2018)	1.99–5.99 Countdown (2021)	Tender, brown, umami Legako et al. (2016)
Tuna, canned	26.8 NZ Food Composition Data (2021)	1.00, Boye et al. (2012)	Not available	6.1 Rahmadi et al. (2021)	2.31 Countdown (2021)	Fishy, oily, hard, salty, rancid Caponio et al. (2010)
Chicken breast, roasted	25.2 NZ Food Composition Data (2021)	1.00 Burd et al. (2019)	2,872 Gerbens-Leenes et al. (2013)	6.9 Rahmadi et al. (2021)	1.09 Countdown (2021)	Juicy, chewy, chickeny Zhuang and Savage (2010)
Peanut butter	22.4 NZ Food Composition Data (2021)	0.70 Arya et al. (2016)	3,740 Vanham et al. (2020)	2.5 Rahmadi et al. (2021)	1.47–1.66 Countdown (2021)	Brown, glossy, roasted/peanutty, sweet, oily, adhesive, grainy Riveros et al. (2010)
Almonds	20.1 NZ Food Data Composition (2021)	0.44–0.48 House et al. (2019)	13,080 Vanham et al. (2020)	2.6 Volpe et al. (2015)	3.10–3.57 Countdown (2021)	Fruity (150 °C) Nutty (170 °C) Burnt, roasted (190 °C) Lipan et al. (2020)
Eggs, boiled	12.2 NZ Food Composition Data (2021)	1.00 Matsuoka et al. (2019)	3,265 Mekonnen and Hoekstra (2012)	4.8 Rahmadi et al. (2021)	0.70 Countdown (2021)	White (albumen), yellow/orange (yolk), sulphury Yimenu et al. (2017)
Tofu	10.6 NZ Food Composition Data (2021)	1.00 DePalma et al. (2019)	926 Usman (2011)	2.0 Rahmadi et al. (2021)	0.97 Countdown (2021)	Light grey colour, sweet and fermented aroma, sweet/bitter/astringent flavour, firm and elastic texture Kamizake et al. (2018)

(continued)

Table 4.1 (continued)

	Nutrition		Sustainability		Taste	
Food products	Protein quantity (g/100 g)	PDCAAS[a]	Water Footprint (L water/kg product)	Carbon Footprint (kg CO_2/kg product)	Price (NZD/100 g)	Sensory profile
Greek Yoghurt	9.5 NZ Food Composition Data (2021)	0.95 (whey) ESHA Docs (2021)	672 Vasilaki et al. (2016)	4.5–6.8 Houssard et al. (2020)	0.47–1.50 Countdown (2021)	Fatty, sour, velvety, grainy, smooth Megalemou et al. (2017)
Beans, canned (*Phaseolus vulgaris*)	7.5 NZ Food Composition Data (2021)	0.75 Hoffman and Falvo (2004)	5,053 Mekonnen and Hoekstra (2011)	2.0 Rahmadi et al. (2021)	0.26 Countdown (2021)	Beany, boiled potato, earthy, smoky, sulphury Mishra et al. (2017)

[a]Protein Digestibility Corrected Amino Acid Score

makes soybean the main component that confer tofu its protein. Unlike other plant material, tofu offers a complete source of protein, with a PDCAAS of 1.00 (DePalma et al., 2019), delivering all essential amino acids in high amounts with high digestibility. Much like peas, soybeans are a legume, which means that they can fix their own nitrogen, thus reducing the need for fertiliser. The water requirements are much lower in comparison to milk powder: 926 vs. 4,745 L water/kg product (Mekonnen & Hoekstra, 2011), accounting for harvesting of soybeans and processing into tofu. Similarly, the carbon footprint is very low. The tofu production results in only 2.0 kg of CO_2 emission as opposed to the 9.0 kg CO_2 released by the production of skim milk powder (Flysjö et al., 2014; Rahmadi et al., 2021). The large differences include factors such as pasture growth, cows' diet and maintenance, milk processing and drying. The top five descriptive factors were obtained from a study by Chung and collaborators (2008): beany flavour comes from soybeans, astringency from the tannins and other plant compounds, hardness and roughness from the pressing time, and the saltiness is most likely from the tofu being stored in brine. Due to tofu being historical and heritage driven food, coupled with soybeans being cheap and easy to produce, it means that the price can be significantly lower (0.97 NZD/100 g) (Countdown, 2021) than skim milk powder.

