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Exploring the frontier of protein development: Novel proteins with insects: A review

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Abstract

In recent years, there has been a surge of interest in edible insects as a novel source of protein for humans, with increased concern about the environmental effect of traditional livestock production and the need for more efficient and sustainable protein sources. Protein is a nutrient-dense protein source that can be extracted and processed into various food products, depending on functional properties and their sensory acceptability. The article's purpose is to express about the different and various protein sources commonly consumed particularly nutrient dense proteins, affect nutritional intake (with a focus on inadequate intake), nutritional adequacy, and dietary quality. In this article, we will look at some of the numerous forms of seaweed and its nutritional worth, as well as the ways for extracting protein from seaweed for use as an edible source. The results of this study show that seaweed is a promising new protein source with high nutritional value and can be extracted and processed into various food products, depending on functional properties and their sensory acceptability. When the nutritional intakes of beef consumers were compared, low-fat beef consumers had greater amounts of protein, vitamins B-6, vitamin B-12, magnesium, and iron. A sustainable source of high-quality protein that may be used in a variety of food products could be edible mushrooms.

Keywords: Protein sources, bioactive peptide, novel protein sources, waste utilization

1. Introduction

Diet quality is defined as the ability to meet recommended nutrient intakes while consuming the recommended amount of energy (Stuart M *et al.*, 2015; Cena H *et al.*, 2020) [14, 20]. When it comes to dietary patterns, it's crucial to consider the impact of protein-containing foods on nutrient intake, adequacy, and overall diet quality. Firstly, the risk or prevention of chronic diseases cannot be predicted solely by the consumption of a single nutrient, such as protein, in a food or food group. Instead, it's the total nutrient intake of the diet consumed within energy needs that plays a crucial role (Stuart M *et al.*, 2015; Cena H *et al.*, 2020) [14, 20]. Secondly, many North Americans' diets are suboptimal, as evidenced by the high prevalence of overweight and obesity, as well as nutrient deficiencies. Furthermore, their dietary consumption patterns may be contributing to these issues (Stuart M *et al.*, 2015, and <https://www.hsph.harvard.edu/nutritionsource/what-should-you-eat/protein/>) [140]. Therefore, it's essential to pay attention to the types of protein sources we consume and how they fit into our overall dietary patterns to ensure optimal nutrient intake and diet quality. (Ogden *et al.*, 2013; Dietary guidelines advisory committee, 2010; US department of Agriculture, 2010, and US department of Agriculture, 2013) [111, 25, 148]. The 2010 Dietary Guidelines for Americans (DGA) have two overarching themes: "Maintaining a long-term calorie balance to achieve and maintain a healthy weight" and "Nutritious foods and a focus on consuming beverages" (USDA, 2010). According to his 2010 DGA, the nutrients which are of consumers health concern are vitamin D, calcium, fibre, and potassium. Pregnant women need to have enough iron and folic acid, while elderly (Those over 50) need vitamin B-12 (US Department of Agriculture, 2010) [148]. Furthermore, the 2010 DGA recommends limiting consumption of saturated fats, solid fats, added sugars, and refined grains (US Department of Agriculture, 2010) [148]. The problem provided by these guidelines, which are covered in this article, is to reduce saturated and solid fats while increasing intake of the nutrient of concern. While many regularly eaten dietary protein, sources contribute significantly to nutritional consumption, they also significantly contribute to saturated and solid fat intake, rendering this dietary guidance insufficient. We believe that if continued instruction to reduce saturated and solid fat intake is not accompanied by significant dietary changes, it may lead to further deficiencies of the nutrient of interest.

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Among other data more North Americans than at the 2010 DGA calorie targets still do not achieve consumption goals for other critical nutrients, certain groups do not eat recommended protein intakes, and it is believed that they should consume significantly more protein. Health improvement (Bauer *et al.*, 2013; Wolfe RR, 2008) ^[10, 154]. Considering that excessive energy consumption may lead to overweight/obesity, following protein requirements (Institute of Medicine, 2005) ^[64] is critical, although certain energy-demanding communities and people may have greater protein intakes (Wolfe RR, 2008) ^[154]. It has been proposed that eating more protein than is suggested promotes satiety and may have some advantages in using other nutrients. Leidy *et al.*, (2014) ^[84] mentioned the supplements for the protein from the different sources. The purpose of this article is to look at how popular protein sources are, especially nutrient-dense protein sources, influence nutritional intake (particularly insufficient consumption), nutritional adequacy, and dietary quality.

