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Single Cell Protein: A “Nutrient Rich” Super Food

Neha Kumari[1*], Abhishek Kumar[2] and Anjli Dhiman[2]
[1]School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab- 141004, India
[2]Netaji Subhash Chander Bose Memorial Govt. College, Hamirpur, Himachal Pradesh 177001, India

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Introduction

Humans and Animals rely on protein for nitrogen and essential amino acids necessary for building structural and functional proteins crucial for survival, including enzymes and hormones. Proteins can also serve as an energy source under extreme conditions. The nutritional quality of a protein is determined by its amino acid composition, with several amino acids being essential and needing to be obtained through diet. Animal and dairy productions have been increasing to meet growing demands, with a focus on healthier foods with optimal amino acid profiles and sustainable production methods. Additionally, protein can be sourced from microbes and algae with high protein content and a balanced amino acid profile, known as single-cell protein (SCP) Figure 1 (Ritala et al. 2017). SCP production involves steps like preparing nutrient media, cultivation, separation, and final processing. Efforts are underway to develop SCP production methods using inexpensive waste materials from various industries.

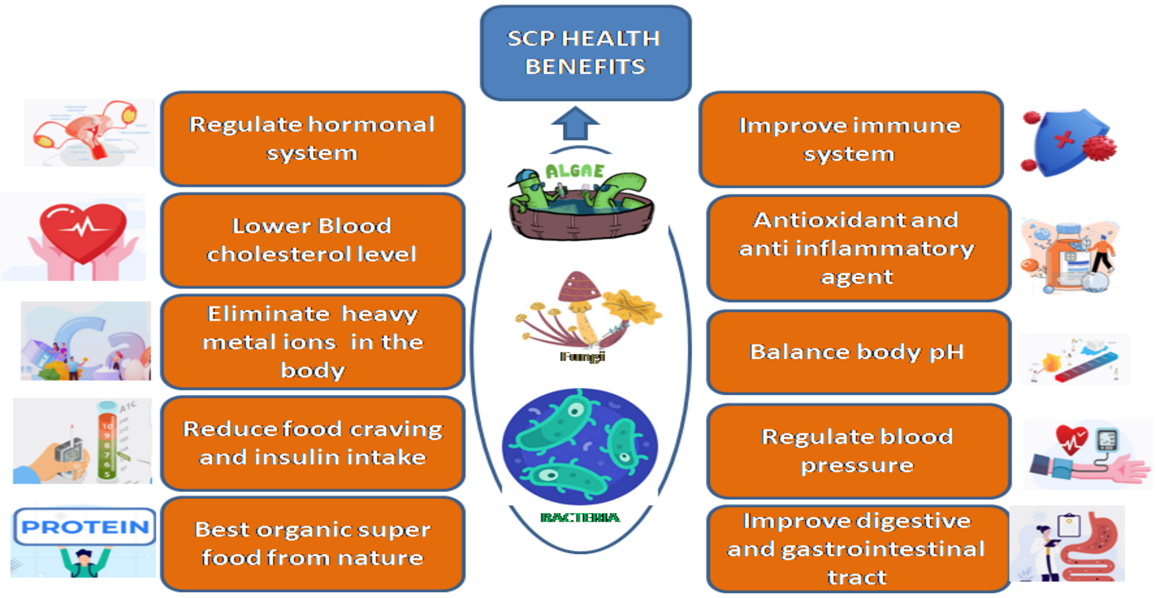

Fig 1 Health benefits of Single Cell Protein (SCPs)

SCP production systems, substrates used and processes:

Algae, filamentous fungi, yeast, and bacteria are viable sources of single-cell protein (SCP). Future advancements may include deriving dietary protein from proteins secreted by engineered microbial cells, such as milk or egg white proteins. Additionally, protein production from animal and plant cell cultures, which are not microbes but not classified as animals or plants either, holds promise for future dietary protein sources.

SCP from algae

SCP derived from algae is characterized by its high protein content, typically ranging from 60% to 70% (Table 1). Additionally, algae offer essential fats, vitamins (A, B, C, and E), mineral salts, and chlorophyll, with relatively low nucleic acid content (3–8%) (Zimberoff, 2017). Algae-based products, such as spirulina, Chlorella, and Dunaliella salina, are increasingly utilized in various forms, including supplements and food ingredients like pastas and snacks. Companies like Euglena, Algaeon, and TerraVia are actively commercializing algae-derived products, primarily for their nutritional value, with a focus on beta-glucan content and whole-cell products. While algae typically rely on CO_2 and light for growth, some products are produced through traditional fermentation methods. Outdoor algae production in open ponds is common but faces challenges like contamination and weather variability. Indoor photo bioreactors are emerging as a solution to ensure consistent algae supply, especially for aquaculture, where algae serve as a source of omega fatty acids, carotenoids, and protein for animal nutrition.