As expected, meat and fish offer large quantities of high-quality protein: 25–30 g/100 g with a PDCAAS score of 1.00 (Table 4.1). The limiting factor is footprint, with water needs in the order of 3–17 times larger than that of plant-based foods. While chicken requires 3 times the amount of water of tofu (2,872 L water for each kg of meat processed), beef reaches the impressive number of 15,712, meaning 17 times more water than tofu (Gerbens-Leenes et al., 2013). This is due to the fact the animals consume plants, such as soya, and later convert it into meat, eggs, and dairy. Therefore, animal-based foods will always require more water than the plant-based counterparts. What is astonishing, is the difference in emissions. For example, the carbon footprint of meat, fish, dairy, and eggs ranges from 4.8 to 24 kg CO_2/kg product (eggs and beef, respectively). This again, is due to the conversion of plant material into meat. These extra steps produce high quality protein but at a cost of the environment. A lower impact choice, within the animal reign, is eggs: on average, 3,265 l water are needed per kg of eggs (Mekonnen & Hoekstra, 2012), producing 4.8 kg CO_2 (Rahmadi et al., 2021). These numbers are moderately high, but closer to those of plant-based foods. Reason laying in chicken's quick conversion of food (they are not ruminants) and abundant production of eggs. In comparison only 0.14–2.6 kg CO_2/kg product are the result of industries producing pumpkin seeds and almonds (Table 4.1).

Almonds look as the least sustainable option among plant-based foods, and not just because of the high footprint (high amounts of water required), but also because of their low protein quality, reported in the range of 0.44–0.48

PDCAAS (House et al., 2019) thus making them a good choice as food in general (energy, fibre, lipids, protein content) but not as source of highly digestible protein.

In this regard, a special mention goes to pumpkin seeds. In the list provided, they are the second highest source of protein: 32.9 g protein/100 g pumpkin seeds (NZ Food Composition Data, 2021). This is common to most nuts and seeds. What is interesting, is the high protein quality: PDCAAS 0.97 (ESHA Docs, 2021). This means that pumpkin protein delivers high levels of all essential amino acids (Vinayashree & Vasu, 2021). Therefore, pumpkin seeds is a potential powerhouse of nutrition. In addition, the environmental footprint of their harvesting and processing is extremely low, even lower than for legumes and nuts: only 336 L water/kg product (Mekonnen & Hoekstra, 2011) and as little as 0.14 kg CO_2 produced for each kg of seeds (Schäfer & Blanke, 2012). This can be explained by the fact the pumpkin seeds are found in a vegetable, pumpkin, which is the actual food product. In some ways, pumpkin seeds can be considered as a by-product of the pumpkin industry. They are found plentiful in pumpkins and contain low levels of moisture. Therefore, processing is minimal, mostly roasting to reduce moisture content and remove any bitterness (Uddin et al., 2016). What is even more fascinating is the versatile functionality of pumpkin protein. It has been shown that pumpkin protein is soluble at mild acidic pH, typical of most foods, comparably to soy protein. Furthermore, pumpkin protein exerts moderate foaming, emulsifying and water absorption properties at high level, similar to those of pea, soy and wheat protein (Vinayashree & Vasu, 2021). The only challenge is sensory: can pumpkin seeds be consumed in similar amounts to dairy, meat, eggs and legumes? Is it feasible to imagine people consuming hundreds of grams of roasted pumpkin seeds? Probably not, unless food innovation were to provide a way to make it more palatable, such as the example of peanut butter, which made peanut consumption easier (Riveros et al., 2010).

4.2 Innovative Food Sources of Protein

As discussed in Sect. 4.2, the main challenges with protein-rich foods are represented by their environmental impact (meat, fish, dairy, eggs), taste (seeds, legumes) and price (beef, nuts). Sustainable food innovation should reduce footprint and price while increasing sensory quality. Numerous options have been proposed: mycoprotein obtained from fermentation, insects, duckweed, legume protein, seaweeds and upcycled ingredients such as spent malt (Table 4.2). Let's investigate one attribute at a time: consumer acceptability, nutrition, sustainability.