Protein levels based on current satiation from meals consumed. Dietary patterns that promote nutrient-dense protein intake to satisfy protein guidelines (Freeland-Graves *et al.*, 2013; Huth *et al.*, 2013) ^[34, 61] are connected with dietary quality, according to the hypothesis. It had a beneficial effect, and nutritional adequacy helped, without surpassing energy need. Additionally, continual recommendations to limit saturated fat consumption might generate 'pressure' to lower intake of numerous regularly used nutrient-dense protein sources, hence influencing dietary quality. (Huth *et al.*, 2013; Freeland-Graves *et al.*, 2013) ^[61, 34]. We understand that there are significant research gaps in the field of protein and nutritional consumption, and these are underlined. It contains a high protein content of 0.80 g with high-quality (RDA). The DRI suggests consuming high amount of protein, however the word is not defined (Institute of Medicine, 2005) ^[64]. Protein quality is defined by the WHO as the amount and proportion of individual amino acids that can be absorbed and used by the body (FAO/WHO/UNU, 2007); however, the Digestible Indispensable Amino Acid Score (DIAAS) is a novel grading system that defines protein quality based on the Digestible Indispensable Amino Acid Score (DIAAS) of ileal digestibility and has been recommended as a replacement for the Protein Digestibility Corrected Score (PDC). The use of the DIASS technique (Which has ramifications for how individual amino acids are treated in terms of adequacy) leads in meal ratings that are similar to PDCAAS but allow for quality evaluations based on ileal digestibility and are not arbitrarily terminated at 1.0. Food and nutrition document, FAO, 2015. Protein sources such as meat, chicken, fish, eggs, isolated soy protein, and dairy foods (milk, cheese, and yoghurt) supply all 9 necessary (essential) amino acids and are considered high-quality protein sources (Institute of Medicine, 2005; FAO food and nutrition paper, 2015) ^[64, 26]. Plants, legumes, grains, nuts, seeds, and vegetables are all examples of plant foods. are considered lower-quality protein sources because their proteins may be insufficient in one or more essential amino acids (FAO food and nutrition paper, 2015) ^[26]. Many researches have looked at protein dietary sources and the percentage of complete consumption of protein from plants and animals sources (O'neil *et al.*, 2012; Berner LA *et al.*, 2013) ^[108, 15]. Researchers have investigated protein intake dietary sources using data from a nationally representative sample of \$2-year-old (n = 17, 386) persons

participating (VL Fulgoni *et al.*, 2011) ^[36] in the NHANES 2007-2010.

Current dietary guidelines, including protein, emphasise the need of consuming a range of meals (Freeland-Graves *et al.*, 2013; Huth *et al.*, 2013; Dietary Guidelines Advisory Committee, 2010; USDA 2010, Pennington *et al.*, 2007, and Marra MV *et al.*, 2009) ^[34, 61, 25, 117, 94]. The 2010 DGA's fundamental principle is that nutrients are obtained through diet, particularly healthy foods (US Department of Agriculture, 2010) ^[148]. Nutrient saturation is a hard-dietary theory that underpins DGA 2010 and the USDA's MyPlate (USDA, 2010; USDA, 2015). Nonetheless, it is recognised that there are no clear definitions of "nutrient-dense" foods, and difficulties in generating these classifications have been identified (Pennington *et al.*, 2007; Miller GD *et al.*, 2009) ^[117, 96]. Yet, "nutrient-dense meals include vitamins, minerals, and other elements in very few calories that may have health benefits." They have low number of solid fats, they eliminate additional solid fats, sweets, and processed carbohydrates, which contribute calories but little vital nutrients or fibre" (US department of Agriculture, 2010) ^[148]. While this definition has been critiqued as unclear (Pennington *et al.*, 2007) ^[117], it is the most often used explanation of nutrient dense food products. Foods with low nutritional value but relatively high energy content (particularly from added sugars) are referred to as energy-dense and nutrient-poor or "empty calories" in contrast to nutrient-dense meals (US Department of Agriculture, 2010) ^[148]. Many nutrient profiling methodologies and nutrition quality indices have been developed and validated or tested against diet quality indicators such as the USDA-derived Healthy Eating Index (HEI), to aid in assessing the nutritional quality of overall diets (Pennington *et al.*, 2007; Miller GD *et al.*, 2009; Fulgoni VL *et al.*, 2009; Drewnowski A, 2009, and Katz DL *et al.*, 2009) ^[117, 96, 37, 27, 76].

Protein is a vital nutrient in the human diet that is required for living. Its primary function in nutrition is to supply the body with enough amino acids, as these amino acids serve as building materials for the body. The quality of protein, also known as the nutritional value of the product, is determined primarily by its amino acid content and physiological use after digestion, assimilation. The number of amino acids needed for protein synthesis determines amino acid metabolism. The amino acid availability is determined by the protein supply, processing techniques, and interactions with other dietary components such as lipids, minerals, and so on. 2019 (Maurya and Kushwaha) Proteins produced from soy, wheat, vegetables, and potatoes are now frequently utilised plant protein sources. Soy is the primary protein source for the creation of animal protein. As animal protein alternatives, insects, algae, duckweed, microbial proteins, mycoproteins, leaf proteins, and canola are likely to join the food industry. There are also several vegetarian alternatives on the market, including seitan, tofu, soybeans, tempeh, Quorn, and lupins without meat, as well as canola oil. Unfortunately, the dietary safety of these alternative protein sources is unknown. 2019 (Maurya and Kushwaha).

