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4. Alternative Proteins - Food and Feed for The Future

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Abstract:

The population boom and rising incomes will increase the global demand for meat in the near future. Sustainable alternative proteins could be a solution to reduce the protein gap i.e., the gap between the protein demand and protein supply. Alternative proteins are proteins sourced from plants, insects, fungi or through tissue culture to replace conventional animal based sources. The different types of alternative proteins are single cell proteins, food analogues, protein isolates, leaf protein concentrate, insect proteins and mushrooms. Single cell protein (SCP) is a generic term for crude or refined sources of protein obtained from unicellular or multicellular microorganisms such as yeast, bacteria, algae and fungi. Food analogue is a manufactured food product, designed to imitate a given food, possessing characteristics equal or superior to that of food. In vitro meat production is an emerging technology where cell culture and tissue engineering are involved to produce animal muscle in a laboratory. Isolates are the most refined form of protein products containing concentrated form of protein. Leaf protein concentrate is a nutritious protein source made by mechanically separating indigestible fibre and soluble anti-nutrients from fresh green leaves of plant. Consumption of insects or entomophagy is another area that needs to be explored. Mushrooms have a protein content ranging from 19.68 per cent to 36.96 per cent of dry weight. Hence, to meet the protein needs of the projected population of 10 billion by 2050, a change in food system with alternative proteins is necessary, which thereby helps to create a sustainable and healthy future with diminished environmental and health impacts.

Keywords:

Alternative proteins, Food analogues, Insect proteins, In vitro meat, Leaf protein concentrate, Mushrooms, Protein isolates, Single cell proteins

4.1 Introduction:

Today, due to population boom and rise in incomes there is an increase in the global demand for animal based proteins. Hence, one of the main concerns about world food supply is the production of proteins. Protein deficiency is one of the major nutritional problems in the world. In developing countries, the prevalence is 12.9 per cent and of developed countries is less than 5 per cent. This problem is not only the result of insufficient food production and inadequate distribution, but also the financial inability of the poor to purchase good quality animal protein. It is a global challenge to address food security and preserve land and water resources due to climate change, population growth and changing diets. Accordingly, interest in sustainable and biodiverse food systems is also on the rise. From a consumer's perspective, purchasing habits that can improve the environment are gaining prominence. Consumers are seeking transparency and sustainability in their food supply. Accordingly, food industries are interested in commercializing products formulated with ingredients derived from environmentally sustainable crops or process.

It is estimated that the livestock production does have an impact on environment, as there is emission of greenhouse gases such as methane and immense deforestation is taking place in the name of livestock production farms. To combat global protein deficiencies and to ensure the future of global food security it is required to make changes in the way we produce our food as well as in what we eat. Thus, food industrialists aim cheaper, high bioavailable and more convenient food products leading to development of novel protein sources. These sustainable alternative proteins could be a solution to reduce the protein gap i.e., the gap between the protein demand and protein supply.

4.2 Proteins:

The term protein is derived from a Greek word "*proteins"* meaning holding the first place. The term was coined by Jons Jacob Berzelius in 1838. Proteins are composed of amino acids and the main functions of protein in our body are body building and as an energy source providing 4 Kcal of energy per gram of protein.

Protein requirements vary with age, physiological status and stress. More proteins are required by growing infants and children, pregnant women and individuals during infections and illness or stress. Animal foods like milk, meat, fish and eggs, and plant foods such as pulses and legumes are rich sources of proteins.

4.3 Types of proteins:

Some of the types of proteins based on biological function other than body building and providing energy are:

- **Structural proteins:** Collagen / fibrous protein present in skin and bone. Also known as fibrous proteins, structural proteins are necessary components of your body. They include collagen, keratin and elastin. Collagen forms the connective framework of our muscles, bones, tendons, skin and cartilage. Keratin is the main structural component in hair, nails, teeth and skin.