4.2.1 Acceptability

Plant-based meals have been a trend that has been booming for a while, especially now with climate change and sustainability also being addressed. Plant-based meals is a global trend and an expected Compound Annual Growth Rate (CAGR) of 7.8% (Associated Press, 2020). Mycoprotein is the raw material for Quorn products. Quorn is a brand that was founded in 1985 and produces a variety of vegetarian and vegan products, with staples such as nuggets and burgers. Mycoprotein is a single-celled protein, derived from fungi, for human consumption (Finnigan et al., 2017). Aerobic fermentation of fungal spores (typically *Fusarium venenatum*) is fermented with glucose and nitrogen. Depending on the type of production, spent grains can be used as a source of glucose and ammonia for nitrogen (Zeece, 2020); this is a great way of recycling food waste. The protein quality is excellent, reaching a PDCAAS score of 0.99 (Finnigan et al., 2017). For a product to be successful, it must appeal to customers in terms of sensory and price. For example, Quorn mince (a vegan alternative to beef mince) is priced at \$2.83/100 g. On the other hand, the average price of premium beef mince from Countdown is \$2.57/100 g (Countdown, 2021). This price difference is insignificant, meaning the median earning consumer would have access to this

Table 4.2 Innovative food sources of protein: raw materials, bioavailability (PDCAAS) and sustainability (water and carbon footprint)

Products	Raw materials	Bioavailability	Sustainability	
		PDCAAS[a]	Water footprint (L water/kg product)	Carbon footprint (kg CO_2/kg product)
Mycoprotein	Mycoprotein	0.99 Finnigan et al. (2017)	500 Smetana et al. (2018)	1.14 Smetana et al. (2018)
Insect flour	Crickets	0.91 Halloran et al. (2017)	420 Halloran et al. (2017)	2.57 Halloran et al. (2017)
Duckweed	Lemenaceae	0.89 Kaplan et al. (2019)	Not available	−3.0 Duckweed absorbs three times the volume produced of CO_2 Mohedano et al. (2019)
Pea protein	Peas	0.68–0.71 Nosworthy et al., 2017	595 Mekonnen and Hoekstra (2011)	0.49 Nette et al. (2016)
Seaweed	Microalgae	0.64 Wang et al. (2020)	960 Martins et al. (2018)	1.72 Martins et al. (2018)
Spent grain Bar	Spent barley malt	0.61 Nitrayová et al. (2018)	1,423 Mekonnen and Hoekstra (2011)	0.29–1.74 Cimini and Moresi (2016); Mussatto et al. (2013)

[a]Protein Digestibility Corrected Amino Acid Score

product. In comparison to beef mince, Quorn mince has a very mild, almost neutral flavour. The appearance resembles fried/steamed mince, however, the texture is slightly chewy. To counteract this, Quorn processes the mycoprotein into convenience products such as nuggets, burgers, and chilli mince. The only drawback is that this product is sold in supermarket freezer sections. Often this is associated with the food being not as fresh (like organic produce) and hence not as beneficial. Mycoprotein has an extremely high PDCAAS value, of approximately 0.996 (Finnigan et al., 2017). However, methionine and cystine are two of the limiting amino acids found in mycoprotein. When the protein digestibility was initially calculated, a value above 1.0 was determined. However, the data suggested that approximately 10% of the glucosamine nitrogen is possibly digested by the small intestine, as intestinal mucus contains some glucosamine. Once the mycoprotein is ready it is seasoned, mixed egg protein, or plant protein, to help bind the mix into a dough-like form. It is then steam-cooked for about 30 minutes, and chilled, before being shaped into a variety of products. Studies have shown the average digestibility of Quorn is approximately 0.91 (Schweiggert-Weisz et al., 2020). This change is minimal and could be due to the processing but is likely calculation discrepancies. Additionally, a study conducted in 2018 showed that it takes about 500 L of water to produce a kilogram of mycoprotein (Smetana et al., 2018). Furthermore, to produce 1 kg of mycoprotein, 1.14 kg CO_2/kg is produced, and 1.72 kg CO_2/kg once it is processed into Quorn mince (Harrison & Johnson, 2018).