Protein is a nutrient that is trending favourably in the marketplace, as demand for plant and animal protein sources increases (Henchion *et al.*, 2017) ^[51]. Clinical evidence of health advantages related with protein consumption beyond the recommended dietary protein intake is growing. These health advantages include B. Improved leg strength (Mitchell

et al., 2017)^[99] or enhanced walking speed (Park *et al.*, 2018)^[115] and better bone density (Houston *et al.*, 2008, Hudson *et al.*, 2020; Mitchell *et al.*, 2017; Oikawa *et al.*, 2018, and Park *et al.*, 2018)^[54, 58, 99, 112, 115]. Kerstetter *et al.*, (2000)^[77]

sustained promotion of high-protein choices in the food market should be predicted, while increased worldwide production of food protein, particularly high-quality animal protein, will contribute to environmental sustainability.

Adolescent overweight is an important concern globally, and various studies have looked into animal protein as a viable way to aid with teenage obesity. Animal protein consumption has been linked to decreased total fat % and body mass index (BMI) in adolescents when compared to adolescents who consume more animal protein. There is no evidence of a link between This article examines the evidence for using animal protein in the diet to mitigate the danger of adolescent obesity (Naghshi *et al.*, 2020; Bernier Jean *et al.*, 2020; Adair *et al.*, 2020)^[103, 16, 1]. Plant proteins other than soy have also shown promise in exercise tests. Joy *et al.*, (2013)^[71] discovered that taking 48 g of rice or whey protein isolate per day on training days enhanced body composition, bench showed comparable gains in press and leg press strength following an 8-week resistance training programme in college-aged people. A study of professional MMA fighters who completed 6 weeks of hard training revealed no difference in body composition outcomes between 75 g of whey protein isolate and rice protein isolate (Kalman *et al.*, 2018)^[74].

Eating insects, also known as entomophagy, is a traditional activity undertaken by a number edible insect consumption in which according to scientific literature, there are over 1,900 edible species worldwide, with two billion people, mostly in Asia, Africa, and South America, consuming them as part of their diet. A number of positive factors favour the expanding use of insects as a sustainable source of animal protein. In comparison to other animals, insects take up less space, have higher fertility, and certain breeds are multiparous, that is, they go through higher than life cycle of one per year. Edible insects are more ecologically friendly than chickens because they produce less greenhouse gases and ammonia. Omnivorous insects may be bred on VGF waste and help to valorize its biomass. Above all, insects may help with protein, food, and feed security (Birgit A Rumpold and O Schlüter, 2015)^[18]. Edible insects, particularly those of the classification Orthoptera (locusts, locusts, and crickets), are high in protein and serve as vital substitute origin of protein worldwide. Insects have a high protein and lipid content and may supply vital amino acids, unsaturated fatty acids, and trace minerals. Mushrooms, algae, leaf protein extract, and a broad range of insects are among unique sources of protein and other nutrients utilised by a restricted set of tribes and societies. Even when dried, they contain high-quality protein and micronutrients (minerals and vitamins). As a result, it may be the finest location for producing proteins and minerals that can be added to a variety of goods to make them more nutritious.

2. Source of protein

There are many sources of protein as the protein is the main source of body muscle formation. Those proteins are broken down into amino acids and smaller components and distributed to the body muscles for structure formation and mass function. The essential amino acids respects to the function of the muscle formation and mass structure

formation.

Table 1: The nine essential amino acids required for the human nutritional diet

S. no.	Indispensable	Dispensable
1.	Valine	Asparagine
2.	Phenylalanine	Glutamic acid
3.	Tryptophan	Serine
4.	Threonine	Aspartic acid
5.	Histidine	Alanine
6.	Isoleucine	
7.	Lysine	
8.	Methionine	
9.	Leucine	