- **Defensive protein:** Proteins in the form of clotting factors prevent the loss of blood and proteins in the form of immunoglobulins protect body from infections. Antibodies, or immunoglobulin, are a core part of immune system, keeping diseases at bay. Antibodies are formed in the white blood cells and attack bacteria, viruses and other harmful microorganisms, rendering them inactive.
- **Storage proteins:** Myoglobin is the protein that stores oxygen in muscles. Storage proteins store mineral ions in body. Iron, for example, is an ion required for the formation of hemoglobin, the main structural component of red blood cells. Ferritin - a storage protein - regulates and guards against the adverse effects of excess iron in your body. Ovalbumin is a storage proteins found in breast milk that play a huge role in embryonic development.
- **Receptor proteins:** Proteins responsible for transmitting nerve impulses. Located on the outer part of the cells, receptor proteins control the substances that enter and leave the cells, including water and nutrients. Some receptors activate enzymes, while others stimulate endocrine glands to secrete epinephrine and insulin to regulate blood sugar levels.
- **Hormonal and genetic regulatory proteins:** Coordinates the activities of different cells and genetic material. Hormones are protein-based chemicals secreted by the cells of the endocrine glands. Usually transported through the blood, hormones act as chemical messengers that transmit signals from one cell to another. Each hormone affects certain cells in your body, known as target cells. Such cells have specific receptors on which the hormone attaches itself to transmit the signals. An example of a hormonal protein is insulin, which is secreted by the pancreas to regulate the levels of blood sugar in your body.
- **Enzymes:** These are the proteins that catalyse chemical reactions in the body. Enzymatic proteins accelerate metabolic processes in cells, including liver functions, stomach digestion, blood clotting and converting glycogen to glucose. An example is digestive enzymes that break down food into simpler forms that your body can easily absorb.
- **Transport proteins:** These are the proteins such as albumin, heamoglobin that transport oxygen and iron through blood. Transport proteins carry vital materials to the cells e.g. hemoglobin, carries oxygen to body tissues from the lungs. Serum albumin carries fats in your bloodstream, while myoglobin absorbs oxygen from hemoglobin and then releases it to the muscles.

4.4 Alternative Proteins:

Alternative proteins are proteins sourced from plants, insects, fungi or through tissue culture to replace conventional animal based sources. These provide a substantial amount of protein, but requires less natural inputs (e.g. water) to produce, compared to the most common and conventional protein sources (i.e. meat and fish).

The advantages or rather need for alternative proteins is that a more sustainable alternative protein would help in diminishing environmental health impacts. Also, a thought to alternative proteins would help in developing products that are similar in sensorial characteristics to that of meat products. Most importantly it helps to reduce the protein gap.

4.5 Types of Alternative Proteins:

A. Single cell proteins
B. Food analogues
C. Protein isolates
D. Leaf protein concentrates
E. Insect proteins
F. Mushrooms

4.5.1 Single Cell Proteins (SCP):

Single cell protein is a generic term for crude or refined sources of protein whose origin is unicellular or multicellular microorganisms[1]. The term single cell proteins were coined by Carroll L. Wilson in 1966.

The advantages of single cell proteins are:

- Fast growth rate
- High yield of production
- Independent of seasons

There are different types of single cell proteins as shown in the Figure 4.1.

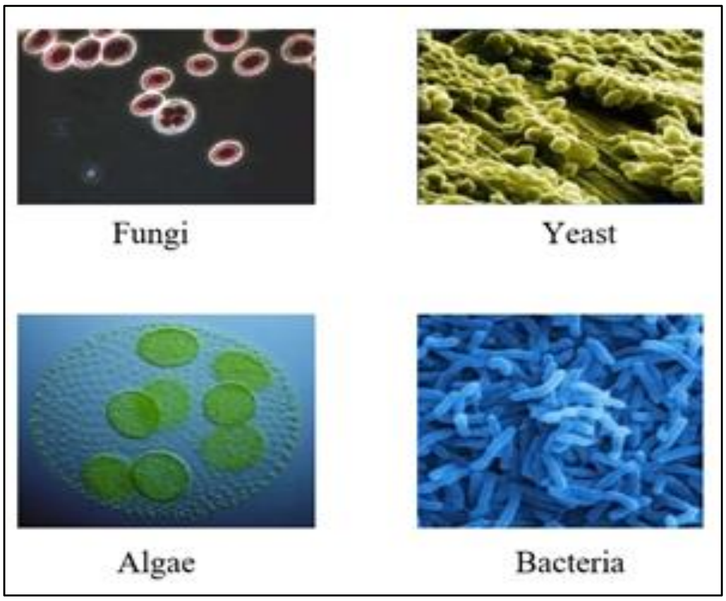

Figure 4.1: Types of SCP

Microorganisms can grow on a wide range of raw materials like low value wastes, cellulose, molasses, raffinose, whey *etc.* Different substrates used for SCP production are detailed in Table 4.1.