The global insect market is expected to have a Compound Annual Growth Rate (CAGR) of 23.8% from 2018 to 2023 (Ebenebe et al., 2020). This may be due to the increasing global population and the search for alternative food sources. Insect farming and rearing are already practiced in countries such as Thailand, Singapore, and China (Amadi & Kiin-Kabari, 2016). By using the CAGR insect farming has the opportunity to provide income to otherwise economically disadvantaged countries. Because of the low labour and production costs in these countries, there would be a symbiotic economic relationship of the low production cost for importers and income for exporters, leading to good price sustainability. The main negative that this product faces are consumer perception, mainly stemming from the Western world. Insects have long been a staple in Asian and African cultures, as the product pushes into the west it may not be as accepted. Burt and collaborators (Burt et al., 2020) found that the consumer acceptance of using cricket flour as a substitute for all-purpose flour in muffins was very low, however, the sensory characteristics were improved by using cricket flour. This highlights the opportunity of cricket flour following consumer acceptance. Crickets have the lowest water requirements of the three protein sources coming in at 420 L of water per kilogram of product and 2.57 kg CO2 produced per kg of crickets (Halloran et al., 2017), this is because crickets can get most of their water from the food they eat. When invertebrates eat fresh fruit and vegetables this is usually enough to sustain their water requirements, if invertebrates require freshwater, the amount is to be so small that they do not drown in it (Inostroza et al., 2016). This is important because although the feed uses water, the fresh-water requirement is very low and therefore sustainable. Insects can transform low value or unwanted organic material into high-quality nutrient food (van Huis & Oonincx, 2017). This means that crickets can feed on food that may not be accepted by the consumer, using food that would otherwise go to waste. Cricket flours have a high PCDAAS score at 0.91, this is similar to that of beef and soy (van Vliet et al., 2015). This means that the proteins and specifically, amino acids in crickets are highly digestible. Crickets have high levels of isoleucine, leucine and valine, the limiting amino acid is tryptophan (Köhler et al., 2019). So, although crickets are a complete protein, they are limited in at least one of the essential amino acids. Another important factor to consider is the effect of processing on the protein content of the products. Cricket powders that were treated with high-temperature processing methods showed sufficient thermostability regarding protein (Montowska et al., 2019). This is important because it means the protein digestibility is not compromised through thermal processing methods.

Another interesting source of protein is duckweed, also known as water lentils. Duckweed is commonly eaten in southeastern Asian countries such as Laos, Myanmar and Thailand and it's gaining attention from researchers and industries across the world (de Beukelaar et al., 2019). Duckweed is a plant belonging to the family *Lemnaceae*, subfamilies of *Landoltia, Lemna, Pirodela, Wolfiella* and *Wolffia* (Bog et al., 2019). Its appearance is round, without roots (Kaplan et al., 2019) resembling green lentils in colour and shape, from which the name water lentils originated. It floats on the water surface of ponds and lakes, sometimes even in low current rivers. Duckweed is one of the fastest growing plants, with the unique ability to produce large quantities of nutrients, with a staggering protein concentration of 20–43% (Appenroth et al., 2017; Bog et al., 2019; de Beukelaar et al., 2019). Furthermore, duckweed protein is highly bioavailable, with a PDCAAS score of 0.89, due to high concentrations of all essential amino acids (lysine, methionine, cysteine, phenylalanine, and tyrosine, in particular) with high digestibility (Appenroth et al., 2017; Kaplan et al., 2019). Due to the limited information available on this food, no data was found on its environmental footprint. The carbon footprint of the duckweed itself can be considered as negative due to its ability to absorb carbon dioxide. Processing into food ingredients may generate carbon emissions. In terms of consumer acceptability, studies have shown high liking for duckweed, with panellists considering this plant material as a vegetable, rather than a protein source, thus increasing its acceptability in vegetable-containing meals (de Beukelaar et al., 2019). Sensory quality and the rapid production of biomass seems to indicate a promising future for duckweed farming, but assessment of its footprint is needed to evaluate its sustainability.