3. Plant and animal-based protein source

Plant-based proteins are increasingly being employed in human nutrition as a health-promoting and cost-effective alternative to animal proteins. Yet, growing prices, restricted availability, biodiversity loss, dangers to human health from numerous illnesses, shortage of fresh water, and sensitivity to climate change have all led to the replacement of animal protein. Sun Waterhouse D *et al.*, (2014)^[142]; Sabate J *et al.*, (2014)^[131]. Moreover, obtaining adequate animal protein is challenging and costly. As a result, plant proteins are the primary source of alternatives for enhancing human nutrition. Plant sources account for 57% of all known dietary protein sources, with the remaining 43% coming from dairy products (10%), shellfish and fish (6%), and meat (18%), as well as other animal products (9%) (FAO, 2010). Several plant protein sources have recently been explored to assure dietary protein supply and fulfil the population's nutritional demands (Iqbal A *et al.*, 2006; Day Y., 2013; Hughes GJ *et al.*, 2014; Wang X *et al.*, 2010)^[66, 23, 60, 152]. Plant-derived proteins may lack certain important amino acids depending on the source. Grains, for example, are usually poor in lysine, while legumes are low in sulfur-containing amino acids like cysteine and methionine. Nosworthy *et al.*, 2017^[107]; Pseudo grains, on the other hand, contain substantial quantities of lysine (such as quinoa and amaranth). Because of changes in soil variety, climate conditions, precipitation, latitude and elevation, agricultural techniques, and cultivars/cultivars, the same plant might contain various nutrients (Goldflus *et al.*, 2006; Liu *et al.*, 2017)^[44, 88]. Humans have employed a variety of traditional plants as protein sources, including beans, peas, and soybeans. Additionally included are novel protein sources (such as proteins derived from insects and algae) (Sa AGA *et al.*, 2020)^[130] as well as unconventional and alternative protein sources (agro-industrial by-products from edible oil extraction and waste during fruit processing) have been found. Moreover, plant protein equivalents of meat, milk, and eggs have been found (Frias *et al.*, 2011)^[35]. Several studies have shown that plant proteins have antitumor, antioxidant, hypoglycaemic, ACE inhibitory, antibacterial, and lipid-lowering effects (Luna-Vital *et al.*, 2015)^[91]. Countries with high legume consumption have been found to have lower risk of diseases such as type 2 diabetes, cardiovascular disease, colon cancer, and other chronic diseases (Sanche *et al.*, 2018). (Campos Vega *et al.*, 2010; Chino *et al.*, 2015)^[19, 132]. Small peptide bioactivities are found in numerous legume proteins, mostly as a result of enzymatic breakdown by various proteases such as pepsin, trypsin, chymotrypsin, alcalase, papain, pancreatin, thermolysis, and flavoursome (Awika *et*

al., 2017) [9]. These peptides have a variety of biological functions. B. It has antioxidant, antifungal, anticancer, and ACE inhibitory properties and is utilised in a number of products such as dietary supplements, functional food

components, and dietary supplements (Awika *et al.*, 2017; Das D *et al.*, 2020) [9, 22]. It is also used for other reasons (Luna-Vital *et al.*, 2015) [91].



Fig 1: Plant based protein source for human health

4. Novel sources

4.1 Insects

Crickets are one of the most often consumed edible insects, containing up to 20% protein by weight as well as significant amounts of iron and calcium (Lenka K, and Anna A. 2016) [85]. Mealybugs, grasshoppers, and silkworms are also high in protein, with certain species containing up to 60% protein by

weight (Giampieri F *et al.*, 2022) [43]. Edible insects are also abundant in good fats, such as omega-3 and omega-6 fatty acids, which are essential for maintaining a healthy brain, heart, and immune system (Giampieri F *et al.*, 2022) [43]. Bugs are also low in carbs and high in fibre, making them a great choice for diabetics or anybody watching their carb intake (Giampieri F *et al.*, 2022) [43].

Table 2: Major Sources of Protein

Source	Extraction Method	Extraction tool	Separation technique	Reference
Insect	Chemical	Sodium hydroxide, nucleic acid	Chemical synthesis	(Fimel Gresiana, 2015)
Algae	Acidic, Alkali and Aqueous method	Centrifugation	Ultrafiltration Precipitation or Chromatography	(Stephen Bleakley, 2017) [139]
Duckweed	Mechanical method	Hydraulic press	Pulverizing, Concentration	(Maaike Nieuwland, 2021) [165]
Rapeseed	Aqueous, alcoholic	Hydraulic press, spray dryer	Chemical synthesis, Spray drying	(Daniela Von Der Haar, 2014)

Insects are a sustainable source of protein since they use significantly less area, water, and feed than cattle and release far less greenhouse emissions (Giampieri F *et al.*, 2022) [43]. For example, crickets use 12 times less food than calves to generate the same quantity of protein. Insects may also

convert carbs into protein. Hundreds of different bug species are eaten in various parts of the world. Crickets, bamboo worms, and water beetles, for example, are popular treats and ingredients in traditional Thai dishes. Traditional cattle farming, on the other

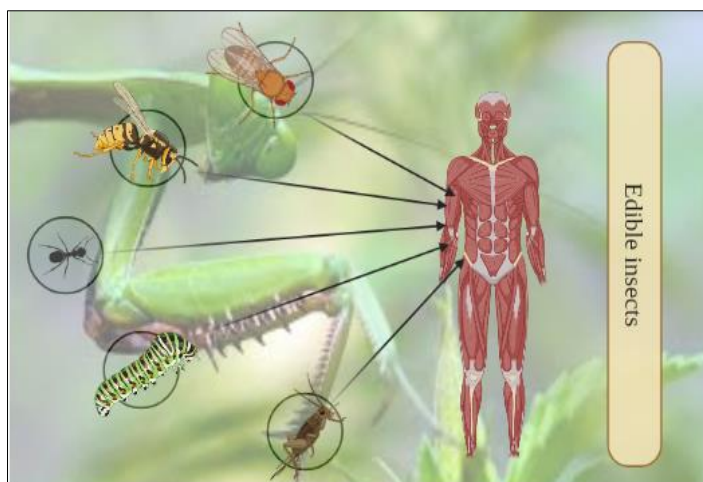


Fig 2: Edible insects as a novel source of protein in enhancement of human health

hand, contributes considerably to greenhouse gas emissions, deforestation, and water pollution (Giampieri F *et al.*, 2022) [43]. Chapulines, or grilled grasshoppers, are a popular snack in Mexico and are often found at local markets. Termites, locusts, and mopanes are often eaten in Africa, usually as a source of protein during times of food shortage. Insects such as ants, beetles, and crickets are among the almost 2,100

species that may be eaten and are beneficial to the environment since they consume less resources than meat production and provide significant health advantages. More than a fifth of the world's population consumes edible insects on a daily basis, and many nations and civilizations have eaten bugs for centuries because they are nutritious and tasty (Annie P. 2019) [4].