Table 4.1: Substrates used for SCP Production

Source	Example
Energy sources	Methanol, methane, ethanol, starch, glucose, maltose and lactose
Waste products	Molasses, raffinose, whey, cellulose, lignin, hemicellulose
Petroleum by-products	Natural gas and CO_2

A. Production of SCP:

The basic requirements for SCP production include pure culture of microorganism, a fermenter, bioreactor, thermostat, cooling device and an equipment for moisture removal.

The general protocol for SCP production is the addition of inoculum and the subsequent substrate along with the nutrients into the fermenter which is then aerated. When the microorganism is grown up to the required extent it is filtered which is then dried producing SCP.

The comparative evaluation of amino acids in SCP and egg reveals that leucine is the amino acid present in highest amount and methionine is the limiting amino acid. In India the SCP production is taking place at National Botanical Research Institute **(NBRI),** Lucknow and at Central Food Technological Research Institute **(CFTRI),** Mysuru.

4.5.2 Food Analogues:

Food analogues are manufactured food products, designed to imitate a given food, possessing characteristics equal or superior to that of food[2]. Different types of food analogues are discussed below.

A. Meat analogue:

Meat analogue, also called a meat substitute, mock meat, faux meat or imitation meat, approximates the aesthetic qualities and/or chemical characteristics of specific types of meat[3].

B. Textured vegetable protein (TVP):

TVP is a fabricated vegetable product mostly used to replace meat. It was invented at “Archer Daniel Midland” company in 1960. The TVP name is a registered trademark of this particular company. TVP is famous for its high protein and low fat content and it resembles meat mostly in terms of chewiness and flavor. There are two different types of TVP which is used as a meat analogue which is shown in the table (Table 4.2) below.

Table 4.2: Types of TVP used as Meat Analogue

Parameters	Dry meat analogue	Wet meat analogue
Moisture content (%)	20 – 40	50 – 70
Texture	Higher in springiness	Higher in hardness and cutting strength
Structure	Sponge like	Fibrous structure like muscle meat
Cooking	Rehydration prior to cooking	Direct cooking with added flavours
Shelf life	Within 6 months	Within 2 weeks
Storage	Dry and store at room temperature	In refrigerator
	Eg. Soy chunks 	

Green gram based meat analogue with 60 per cent green gram, 30 per cent wheat and 10 per cent soyabean could be prepared[4]. The method of preparation begins with pretreatment such as roasting of green gram for 15 minutes and soaking all the grains for 12 hours. To the pretreated grains, spices are added to enhance flavour. The mixture was then ground and steamed for 40 minutes which ones cooled were cut into desired shapes and kept for drying at 65°C for 18 hours. The organoleptic evaluation revealed great acceptance especially in terms of taste, texture and flavour. The nutritional quality evaluation (Table 4.3) showed that the developed meat analogue had a protein content of 26 g.

Table 4.3: Nutritional Quality Evaluation of Green Gram Based Meat Analogue

Nutrient	Amount (in 100g)
Protein (g)	26
Fat (g)	1.23
Dietary fibre (g)	1.82

Nutrient	Amount (in 100g)
Calcium (mg)	276
Potassium (mg)	72
Iron (mg)	1.89

C. Tempeh:

Tempeh is a compact and sliceable mass of cooked particles of raw materials covered, penetrated and held together by dense non-sporulated mycelium of *Rhizopus spp*[5]. The origin of tempeh was in Indonesia and the most isolated culture from tempeh is *Rhizopus oligosporus* and the most commonly used raw material for production of tempeh is soybean. The production process of soyabean based tempeh begins with the washing of soyabeans which is then boiled for 30 minutes. After boiling the soyabeans are washed again and is kept for soaking overnight. Then the dehulling is done and its surface is dried. To this vinegar is added and the inoculation is done after which it is kept for incubation at 32 C for 48 hours finally producing tempeh[6]. Tempeh could also be developed from raw materials other than soyabean such as green gram and combinations of green gram and rice. It was observed that the *invitro* digestibility of protein was higher for tempeh prepared with 100 per cent green gram than combinations of green gram and rice as well as 100 per cent soya bean and products such as tempeh roast, tempeh chips and soup mixes could also be developed from green gram based tempeh[7].