Pea protein is expected to show a 12% CAGR from 2021 to 2026 (Arteaga et al., 2021). Additionally, pea protein is a viable and functional protein source that contains around 70% w/w protein (Qamar et al., 2019). This is important because it shows that the pea protein extraction process is effective and proves viability and scalability. Pea protein also has good sensory characteristics apart from some bitter notes which may play a role in the way they are added to food (Arteaga et al., 2020). Careful use of pea protein additions in food should be able to mitigate these flavours and lead to full consumer sensory acceptability. Peas have a moderate water requirement: it takes 595 L of water to produce one kilogram of product. This is exponentially higher than the water requirement for cricket production. The water use for peas also may be high because the farmers producing peas need to keep the availability of water high. This is because if the pea plant becomes water-stressed during key developmental and growth stages there will be a reduction in the yield of the seed (Martin et al., 1994). It only produces 0.49 kg CO_2 per kg peas (Nette et al., 2016). Lastly, peas have a moderate PCDAAS (0.68–0.71) depending on varieties. Peas contain all the essential acids however they are not complete due to their low levels of methionine (Gorissen et al., 2018). Like grains, legumes such as peas carry anti-nutritive factors which may lead to decreased absorption of protein. An example of an amino acid inhibitor in peas are trypsin inhibitors, trypsin inhibitors work by being a competitive substrate for trypsin and reduce protein digestion, however, cooking, soaking, and processing peas help to remove these factors (Wang et al., 1998). This is an important factor to consider during processing to enhance the bioavailability of the protein in peas. The protein content of peas is not affected by thermal or high-pressure processing such as the extrusion process (Alonso et al., 2001). This aids the extraction of pea protein as it allows a range of processes to be used without compromising the amino acid profiles.

Microalgae are a unique photosynthetic organism made up of phycobiliproteins (Bleakley & Hayes, 2017). Microalgae are fermented in bioreactors and can be fed with spent grains, okara, and molasses. Once again, this is a way of recycling industrial food waste, whilst providing the microalgae with sources of carbon and nitrogen. An expected CAGR of 6.5% is estimated for microalgae, and maybe once this company goes global, more products will be available (Yahoo Finance, 2021). This novel technology can produce a white powder that is odourless and can be used as a base for plant-based milk. Whereas, the brown powder has a seaweed aroma and provides an umami flavour; this can be used as a meat replacer for seafood and chicken products. Currently, the initial price of protein flour is just over NZD 4.00/100 g; with prices expected to drop to $0.84 within 3 years, and then further dropping to $0.28 within 10 years as production scales up (Begum, 2020). Fermentation of microalgae results in a high PDCAAS of 0.81, however, the limiting amino acids in *Chlorella spp*. is histidine and isoleucine (Wang et al., 2020). Even though the amount of histidine and isoleucine is restricted, microalgae are digestible; partially because nutrients become more bioavailable after fermentation. Fermentation reduces the levels of non-nutritive compounds that inhibit digestive enzymes (e.g., trypsin and chymotrypsin inhibitors) and promote protein crosslinking (e.g., phenolic and tannin compounds), additionally production of microbial proteases partially degrades and release some of the proteins (Çabuk et al., 2018). When microalgae are processed into flours, like Sophie's Bionutrients, the cell walls are mechanically ruptured. A study conducted in 2020 investigated PDCAAS of various algae and showed that mechanically ruptured cell walls significantly improved digestibility (Wang et al., 2020). This is because the cellulose cell wall of algae cannot be digested by humans; so true protein digestibility was initially 0.64 which then increased to 0.81 once *Chlorella Sorokiniana* algae were mechanically ruptured. A study on the water footprint of growing microalgae in multi-tubular photobioreactor was conducted showing total water of approximately 0.96 m^3/kg dry biomass, which is 960 L required to produce 1 kg of dry biomass (Martins et al., 2018). Furthermore, 90% of the water can be recycled in production or can be upcycled into biofuel (Martins et al., 2018). Along with this study, a carbon footprint of the microalgae production was done in the same pilot-scale multi-tubular photobioreactor. It was discovered that a total of 1.72 kg CO_2/kg dry biomass is created (Martins et al., 2018).