Table 3: Composition of different types of insects

Types of insects	Scientific name	Protein content (%)	Fat (%)	Reference
Larvae	<i>Allomyrina dichotoma</i>	54.18	20.24	(Ghosh <i>et al.</i> , 2017) [42]
	<i>Protaetia brevitarsis</i>	44.23	15.3	(Ghosh <i>et al.</i> , 2017) [42]
	<i>Tenebrio molitor</i>	46.44	32.7	(Ravzanaadii <i>et al.</i> , 2012) [123]
Beetle	<i>Heteroligus meles</i>	38.1	32	(Jonathan 2012) [70]
	<i>Rhynchophorus</i>	50	21.1	(Jonathan 2012) [70]
Cricket	<i>Gryllus bimaculatus</i>	58.3	11.8	(Ghosh <i>et al.</i> , 2017) [42]
	<i>Teleogryllus emma</i>	55.6	25.1	(Ghosh <i>et al.</i> , 2017) [42]

In recent years, edible insects have also gained popularity in Western countries as people become more aware of their nutritional and environmental benefits. In the United States, edible insects are now available at specialty food stores and online retailers and are increasingly being used as ingredients in energy bars, chips, and other snacks (Gahler, R. 2018) [40]. In recent times, the European Union legalised that insects can be used as a novel food source, paving the path for increased consumption and production of edible insects in Europe (European Parliament and Council. 2015) [29]. Despite the many benefits, there are still challenges to be overcome to increase the consumption of edible insects. One of the biggest hurdles is the "yuck" factor - many people in Western countries are simply not used to the idea of eating bugs. However, as more people become aware of the environmental and nutritional benefits of edible insects, this perception is slowly changing. Another challenge is the lack of regulations and standards for the breeding and processing of insects. In many countries, there are no specific regulations for the production and sale of edible insects, which can lead to inconsistencies in quality and safety. However, efforts are underway to establish guidelines and standards for insect breeding and processing, which will help increase consumer confidence in edible insects (Van Huis *et al.*, 2013) [151].

4.2 Algae

Seaweed and microalgae provide more protein per unit area than crops grown on land such as pulse legumes, wheat, and soybean, (1.1 tonnes/Ha/year, 1-2 tonnes/Ha/year, and 0.6-1.2 tonnes/Ha/year, respectively). Terrestrial agriculture currently uses around 75% of whole world freshwater, with animal protein in particular requiring 100 times more water than the same quantity of protein composed of plant reassets (Stephen Bleakley, 2017) [139]. The ideal proteins, according to Boisen's research, may vary significantly depending on digestibility and the availability of critical amino acids. Animal protein evaluations are often considered as complete proteins because they include a high concentration of essential amino acids (EAAs) that the human body cannot biosynthesize. Because they lack one or more critical amino acids, such as histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine, young plant proteins are often viewed as an insufficient protein source. Plant-based proteins are often more difficult to digest than animal proteins

due to their high concentration of insoluble polysaccharides. Algae are widely considered as a viable protein source, with EAA content satisfying FAO standards and often being comparable to other protein resources such as soybean and egg (Stephen Bleakley, 2017) [139]. The decline in huge consumption of marine algae has led in a scarcity of *in vivo* study on ileal digestion of algae, restricting the evaluation of algal protein best across diverse algae species and with varied protein reassets (Míurcová, 2010) [98]. Despite this, tryptophan and lysine are amino acids that are usually limited in the majority of algae species. Aspartic acid and glutamic acid account for a disproportionately large percentage of all amino acids in many seaweed species, contributing significantly to the distinctive 'umami' flavour associated with seaweed (Stephen Bleakley, 2017) [139]. Such amino acids, for example, are said to account for 22%-44% of general protein subordinates in *Fucus* sp. and (26 - 32%) in *Ulva* sp. Algal proteins are traditionally extracted using aqueous, acidic, and alkaline techniques, followed by many rounds of centrifugation and recovery methods such as ultrafiltration, precipitation, or chromatography (Kadam, 2016) [73]. Algae proteins were traditionally extracted using water, acid, and alkaline procedures, centrifuged numerous times, and recovered using the methods like chromatography, or precipitation, ultrafiltration (Kadam, 2016) [73]. Algae are a varied collection of aquatic creatures that have long been valued as a source of nutrients such as proteins, vitamins, and minerals. Seaweed has emerged as a potential new source of protein for humans in recent years. Because of their high protein content, they may be harvested and processed into a variety of food items. In this article, we'll look at some of the numerous forms of seaweed and its nutritional worth, as well as the ways for extracting protein from seaweed for use as an edible source. Spirulina is one of the most often utilised algae for protein extraction. Spirulina is a blue-green algae that may be produced in both freshwater and saltwater. It has a high protein content, with some strains having up to 70% protein by weight (Khan *et al.*, 2005) [78]. Spirulina is also high in vital amino acids, vitamins, and minerals, making it a very healthy diet.