SCP from fungi

Commercially available products from fungi like Saccharomyces, Fusarium, and Torulopsis typically contain 30–50% protein (Table 1) (Anupama and Ravindra, 2000). These fungal SCPs boast a favorable amino acid profile, with high threonine and lysine content but relatively low methionine content, albeit still meeting FAO/WHO recommendations. Some fungal products, such as Marmite®, exhibit even lower methionine content. Fungal SCPs also provide B-complex vitamins and dietary fiber from glucans in their cell walls. Mycoprotein from *Fusarium venenatum*, utilized in Quorn™ products, has shown potential health benefits, including reducing LDL cholesterol and favorably affecting blood glucose and insulin levels.

Although fungi have moderate nucleic acid content (7–10%), it requires processing to reduce levels suitable for human consumption. The Quorn™ brand, known for its mycoprotein-based meat substitutes, has been extensively marketed for human nutrition and was acquired by Monde Nissin Corp in 2015. Brewer's yeast extracts, like Marmite®, Vegemite®, and Torula, provide protein and essential B vitamins, while Torula, rich in glutamic acid, has been used as a flavor enhancer. Additionally, a process called "Pekilo" uses filamentous fungus to produce SCP from sugars found in paper mill effluents, demonstrating potential for sustainable animal feed production.

Quorn™ and yeast spreads such as Marmite® are manufactured using glucose derived from starch, while the Pekilo process employs lignocellulosic sugars. Beyond these carbon sources, alkanes and methanol have also been utilized for single-cell protein (SCP) production by yeast and filamentous fungi. Methylotrophic yeasts like *Komagataella pastoris* (formerly known as *Pichia pastoris*) are capable of producing biomass and protein from methanol.

SCP from bacteria

Bacterial single-cell protein (SCP) typically boasts a protein content ranging from 50% to 80% on a dry weight basis, with essential amino acid levels meeting or exceeding FAO recommendations (Table 1). Some bacterial SCPs exhibit higher methionine content, up to 3.0%, compared to algal or fungal SCPs. However, bacterial SCPs, similar to fungi, have high nucleic acid content (8–12%), particularly RNA, requires processing before use as food or feed (Nasseri et al. 2011).

Alongside protein and nucleic acid, bacterial SCPs provide lipids and B-group vitamins. Imperial Chemical Industries developed Pruteen, an SCP for animal feed obtained from methanol using *Methylophilus methylotrophus* bacteria, containing up to 70% protein and utilized in pig feed. Although, due to competition from cheaper animal feeds in the late 1970s, production ceased. Methane is now emerging substrate of interest for SCP production, with products like FeedKind® and UniProtein® finding application in feed for animals.

Table 1 Algae, fungi and bacteria producing SCPs, their protein content on specific substrates

Algae	**Protein content (%)**	**Fungi**	**Substrate used**	**Protein content (%)**	**Bacteria**	**Substrate used**	**Protein content (%)**
Aphanizomenon flos-aquae	60–75	*Aspergillus flavus*	Rice bran	10	*Bacillus cereus*	Ram horn	68
Aphanothece microscopica	42	*Aspergillus niger*	Potato starch processing waste Waste liquor	38-50	*Bacillus pumilis*	Potato starch processing waste	46
Arthrospira maxima (Spirulina maxima)	60–71	*Aspergillus oryzae*	Rice bran (deoiled)	24	*Bacillus subtilis*	Ram horn	71
Arthospira platensis (Spirulina platensis)	46–63	*Candida crusei*	Cheese whey	48	*Corynobacterium ammoniagenes*	Glucose + fructose	61
Chlorella pyrenoidosa	45	*Candida tropicalis*	Molasses Bagasse	56 31	*Escherichia coli*	Ram horn	66
Chlorella sorokiana	46–65	*Penicillium citrinum*	Rice bran	10	*Methylococcus capsulatus, Ralstonia sp.*	Methane (Natural gas)	67–73
Chlorella spp.	62–68	*Saccharomyces cerevisiae*	Orange pulp, molasses, brewer's spent grain	24	*Rhizospheric diazotrophs* (whole microbial community)	Brewery waste water	>55
Chlorella vulgaris	42–55	*Trichoderma harzianum*	Cheese whey filtrate	34	*Rhodopseudomonas palustris*	Latex rubber sheet wastewater	55-65

Euglena gracilis	50–70	*Trichoderma viridae*	Citrus pulp	32	*Cupriavidus necator*	Synthetic growth medium	40-46
Scenesdesmus obliquus	30–50	*Kluyveromyces marxianus*	Cheese whey	43	*Methylomonas sp.*	Methane salt broth	69

SCP key processing

The processing requirements for single-cell protein (SCP) vary depending on the substrate material and the intended application in feed or food. In the following section, we examine the key processing steps essential for SCP production.