Brewers spent grains are a byproduct of wort extraction from beer brewing and are rich in hydrophobic protein, fiber, and trace minerals (Ikram et al., 2017). The spent grains are seen as an opportunity to upcycle and use as an adjunct to fortify foods, leading to its rise in uses such as the ReGrained extruded snacks. Stojceska et al. (2008) found that by adding spent grains to extruded snacks the protein content could be enhanced significantly. Although barley uses a lot of water to produce these snacks, they are environmentally friendly as they are upcycled from material that would otherwise be seen as waste. This makes snacks price effective and environmentally sustainable, ReGrained itself boasting multiple certifications such as Non-GMO and Organic (Regrained, 2021). This leads to consumer acceptance; however, the sensory characteristics may need to be enhanced to gain preference. Although an older study, it was found that an increase in the addition of brewers spent grains to 15% of the extrudate deteriorated the sensory characteristics of the product (Makowska et al., 2013). This means that spent grains may deliver some negative organoleptic properties and care should be taken to negate this. Barley has a low PCDAAS score at 0.61. This means that although high amounts of protein can be put into food there may be limiting amino acids or low digestibility. In addition, soluble protein dissolve in water (to make beer), thus leaving only some protein in the spent grains. This may be due to cereals containing anti-nutritive factors such as polyphenolic tannins which bind to proteins and enzymes and in turn, reduce the bioavailability and absorption of protein (Björck et al., 2012). This is important to consider when using barley in food products specifically with nutritive protein claims. Barley has an almost complete amino acid profile with lysine being the limiting amino acid, interestingly, brewers spent grains contains high amounts of lysine and histidine and low amounts of threonine, tryptophan, and methionine (Lynch et al., 2016; Sauer et al., 1974). This implies that the thermal process of wort extraction affects the amino acid profile of barley. Lastly, barley has the highest water use for the three protein sources, it takes 1,423 L of water to produce one kilogram of product. Unlike peas, cereals like barley have a shallow rooting system and don't have the same access to the volume of water. Much like peas, however, the yield of barley is dependent on the plant not undergoing water stress. When barley becomes water-stressed, the time for ear emergence increases and in turn decreases the yield of the ear (González et al., 2008). Because of this, it is important to keep water in the soil when growing barley, and because of the shallow roots, there is a higher water use than peas.

4.3 Conclusions

In closing, protein is an essential macronutrient that can be obtained from a wide variety of foods. They support the development of healthy muscles, bones, skin and hair, while providing energy and modulating human metabolism. Quantity as well as quality are important. All nine essential amino acids should be present in a diet, not necessarily in each meal, but definitely in a daily plan. Also, the matrix is crucial. Different protein-rich foods exert different health effects on human, either positive or negative, based on the amount consumed and based on the matrix (fats, phytochemicals, hormones and so on). Traditional sources of protein include dairy (milk, milk powder, cheese and yoghurt), eggs, meat (beef, poultry), fish, legumes, seeds and nuts. Recently, consumer attention has shifted toward alternative protein such as mycoprotein, insects, duckweed, legume protein, algae and upcycled ingredients (spent grains, defatted flours). Animal sources often match excellent bioavailability (high PDCAAS score) with low sustainability (high water and carbon footprints). Plant protein offer plenty quantity and are more sustainable but sometimes less complete in essential amino acids. Exceptions are available (soy, pumpkin seeds) but limited by their sensory profile. Therefore, a multidisciplinary approach is encouraged. Consumers should choose more plant-based protein from a variety of sources (to achieve complete and balanced amino acid intake). Second, food manufacturers should improve their technology to fully unlock the potential of nutrient-dense foods such as pumpkin seeds. In addition, they may open to new, less explored options, such as mycoprotein, duckweed, and perhaps insects and upcycled ingredients (the last two might face more consumer adversity). Overall, protein sources are numerous and quality is available. It is a matter of reducing the environmental footprint and choosing from a wide variety of options, preferably plant-based.

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