One more algae that has gained attention as a source of protein is chlorella. Among the freshwater green algae Chlorella is the only single-celled green algae which is considered as protein rich retains around 60% of protein by

weight (Becker EW. 2007) ^[12]. Chlorella is also rich in vitamins, minerals, and antioxidants, making it a popular dietary supplement. Dunaliella is a type of algae rich in nutrients, especially protein. Dunaliella is a saltwater alga known for its ability to produce high levels of beta-carotene, a precursor to vitamin A. It is also high in protein, with some strains containing up to 30% protein by weight (Ben-Amotz *et al.*, 2009) ^[14]. To extract proteins from algae, several methods can be used, including centrifugation, filtration, and enzymatic hydrolysis. The centrifugation process involves spinning the algae at high speed to separate the protein from the other components. Filtration involves passing algae through a membrane or filter to remove proteins. Enzymatic hydrolysis involves breaking down algae using enzymes to release proteins. One study investigated the protein extraction efficiency of different methods using spirulina as an algae source. Research shows that enzymatic hydrolysis is the most effective method, with protein extraction efficiency up to 97% (Silva FP *et al.*, 2012) ^[137]. Another study compared the protein content and extraction efficiency of three different algae – spirulina, chlorella and Scenedesmus – using a combination of enzymatic hydrolysis and centrifugation. The study showed that spirulina had the highest protein content and extraction yield, followed by green algae and Scenedesmus (Rinalducci S *et al.*, 2018) ^[125]. Algae protein can be used in many food products, including protein powders, energy bars and snacks. One study looked at the acceptability and nutritional value of a snack made with spirulina and cornmeal. Research shows that this snack is high in protein, vitamins, and minerals and is well accepted by consumers (Oliveira AC *et al.*, 2019) ^[113]. In conclusion, seaweed is a promising new protein source with high nutritional value and can be extracted and processed into various food products. Spirulina, chlorella, and dunaliella are just a few examples of different types of protein-rich algae. The algae protein can be extracted from it by means of enzymatic hydrolysis which is considered as the most efficient method for extraction and that protein can be incorporated into many foods for enrichment and fortification as several research are emerging on specialized topic of algal protein as it is constrained as the sustainable and nutritious source of protein in the global market and diet.

4.3 Duckweed

As per the observations studied by Sree *et al.*, 2016 ^[138] duckweeds are a small circle of relatives of aqueous floating monocotyledons, with a species of 37 distributed globally. According to research papers by Sree *et al.*, (2016) ^[138] and Ziegler *et al.*, (2015) ^[160], certain plant life, such as Wolffia arrhiza, are the fastest growing angiosperms and can cover ponds or lakes in a matter of days under favorable growth conditions. These plants have been studied for their potential use as a substitute for soy in animal feed, including Japanese quails, striped catfish, rohu, carp, and broilers. In a recent study by Appenroth *et al.*, (2017) ^[5], the dietary reputation of the whole plant family, Lemnaceae, was analysed, and the fastest growing species, Wolffia microscopic, was examined for its protein content, amino acid spectrum, starch content, fats content, and fatty acid distribution. Duckweeds have the potential to be used as new crop plants and can grow efficiently on wastewaters, taking up nutrients and cleaning up the water (Su *et al.*, 2014; Cui and Cheng, 2015; Fujita *et al.*, 2015) ^[162].

Analytical procedures for determining protein from duckweed are being researched (Klaus *et al.*, 2018) ^[79]. The freeze-dry weight (FDW) of clean duckweeds was measured after vacuum freeze-drying for 48 hours, according to a study by ncbi.nlm.nih.gov. The freeze-dried material was then finely ground and homogenized using a laboratory mill before being aliquoted for further testing. Another study by sciencedirect.com determined the nutritional value and antioxidant capacity of Wolffia arrhizal, with the moisture content being over 95% of fresh weight (FW), and the crude protein (CP) being 50.89. The dry weight of duckweed was also found to contain an average of 1% phosphorus (dry weight) and removes 113 mg, according to digitalcommons.usu.edu. Finally, frontiersin.org reported that greater duckweed was collected, cleaned, dried, and ground to examine the effect of Spirodela polyrhiza on the final weight and SGR of common carp fed diet SP20. Every previous studies were carried out in a similar way of freeze-dried fabric, and the results were correlated with FDW. Ash, dry matter, general lipids, and general protein had all been investigated (Appenroth *et al.*, 2017) ^[5]. The distribution of minerals, amino acids, tocopherols, and fatty acids has been examined and experimented with statically (Klaus *et al.*, 2018) ^[29].