Degradation of Cell Wall in SCP Products:

Some single-cell proteins (SCP) are utilized as whole cell preparations, while others undergo cell wall breakdown to enhance protein accessibility (Trevelyan,1976). SCP like Quorn™ can be consumed without cell wall degradation, allowing chitin and glucan from fungal cell walls to contribute dietary fiber. Euglena-derived SCP, owing to proteinaceous pellicles instead of cell walls, does not require disruption, making them more readily digestible. Various methods, including mechanical forces (crushing, grinding, etc.), hydrolytic enzymes, and chemical detergents, are used for cell wall disruption. However, this process may impact the quality and quantity of SCP components. Products such as Marmite® and Vegemite® are cell extracts obtained by heating cells to 45–50°C, allowing intracellular enzymes to partially hydrolyze the cell wall and reduce proteins to smaller peptides.

Removal of Nucleic Acids from SCP Products:

Algae are typically low in nucleic acid content, but rapidly proliferating bacterial and fungal species exhibit high RNA content. RNA levels and degradation are influenced by growth conditions, growth rate, and carbon-nitrogen ratio. High nucleic acid amountin SCP usedfor human intake poses health risks due to increased uric acid concentrations, leading to conditions like gout and kidney stones. Methods to decrease RNA content include utilizing endogenous RNA-degrading enzymes (ribonucleases) activated through heat treatment, or adding ribonucleases directly during SCP production. Improved methods involve higher temperatures for shorter durations to minimize biomass loss. However, increased temperatures require additional energy input.

Safety regulations of SCPs

Like any feed or food product, single-cell protein (SCP) must adhere to safety regulations during production and use. Regulatory frameworks in most regions ensure the safety of feed and food for consumption. These regulations typically differentiate between food for humans, feed for animals, and various additives such as preservatives or colorants. International standards overseen by organizations like the Joint FAO/WHO Expert Committee on Food Additives govern internationally traded products. Regulations vary based on the intended purpose of the SCP, whether as food, feed, or additives. Some products may enter the market as additives rather than SCP, limiting their potential usage and value as a protein source.

Key concerns during SCP production include managing RNA content, microbial toxins, potential allergic reactions, and contaminants from feedstock such as heavy metals. Technologies have been evolved to reduce RNA content to safe levels. Selecting production organisms and optimizing process conditions mitigate toxin production, though fungi producing mycotoxins are unsuitable SCP sources due to associated health risks ranging from allergies to carcinogenesis. Bacteria, like Pseudomonas spp. and *Methylomonas methanica*, may also produce toxins, limiting their use. Utilizing diverse waste materials for SCP production offers cost and sustainability benefits, but safety concerns necessitate careful consideration of feedstock origins. Products like Quorn™ ensure safety by utilizing chemically defined mediums and adhering to Good Laboratory Practice (GLP) standards. Public perception and acceptance of waste-derived foods are crucial factors when integrating SCPs into human diets.

Future Prospects for User-Genetically Modified Organisms (GMOs) in SCP Production

The use of genetically modified organisms in food and feed faces limited public acceptance in Europe, despite greater acceptance elsewhere. Genetic modification offers numerous advantages for SCP production. For instance, DuPont has engineered yeast to produce long-chain omega-3 fatty acids, essential for human health. Genome sequencing and genetic engineering enable the disruption of genes involved in toxin production, enhancing SCP safety. Compared to traditional mutagenesis, genetic modification offers quicker and more precise gene disruption. Emerging technologies like Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) facilitate specific genome editing without introducing new DNA. Genetic modifications can also enhance the nutraceutical value of SCP biomass by optimizing amino acid composition or increasing vitamin and fatty acid content. Tailor-made SCPs with personalized nutritional profiles are possible through genetic engineering. Additionally, genetic modifications can improve protein harvesting methods, such as enhancing flocculation or designing cells with enzymes that degrade cell walls upon specific stimuli, ensuring proteins are released without cell walls.

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