D. Tofu:

Tofu is another meat analogue also known as bean curd is prepared by coagulating soy milk and then pressing the resulting curds into solid white blocks of varying softness. It has a subtle flavour it can also be used in savory and sweet dishes. It is even possible to produce tofu with probiotic bacteria that has acceptable sensory characteristics by inoculating *Lactobacilus casie*, incubated at 37^0C for 2 hours with $CaSO_4$ which is the recommended treatment for commercial production. The protein content in normal tofu is 45 per cent and the probiotic incorporated tofu is 48.2 per cent[8].

E. In Vitro Meat:

In vitro meat in simple terms is the process of culturing muscle like tissue in liquid medium. The application of in vitro meat has been successful on producing ground and processed foods such as hamburgers. The goal of the industries involved in production of in vitro meat is to create traditional meat pieces such as chicken wing or beef steak.

The idea of developing cultured meat was predicted by Winston Churchill in 1920. Later in 1950 a Dutch researcher, Willian Van Eelen developed the idea of using tissue culture to develop *in vitro* meat. In 1995, the US Food and Drug Administration approved the commercial production of invitro meat and in 1999 William Van Eelen received the first patent. In 2013 Mark Post presented and sampled the first lab grown hamburger which received great appreciation and critical evaluation.

For the production process, first a technician takes cells from a live animal. Then these cells are grown up in a lab to permanently establish a culture called a cell line. Once a good cell line has been established, a sample is introduced into a bioreactor which is essentially a culture medium containing all the materials the cells need to grow. The cells in the bioreactor grow and multiply exponentially and are then harvested. Once harvested, the meat cells can be formed into any number of items such as patties and sausages.

The advantages of invitro meat when compared to that of livestock production are:

- Possibility in production of exotic meat
- For the same mass of meat, the nutrients and time needed for growing *in vitro* meat is less.
- There is better control over meat composition and quality
- The water, energy and land requirement for the production of invitro meat is less which could possibly alleviate the environmental burden

The possibility of requirement of less resources and energy for the production *of in vitro* meat was proved through a study conducted at Oxford University's Wild Conservation Research Unit and the results are depicted in the figure 4.2 and it suggests that when compared to livestock production, the energy and the land required for growing *in vitro* meat are 35 - 60 per cent and 80 - 95 per cent less respectively[9].