4.4 Spirulina

Spirulina is a multicellular and filamentous blue-inexperienced alga, which has won significant interest in the fitness care and meals quarter as a protein and diet complement (Ruma Arora Soni *et al.*, 2019) ^[127]. (Akshita Sharma *et al.*, 2019) ^[2] pronounced from the studies that spirulina grows in water, may be harvested, and processed easily and has a completely excessive content material of micronutrients and macronutrients. It includes numerous styles of amino acids, in general valine, leucine, tryptophan, methionine, phenylalanine, threonine, lysine (Pyne *et al.*, 2017) ^[128]. According to (Huesemann *et al.*, 2016) ^[59] research have demonstrated that the spirulina species want mild and temperature as their predominant elements of boom in nutrient-operated out of doors ponds (N, P, CO₂, etc.) and properly combined conditions. (Akshita Sharma *et al.*, 2019) ^[2] made a examine on spirulina that alkaline saline water is favorable for the best manufacturing of *Spirulina Platensis*. *Spirulina Platensis* can't grow in darkish on media which comprise natural carbon compounds and decrease carbon dioxide in mild. (Ruma *et al.*, 2019) ^[127] have concluded from their experimental studies that Spirulina is said as a non-toxic, nutritious meals, due to its richness in minerals, protein, and essential fatty acids. It is a healthful strength complement this is mainly beneficial for low-calorie meals. (Akshita Sharma *et al.*, 2019) ^[2] stated spirulina carries a protein attention of excessive quantity among 55 - 70% via way of means of dry weight and carries all the critical amino acids that are very beneficial for the higher yield of the biomass from *Spirulina Platensis*.

Spirulina is an excellent source of press, a mineral required for the formation of haemoglobin, the protein found in red blood cells that transports oxygen throughout the body. Frailty, a condition characterised by fatigue, weakness, and shortness of breath, might result from a lack of press (Mazokopakis *et al.*, 2014) ^[166]. According to research, spirulina is a good source of iron, and its bioavailability is equivalent to that of other iron-rich foods (Karkos *et al.*,

2011; Selmi *et al.*, 2011) ^[163]. Spirulina is one of the few plant-based sources of vitamin B12, which is essential for proper neurological function and the production of red blood cells. Vitamin B12 deficiency may cause anaemia and neurological problems (Watanabe *et al.*, 2014) ^[164]. While spirulina includes a form of vitamin B12, it is not in the dynamic frame and may not be appropriate for those who are deficient in vitamin B12 (Watanabe *et al.*, 2014; Kwak *et al.*, 2019) ^[164]. Other nutrients included in spirulina include magnesium, calcium, potassium, and zinc (Zhang *et al.*, 2023)

^[159]. These minerals are necessary for a variety of biological functions, including muscle and neuron function, bone health, and resistance work. In conclusion, spirulina may be a unique source of protein that is gaining ubiquity because to its many health advantages. It is also high in cancer-fighting compounds, press, and other minerals, making it a kind of super food. However, it is important to remember that spirulina may not be ideal for those with certain health concerns, and it is suggested that you get advice from a healthcare expert before including it into your diet.

Table 4: Nutrient composition of Spirulina

Macronutrient composition	Amount (%)	Reference
Protein	60 - 69%	(Akshita sharma <i>et al.</i> , 2019) ^[2]
Carbohydrate	16- 20%	(Akshita sharma <i>et al.</i> , 2019) ^[2]
Fat	5 - 7%	(Akshita sharma <i>et al.</i> , 2019) ^[2]

4.5 Fungal protein

The world's largest growing population keeps growing and is expected to exceed 9 billion by 2050, implying an increase in food consumption. According to FAO (Food and Agricultural Agency), agricultural output would need to rise by 70% by then to fulfil demand (Paillard *et al.*, 2014) ^[129]. As a result, new protein sources that can generate rich quality of protein in limited time period and area are needed. Protein is acknowledged as a crucial ingredient for sustaining good health. Proteins, in addition to providing energy, serve a variety of critical tasks in the body, whether as constituents of enzymes, hormones, or tissues (Bhutta *et al.*, 2013) ^[157]. Edible mushrooms are a potential alternative for acquiring high-quality protein, with the majority of them possessing a full necessary amino acid profile (Bach *et al.*, 2017) ^[30]. They are usually speedier and less expensive than animal-based products and plant proteins. Another significant benefit is that it can be grown on a wide range of substrates, including wood, paper, and agricultural waste, making it ecologically benign (Lavelli *et al.*, 2018) ^[149]. The goal of this study is to compare mushroom protein to traditional protein sources in terms of protein quality, digestibility, bioavailability, economics, and the environment. Since there is little literature on the use of fungal proteins in the creation of meals goal of this evaluation is to look into the safety of food for human consumption and viability of exploiting this underused protein source. It might be especially useful in the creation of novel products with increased functional properties while adding value to protein.