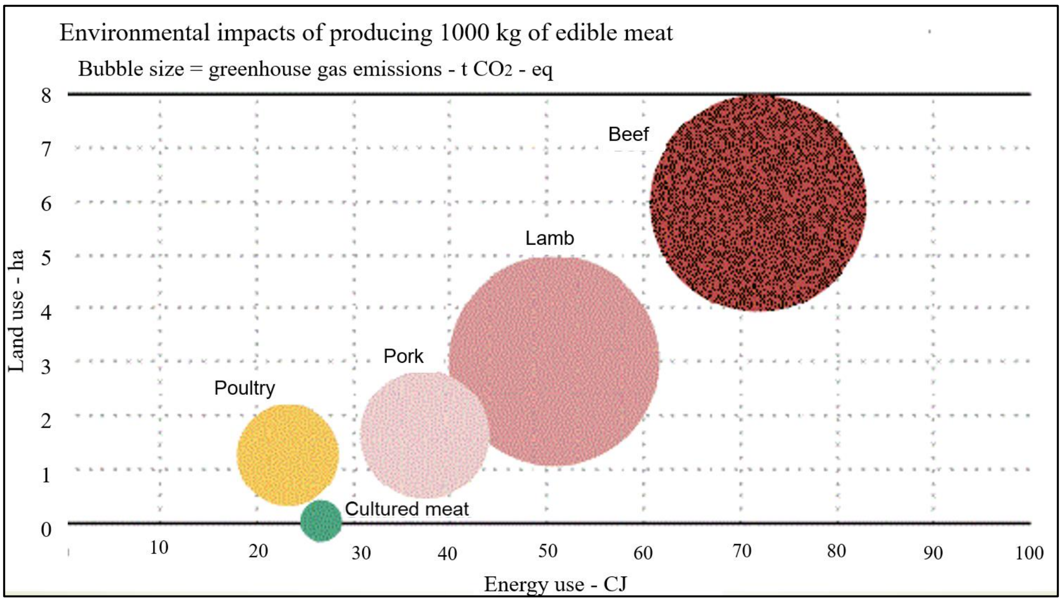

Figure 4.2: Environmental Impact of Producing Meat

The first cultured meat to get approval for sale was produced by the company "**Eat Just Inc.**" under the brand name "**Good Meat**" in the year 2020 at Singapore. The product was chicken nuggets and it is sold for a price of $ 17.

Figure 4.3: In vitro meat

Figure 4.4: Chicken nuggets from cultured meat

4.6 Dairy Analogues:

Dairy analogue is an imitation product that is designed or structured to mimic or offered as an alternative/replacement to a milk or milk product by partial or full substitution with other components from non-dairy sources.

A. Cheese analogue:

Development of cheese analogues involves the use of fat and/or protein sources other than those native to milk, together with a flavor system simulating as closely as possible that of the natural product.

The major advantages of cheese analogues are that

- It helps to meet special dietary needs
- Manufacturing is simple
- Cost effective

The production of cheese analogue first the temperature of vegetable fat is raised to around 70^0 C and to this stabilizer such as sodium phosphate or sodium citrate is added forming an emulsion. Then protein, salt, acid and flavor are added and then it is refrigerated and stored. The protein content is comparable and the fat content is much less in cheese analogue when compared to that of natural cheese which makes cheese analogue a healthier alternative[10] (Table 4.4).

Table 4.4: Nutritional Qualities Comparison of Natural Cheese and Cheese Analogue

Nutrients	Natural cheese	Cheese analogue
Protein (g)	23.06	20.13
Fat (g)	24.56	15.23

Nutrients	Natural cheese	Cheese analogue
Calcium (mg)	0.62	0.39
Ash (%)	3.18	3.91

The beneficial aspect of synthetic cheese analogue is that many such products can be produced with high shelf life. Cheese analogue was functionally more stable during refrigerated storage than natural cheese. Such stability makes analogues very attractive to the food processing and service industries. They have consistent quality, without seasonal variations and they can be varied to meet desirable quality characteristics. Production can be scheduled to meet sales needs, eliminating or significantly reducing storage and refrigeration costs[11]. The different quality attributes of cheese analogue are given in table 4.5.

Table 4.5: Advantages of cheese analogue

Quality attributes	Natural cheese	Cheese analogue from soy protein
Shelf life	Low	High
Seasonality	Affects quality	Do not affect quality
Refrigerated storage	Low stability	High stability
Cost of production	High	Low
Nutritional quality variation	Can't design	Can design

B. Vegan paneer:

Vegan paneer is another dairy analogue with base ingredients soy and groundnut. The best vegan paneer is prepared with the addition of 10 per cent ground nut milk to 90 per cent soy milk due to the ability of groundnut milk to mask the bean flavor of soy. The protein content of soy-groundnut paneer is 15.5 per cent which is comparable to that of soy paneer[12].

4.7 Protein Isolates:

Isolates are the most refined form of protein products containing the concentrated form of protein but unlike flour and concentrates they contain no dietary fibre[13]. It is digestible, can be easily incorporated into different food products. Protein isolates are widely produced from cereal bran, deoiled cake of legumes, nuts and oil seeds.

For the production of protein isolates first the defatted nuts or seeds are taken along with water in the ration 1:10. Then the pH is adjusted to 9.5 and the slurry is centrifuged. The next step is precipitation after which the precipitate is taken and neutralized. It then undergoes freeze drying and is ready for storage[14].

Some of the examples of legumes, nuts and oilseeds used for protein isolation are rapeseed, groundnut, hemp seed, pea, sunflower seed, soybean *etc.* The comparison of essential amino acids of oilseeds such as chia, hemp and sesame depicts that leucine is the highest amino acid followed by valine and the methionine content is comparable to that of chicken[15] (Figure 4.5).

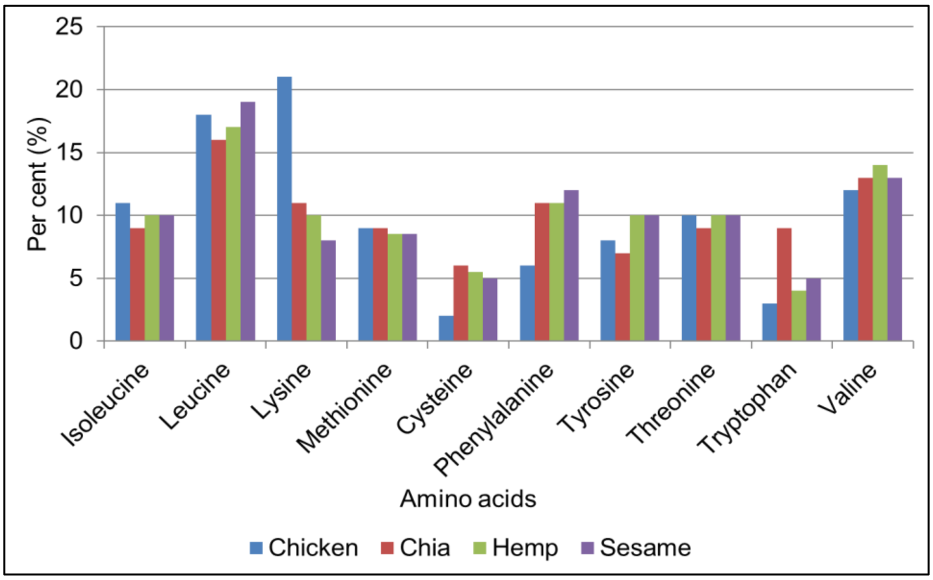

Figure 4.5: Comparison of Essential Amino Acids of Oilseeds with Chicken

The therapeutic benefits of rapeseed are due the presence of bioactive protein peptides that shows Angiotensin Converting Enzyme (ACE) inhibitory activity. This ACE is a key enzyme that regulate the blood pressure. In disease conditions the ACE increases which develops undesirable high blood pressure. Hence rapeseed could be a natural peptide that can act as an ACE inhibitor for the treatment of hypertension. Also rapeseed contains phenolic compounds such as sinapine which shows antioxidant activity[16].

Cereals are edible seeds from agricultural grasses and bran is the skin of an edible seed. Bran is obtained while dehusking and polishing whole grain in order to obtain polished grain. The different proteins form in cereal brans are albumin, globulin, prolamin and glutelin and the major sources are wheat, maize, oat and rice. Products such as rice bran based cookies is being developed from the rice variety *Rakthasali* depicting the successful incorporation of rice bran with the protein content in rice being 11.36g and in the rice bran cookies was 8.78 g/ 100 g[17].

4.8 Leaf Protein Concentrate:

Leaf protein concentrate (LPC) is a nutritious protein source made by mechanically separating indigestible fiber and soluble antinutrients from much of the protein in certain fresh green leaves of plant[18]. It is a good source of amino acids and polyphenols and the its production cost is also low.

For the production of LPC the leaves are collected and its moisture and pH are adjusted which is an important step for better protein extractability. The moisture should be greater than 95 per cent and pH should be greater than 7.5. To achieve this, the leaves are to be blended with distilled water.

The pulp is then filtered and to get maximum juice out of the cake, it is pressed further. Then the filterate is acidified with drops of HCl and heated till the protein coagulate. It is again filtered and it is then oven dried forming protein concentrate[19].

Among common plant leaves such as pumpkin, amaranth, sugar been, sweet potato, cowpea and cabbage, pumpkin leaves recorded the highest protein yield (11.75 %) followed by amaranth leaves (10.7 %). The protein from amaranth is very smooth and less fibrous. The protein contents in the leaves of sugar been, sweet potato, cowpea and cabbage were 8.85 per cent, 7.85 per cent, 6.95 per cent, and 5.60 per cent respectively[19].