Fig 3: Fungus as a source of protein (Mushroom)

The authors' stated carbohydrate (4 kcal g-1), fat (9 kcal g-1), and protein (4 kcal g-1) contents were used to calculate energy values for mushrooms. Mushroom protein content ranges from (18.8 - 36.96) % by dehydrated weight. As per research by scientificliterature.org, 100 g of mushrooms on a dry weight basis can cover from 29.41% to 66.00% of the Recommended Dietary Allowance (RDA) for males and 35.80% to 80.35% for women. Additionally, mushrooms are a good source of protein, minerals, polysaccharides, unsaturated fatty acids, and secondary metabolites, according to intechopen.com. Mushrooms contain approximately 20-40 g/100 g of dry weight basis of high-quality protein content, as reported by intechopen.com. Furthermore, verywellfit.com reported that one cup of mushrooms (70g) provides 15 calories, 2.2g of protein, and 0.2g of fat. Mushrooms are also a good source of copper, B vitamins, potassium, and iron (J.R. Lupton *et al.*, 2002) ^[69]. As previously stated, various variables influence the chemical makeup of mushrooms, and hence the protein content of mushrooms. Mushroom maturity and proportions are two of these parameters. Research that looked at the amounts of crude digestible and indigestible proteins in the cap and stem at four phases of *Pleurotus ostreatus* growth discovered that in the second stage, when the cap reached a diameter of 5, crude and digestible proteins reached 8 cm and thereafter fell. Caps have been discovered to have a greater protein content than stems. It's worth noting that the protein content of the substrate has an impact on the ultimate concentration of mushroom fruiting bodies. Mushrooms with high protein content is cultivated with the help of nutrients added to it as wheat bran, wheat stalks, and beer grains (Lavelli *et al.*, 2018) ^[149]. Of all mushrooms, *P. ostreatus* had the greatest PER. It's more precious than beef jerky and the most valued non-fungal food. A. PERs were similarly greater in *bisporus* (Portobello) and *Agaricus brasiliensis* than in jerky. A. *Pleurotus djamor*, *Pleurotus eryngii*, and *Pleurotus ostreatus* (white oyster) displayed lentil-like PER, *Flammulina velutipes*, and *L.* Among mushrooms, *edodes* has the lowest PER, equal to whole milk. All mushrooms outperformed grains in terms of PER (white rice and oats). The immense protein levels seen in least-calorie meals are beneficial for people who need to lose weight while still achieving their daily protein needs. Edible mushrooms have the significant benefit of growing swiftly in confined spaces, as per ncbi.nlm.nih.gov. Although the fruiting body of mushrooms is typically consumed as a food or food additive, the mushroom mycelium possesses

almost the same beneficial characteristics and takes less time and space to liquefy (H. Rathore *et al.*, 2019) [49]. It may be made *in vitro* using a medium. Mushroom mycelium is a

potential source of bioactive compounds as mentioned by Mdpi.

Table 5: Functional properties of protein (Wouter *et al.*, 2016) [39]

Functional property	Hydrophobic groups	Ionizable groups	Desired Functional property in food products
Solubility	↑↓	↓	Meals and drinks
OAC	↓	↑	Meat, pastry, and bakery goods
WAC	↑	↓	Low-fat snacks, pastries, and bakery items
Gelling	↑	↑	Charcuterie, jam, and yoghurt
Emulsifying	↓	↑	Dressing, sausages, pastries, and sweets
Foaming properties	↓	↑	Beer, bread, ice cream, cake, meringue

5. Conclusion

Novel protein sources, such as insects and algae, should be increasingly used in Europe to replace proteins of animal origin. Research has concluded that spirulina is the most widely used new protein source and contains all the essential amino acids needed for the recommended daily intake. Edible mushrooms are a potential source of high-quality protein that can be used in various food products, depending on their functional properties and sensory acceptability. Additionally, bioactive peptides found in edible mushrooms can be used to address medical and biotechnological issues. The biological value of a protein can be determined by measuring the amount of nitrogen consumed and excreted. Recent reports suggest that mushroom bioactive proteins and peptides, such as lectins and fungal immunomodulatory proteins, have health-promoting potential. Protein concentrates, hydrolysates, and peptides from mushrooms are being used to improve human health. Mushrooms are also natural sources of valuable food molecules such as ergosterol, and they have low fat content and high fiber and protein contents. Plant-based protein products and diets are growing in popularity due to their health and physical function benefits. However, plant protein nutritional quality should be considered when planning an appropriate diet. Plant protein is becoming increasingly popular due to its health-related functions, but there are potential safety issues. People in developing countries face protein and mineral deficiencies due to low levels of legumes, grains, zinc, iron, calcium, and magnesium. Animal protein is less environmentally sustainable and prone to disease and negative health impacts. Plant-based protein products are becoming popular due to their many advantages over animal proteins, but modified methods are needed to improve their nutritional quality.

Data availability

Data will be made available on request.

Author contribution

Writing original draft, Balasharan R; Conceptualization and Supervision, Rahul salve, Review, Editing and Supervision, Piyush Kashyap, Rahul salve.

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Conflict of interest statement

The authors declare no conflicts of interest.

Ethics statement

None declared.

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