4.9 Insect Proteins:

Insect consumption or entomophagy is not a new concept but has been traditionally followed in different parts of the world such as Africa, Asia and Latin America. The most widely consumed insects are caterpillars, palm weevil larvae, termites, stink bugs and grasshoppers. For the commercial production, house cricket, yellow mealworm and palm weevil are the ones mostly being used. The usual negative emotional response toward acceptability of insects as food by consumers is gradually fading away, as many insect containing food products are finding their ways into the market.

More than 1900 species have reportedly been used as food. Insects are a highly nutritious and healthy food source with high fat, protein, vitamin, fibre and mineral content. The nutritional value of edible insects is highly variable because of the wide range of edible insect species.

Even within the same group of species, nutritional value may differ depending on the habitat in which it lives, and its diet. For example, the composition of unsaturated omega-3 and six fatty acids in mealworms is comparable with that in fish (and higher than in cattle and pigs), and the protein, vitamin and mineral content of mealworms is similar to that in fish and meat. To make insects commonly used as human food, it is necessary to develop the technology which will allow large scale productions at a reasonable cost.

The processing methods that can be applied on insects for consumption are drying, pulverizing or grounding, can be converted into paste, heating methods (cooking, boiling, frying, roasting, toasting, extrusion and canning) can be used and for preservation freeze drying or vacuum packing can be done after degutting.

The incorporation of silkworm pupae and locust replacing skim milk powder in high energy biscuit is observed to have the protein content comparable to that of skim milk powder when it was replaced with 15 per cent edible insect powder. Also, vitamin c content was also very high with the incorporation of insect powders. The use of insects to replace skim milk powder would reduce the overall cost of production of high energy biscuits[20].

The protein content in different insect forms[21] are depicted in the Table 4.6 and some of the commercially available insect products are shown in Figure 4.6.

Table 4.6: Protein Content in Various Insect Forms

Insect forms	Protein (%)
Termites	23.3
Caterpillars	38.1
Adult weevils	30.3
House fly pupae	63.1
May beetle larvae	11.1
Bee	18.1
Silkworm	23.1
Grasshopper	46.1

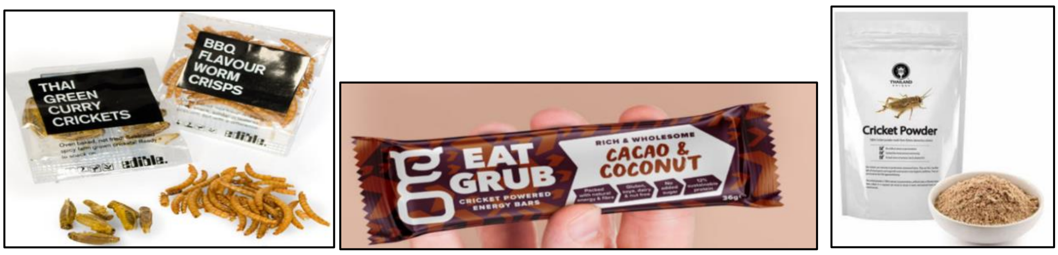

Figure 4.6: Insect Products available in International Market

4.10 Mushrooms:

Mushrooms have a protein content ranging from 19.68 per cent to 36.96 per cent of dry weight. It contains several bioactive compounds that providing antifungal, antibacterial, antioxidant and antiviral properties in mushroom. The production of status of mushroom in India, shows that white button mushroom is cultivated highest (73 %) followed by Oyster mushroom (16 %), Paddy straw mushroom (7 %), milky mushroom (3 %). Production of all other types of mushrooms constituted to (1 %). The protein content of composite maize flour was found to increase from 6.9 g/100 g to 19.32 g/100 g when oyster mushroom was added up to 50 per cent[22].

A. Limitations:

- Less acceptance
- Allergenic factors
- Ant nutritional factors
- Lack of production and extraction process
- Toxicity testing and detailed long term studies are scanty

4.11 Conclusion:

To meet the protein, need of projected population of 10 billion by 2050 is a challenge. Change in food system could be a solution for which innovation and experimentation in food especially in alternative proteins is essential in order to meet the goal of creating a safe, sustainable, affordable and healthy future.

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